Hypertension 2018 Scientific Sessions Abstracts

001

Loss of ET_B Receptor Function Activates Inflammasome Signaling Pathways and Dendritic Cells in the Renal Outer Medulla During Type 1 Diabetes

Authors: Carmen De Miguel, David M Pollock, Jennifer S Pollock, Univ of Alabama at Birmingham, Birmingham, AL

Renal inflammation is a hallmark of diabetic kidney disease. Endothelin-1 (ET-1), a potent vasoactive peptide that acts through two receptors (ET_A and ET_B), has been implicated in diabetes and is upregulated in patients with diabetic nephropathy (DN) and in animal models of diabetic kidney injury. ET_B receptors are highly expressed in renal outer medulla (OM). ET-1 exerts pro-inflammatory actions in the kidney; however, the mechanisms by which ET-1 mediate these effects are unclear. The present studies were designed to determine the role of the ET_B receptor in priming inflammasome signaling pathways, as well as its role in the activation of dendritic cells (DCs), in the renal OM during type 1 diabetes. Diabetes was induced in ET_B deficient (ET_B def) and transgenic (TG) control rats by i.v. injection of streptozotocin (STZ). 10 wk later, 24-h urine and kidneys were collected for analysis of kidney damage. Diabetic ET_B def rats presented exaggerated kidney damage compared to diabetic TG controls, with greater excretion of protein, albumin and ET-1, cortical expression of KIM-1 (respectively: 90±12 vs. 45±6 mg/day, 41±8 vs. 12±4 mg/day, 18±1 vs. 15±1 pg/day, 12±1 vs. 7±1 pg/mg prot; n=4- 6/group, $p<0.05$), and excessive kidney fibrosis, tubular dilation and cell death. Immunohistochemistry for the DC activation marker CD83 also demonstrated exaggerated activation of DCs in renal OM of diabetic ET_B def rats. Expression of inflammasome genes in renal OM was assessed by RT-PCR array. Diabetes led to upregulation of NLRP5 (4-fold increase vs. TG controls; n=3/group; $p<0.05$) and IL-1β (~3-fold increase vs. TG controls; n=3/group; $p<0.05$) in OM of ET_B def rats. In addition, PSTPIP1, a negative regulator of the inflammasome, was decreased in OM of diabetic ET_B def rats vs. TG controls. Together, these results demonstrate that dysfunction of the ET_B receptor leads to worsening of diabetic kidney injury and overactivation of the inflammasome and DCs specifically in the renal OM. These results highlight the protective role of ET_B receptors against the development of diabetes- induced kidney inflammation and damage. Funded by NIH T32 DK007545 to CDM and P01 HL69999, P01HL136267, AHA SFRN 24450002 and U01HL117684 to DMP and JSP.

Disclosures: C. De Miguel: None. **D.M. Pollock:** None. **J.S. Pollock:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

002

Macula Densa SGLT1-NOS1-TGF Pathway -- A New Mechanism for Glomerular Hyperfiltration in Diabetes

Authors: Jie Zhang, Shan Jiang, Jin Wei, Lei Wang, Ruisheng Liu, The Univ of South Florida, Tampa, FL

Glomerular hyperfiltration is a common observation in early diabetes and considered as risk factor for diabetic nephropathy. The mechanisms underlying glomerular hyperfiltration have not been fully clarified. In the present study, we tested a novel SGLT1-NOS1 -TGF (sodium-glucose cotransporter 1 - neuronal nitric oxide synthase – tubuloglomerular feedback) pathway with a hypothesis that tubular glucose is sensed by SGLT1 of the macula densa, which enhances macula densa NOS1 activity and blunts TGF response, therefore increasing glomerular filtration rate (GFR). NO generation by the macula densa, measured by DAF-2 DA in isolated perfused juxtaglomerular apparatus (JGA), increased by 78.4±9.1% when tubular glucose (glucose) was increased from 100 mg/dl to 300 mg/dl (n=4, $p<0.01$ vs 100

mg/dl). A selective SGLT1 inhibitor KGA-2727 (10^{-6} M) blocked this glucose-induced NO generation. Human kidney biopsy tissues were cultured in normal glucose (100 mg/dl) and high glucose (300 mg/dl) media for 30 minutes with hanging drop technique. Protein levels of P-NOS1^{ser1417} increased by 63.1±9.2% and P-NOS1^{ser847} decreased by 47.4±5.9% (n=4, p<0.01 vs normal glucose) with high glucose. TGF response was significantly reduced from 3.8±0.2 µm to 2.4±0.2 µm (n=4, p<0.05 vs 100 mg/dl), measured in isolated and double perfused JGAs when tubular glucose was increased from 100 mg/dl to 300 mg/dl. The glucose-induced TGF inhibition was blocked in the presence of SGLT1 inhibition (n=4). GFR, measured in conscious mice with a single bolus injection of FITC-inulin, increased by 19.1±3.5% in response to an acute intravenous injection of 50 µl 2M glucose in C57BL/6 mice (n=4, p<0.01 vs baseline). Then, we repeated the experiments in macula densa specific NOS1 KO (KO) mice. Both glucose-induced TGF (ΔP_{sf} was from 7.8±1.3 to 7.3±2.1 mmHg) and GFR (from 223±15.7 to 240±6.9 µl/min) (n=5) were blocked in the KO mice. We conclude that increase of tubular glucose concentration activates NOS1 by phosphorylation of NOS1^{ser1417} via SGLT1 in the macula densa, which increases GFR by inhibiting TGF response.

Disclosures: J. Zhang: None. **S. Jiang:** None. **J. Wei:** None. **L. Wang:** None. **R. Liu:** None.

Funding: No

Funding Component:

003

Mrap2 Dissociate Mc4r-Mediated Sympathetic Control of Metabolism and Blood Pressure

Authors: Deng-Fu Guo, Balyssa Bell, Donald A Morgan, Huxing Cui, Julien A Sebag, Kamal Rahmouni, The Univ of Iowa, Iowa City, IA

The brain melanocortin-4 receptor (MC4R) plays an important in the control of food intake and energy expenditure by increasing thermogenic sympathetic nerve activity (SNA). In addition, brain MC4R-mediated activation of SNA subserving cardiovascular organs such as the kidneys increase blood pressure. The melanocortin receptor accessory protein 2 (MRAP2) regulates the activity of the MC4R and other G-protein coupled receptors, but its specific role in MC4R-containing neurons is not clear***. We hypothesized that MRAP2 in MC4R neurons is essential for the regulation of SNA, energy homeostasis and blood pressure.*** We generated mice (MRAP2^{MC4R}) that lack MRAP2 in MC4R neurons by crossing MRAP2$^{fl/fl}$ mice with MC4R^{Cre} mice and confirmed Cre recombinase activity in MC4R neurons using td-Tomato reporter mice. Interestingly, the MRAP2^{MC4R} mice displayed obesity as indicated by the increased (p<0.05) body weight (males: 40.1±2.2 vs 32.9±0.9g in littermate controls, females: 28.6±2.8 vs 24.2±1.3g in controls at 16 weeks of age) and fat mass, but not lean mass. Weights of fat pads and liver were also significantly elevated in the MRAP2^{MC4R} relative to littermate controls. Food intake was increased in the MRAP2^{MC4R} mice relative to littermate controls (males: 4.7±0.3 vs 3.9±0.3g and females: 3.5±0.2 vs 2.8±0.2g). Glucose and insulin tolerance tests revealed impaired glucose tolerance and insulin-induced glucose clearance in MRAP2^{MC4R} mice compared to littermate controls. Baseline brown adipose tissue (BAT) SNA tended to be lower in MRAP2^{MC4R} mice relative to controls. Moreover, intracerebroventricular (icv) injection of MTII (MC4R agonist, 1 µg) increased BAT SNA in control mice (107±31%) but not in MRAP2^{MC4R} mice (18±24%, p=0.02). On the other hand, icv the MTII (1 and 2 µg)-induced increase in renal SNA (p=0.2) and blood pressure (p=0.4) was not different in MRAP2^{MC4R} (100±32% and 15±8mmHg, respectively) relative to controls (74±24% and 12±6mmHg). These findings support a critical role for MRAP2 in uncoupling neuronal MC4R-mediated control of regional sympathetic traffic impacting energy metabolism versus blood pressure.

Disclosures: D. Guo: None. **B. Bell:** None. **D.A. Morgan:** None. **H. Cui:** None. **J.A. Sebag:** None. **K. Rahmouni:** None.

Funding: Yes

Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

004

Caloric Restriction Prevents the Increase in Body Weight but not Hypertension in ALMS1 (Alström syndrome 1) Knockout Rat

Authors: Keyona N King-Medina, Wayne State Univ Sch of Med, Westland, MI; Pablo A. Ortiz, Henry Ford Hosp, Hypertension and Vascular Res Div, Dept of Internal Med, Wayne State Univ Sch of Med, Detroit, MI

In humans, mutations in the ALMS1 gene cause obesity, diabetes and kidney disease. The mechanisms causing these alterations are unclear. We generated ALMS1 knockout (KO) rats in the Dahl salt-sensitive (SS) genetic background and found that ALMS1 KO are hypertensive (telemetry or tail-cuff) and develop obesity and insulin resistance. We found that ALMS1 is expressed in the kidney where it regulates thick ascending limb NaCl reabsorption. However, it is unclear whether the hypertension in the ALMS1KO occurs prior to or after the development of obesity and insulin resistance. W*e hypothesize that hypertension in the ALMS1 KO rats is primarily caused by a renal defect and not secondary to obesity*. We studied the effect of control feeding or caloric restriction (CR) in body weight (BW), blood glucose, glucose tolerance test (GTT) and systolic blood pressure (SBP) by tail-cuff in ALMS1 KO and wild-type Dahl SS (WT) littermate rats. A control group of ALMS1 KO and WT rats received 30 g/day regular chow (0.4% Na). A caloric restricted (CR) group received 20% less food (24 g/day) of a custom diet with 20% higher Na, vitamins and minerals, thereby matching Na intake. At 7 weeks of age, ALMS1 KO rats had similar BW (KO: 209±13 g vs. WT: 182±15 g), baseline glucose (KO: 88±3 vs. WT: 75±7 mg/dL, N.S.) and GTT. Importantly, SBP was higher in ALMS1 KO rats (KO: 156±5 vs. WT: 143+3 mmHg, $p<0.05$). By 11 weeks of age, KO rats had a greater BW (KO: 393±9 vs. WT:344±5 g, $p<0.05$) and higher SBP (KO:186±7 vs. WT:162±7 mmHg, $p<0.05$). Baseline glucose was higher (KO:101±5 vs. WT:65±4 mg/dL, $p<.001$) and peak levels of glucose during GTT were higher (KO:464±21 vs. WT:341±33, mg/dL, $p<.0.05$) in KO rats. Caloric restriction for 4 weeks prevented the difference in BW between KO and WT (KO:359±8 vs. WT:340±3 g, N.S.) and lowered BW compared to control feeding (KO control: 393±9 vs. KO- CR: 359±8 g, $p<0.05$). However SBP remained higher than in WT (KO:178±9 vs. WT:151±3 mmHg, $p<0.05$) and was not different from KO fed control diet. GTT was also not different between KO control and KO-CR. We conclude that the hypertension in rats with ALMS1 deficiency occurs prior to obesity and metabolic syndrome, and that CR prevents the increase in BW but does not prevent hypertension or improve insulin resistance in this rat model.

Disclosures: K.N. King-Medina: None. **P.A. Ortiz:** None.

Funding: No

Funding Component:

005

Hemodynamic and Renal Changes in Obese Patients 12 Months After Bariatric Surgery

Authors: Anna Oliveras, Ana M Granados, Sara Alvarez, Albert Goday, Laia Sans, Susana Vazquez, Anna Faura, Julio Pascual, Hosp del Mar, Barcelona, Spain

Aim: to study changes in central and peripheral 24h-BP, as well as in estimated glomerular filtration rate (eGFR) and albuminuria (Alb), 12 months after bariatric surgery (BS). **Methods**: Sixty-two patients (39% hypertensives) with severe obesity (BMI 42.7±5.6 Kg/m^2) were evaluated prospectively before (0) and 1,3,6 and 12 months after undergoing BS. Peripheral and central BP parameters were determined by the Mobil-O-Graph device. Renal function was evaluated by eGFR and Alb. Generalized estimation equations were used to evaluate repeated measures in the variables analyzed.

Results: at 12 months weight (-35.7±9.9 Kg) and waist circumference (-27 ± 9.2 cm) decreased (p<0.001 for both). The Table shows a significant decrease in only systolic BP, in both the 24h and daytime periods, but not in the nocturnal period. There was a decrease in plasma aldosterone (Ald) levels (mean,95%CI: -36.7 pg/mL, -60 to -13.4, p=0.003). After adjusting for age, BP_0, variation (Δ) of weight, Δ Ald and having or not HT at baseline, the only significant covariables in Δ 24h-SBP and Δ day-SBP were previous HT and Δweight. There was a decrease in eGFR (mean,95%CI: -5.6 mL/min/1.73m^2, -9.4 to -1.8, p=0.005) and Alb (Z=-2.04,p=0.042). According to the constructed variance analysis models, the Δ Alb was dependent on Alb_0 and on Δ night-SBP, but not on the baseline hypertensive state, on the Δ eGFR nor on the Δweight (adjusted r^2=0.606). Δ eGFR was independent of baseline eGFR and Δ weight. **Conclusions:** patients with morbid obesity significantly reduce heart rate and 24h- and day- SBP, both central and peripheral, 12 months after undergoing BS. In addition, there is a significant decrease in plasma Ald, eGFR and Alb.

	Change at 12 months post-BS Median (95% CI)	p	Change at 12 months post-BS Median (95% CI)	p
	PERPHERAL		CENTRAL	
Office-SBP	-7,4 (-11.0 a -3.8)	<0.001	-7,2 (-10.6 a -3.8)	<0.001
Office-DBP	-6.5 (-11.1 a -2.0)	0.001	-4.8 (-7.4 a -2.2)	0.001
24h-SBP	-2.7 (-5.1 a -0.3)	0.03	-3.3 (-5.7 a -0.9)	0.008
24h-DBP	-0.9 (-2.5 a 0.7)	NS	-1.4 (-3.1 a 0.3)	NS
Daytime-SBP	-3.2 (-2.4 a -5.8)	0.02	-3.4 (-6.0 a -0.9)	0.009
Daytime-DBP	-1.3 (-3.0 a 0.5)	NS	-2.4 (-4.3 a -0.5)	0.02
Nighttime-SBP	-0.2 (-3.6 a 3.1)	NS	-1.0 (-4.4 a 2.3)	NS
Nighttime-DBP	1.7 (-1.1 a 4.5)	ns	3.0 (0.7 a 5.3)	0.01

Disclosures: A. Oliveras: None. **A.M. Granados:** None. **S. Alvarez:** None. **A. Goday:** None. **L. Sans:** None. **S. Vazquez:** None. **A. Faura:** None. **J. Pascual:** None.

Funding: No

Funding Component:

006

A Dual Role of 12/15-lipoxygenase in Lipopolysaccharide-Induced Acute Renal Inflammation and Injury

Authors: Ahmed A Elmarakby, Ahmed S. Ibrahim, Mohamed A. Katary, Ahmed M. Abd El Razek, Nehal M, Elsherbini, Mohamed Al-Shabrawey, Augusta Univ, Augusta, GA

Recent studies suggest a potential role of bioactive lipids in lipopolysaccharide (LPS) induced acute kidney injury. The current study was designed to determine the profiling activities of various polyunsaturated fatty acid (PUFA) metabolizing enzymes, including lipoxygenases (LO), cyclooxygenase and cytochrome P450 in the plasma of LPS-injected C57BL/6 mice (4 mg/kg i.p) using LC-MS. Heat map analysis revealed that out of 126 bioactive lipids screened, only the 12/15-LO metabolite, 12-HETE, had a significant (2.24 ± 0.4) fold increase relative to control (P= 0.0001). We then determined the role of the 12/15-LO in LPS-induced acute kidney injury using genetic and pharmacological approaches. Treatment of LPS injected mice with the 12/15-LO inhibitor, baicalein significantly improved creatinine clearance (0.05±0.01 ml/min in baicalein treated vs. 0.02±0.004 ml/min in untreated LPS injected mice) and reduced albumin to creatinine ratio (54±18 µg/mg in baicalein treated vs. 143±31 µg/mg in untreated LPS injected mice) in LPS injected

mice. Baicalein treatment significantly reduced markers of renal inflammation {urinary thiobarbituric acid reactive substance (TBARs), urinary monocyte chemoattractant protein-1 (MCP-1), renal intercellular adhesion molecule 1 (ICAM-1), and tumor necrosis factor-α (TNF-α) levels}. Knocking-out of 12/15-LO also reduced markers of renal inflammation and injury elicited by LPS injection. Next, we sought to divert the role of 12/15-LO from being pro-inflammatory to anti-inflammatory via dietary supplementation with docosahexaenoic acid (DHA) and generation of anti-inflammatory metabolite, resolvin D2 (RvD2). DHA-treatment significantly reduced marker of renal injury and inflammation in LPS-injected mice whereas DHA treatment failed to provide any synergistic effects in reducing renal inflammation and injury in LPS injected 12/15-LO knock-out mice by increasing plasma RvD2 levels. This was further confirmed by exogenous RvD2 administration which significantly reduced the elevation in renal injury and inflammation in LPS injected mice. In conclusion, our data suggest a double-edged sword role of 12/15-LO in LPS-induced acute renal injury, depending on the type of substrate available for its activity.

Disclosures: A.A. Elmarakby: None. **A.S. Ibrahim:** None. **M.A. Katary:** None. **A.M. Abd El Razek:** None. **N.M. Elsherbini:** None. **M. Al-Shabrawey:** None.

Funding: No

Funding Component:

007

Exogenous Delivery of Alpha-Calcitonin Gene Related Peptide Inhibits Apoptosis and Oxidative Stress and Protects Against Pressure-Induced Congestive Heart Failure

Authors: Ambrish Kumar, Scott Supowit, Jay D Potts, Donald J DiPette, Univ of South Carolina, Columbia, SC

Congestive heart failure (CHF) is the leading cause of mortality in men and women world-wide, despite multiple but limited treatment modalities. Our laboratory and others have established that α-calcitonin gene-related peptide (αCGRP) plays a significant protective role in several cardiovascular diseases. αCGRP is a 37-amino acid neuropeptide and potent vasodilator. In αCGRP-knockout mice, pressure overload significantly exacerbates cardiac hypertrophy, dysfunction and fibrosis. The present study was performed to determine if exogenous delivery of native αCGRP was cardio-protective against pressure overload-induced CHF. Transverse aortic constriction (TAC) was performed to induce pressure overload CHF in nine week old C57/BL6 mice. One group were sham-treated, one TAC alone, and one TAC plus αCGRP (4 mg/kg bwt/day) via an implanted mini-osmotic pump. All mice had serial echocardiography performed and were sacrificed and hearts collected after 28 days. ELISA data showed that αCGRP levels in the TAC left ventricle (LV) were significantly lower compared to sham LV, while αCGRP in the TAC-CGRP LVs was similar to sham levels. TAC alone significantly decreased fractional shortening (FS) and ejection fraction (EF), and increased cardiac hypertrophy, apoptosis, and fibrosis in the LV compared to sham mice. αCGRP in the TAC mice significantly preserved LV FS and EF, (FS ±SEM: sham 46.2±1.8%, TAC 25.4±1.1%, and TAC-CGRP 36.6±1.2%; EF ±SEM: sham 78±1.9%, TAC 51.3±1.5%, and TAC-CGRP 67.4±1.5%) and attenuated apoptosis (measured by cleaved caspase-3 and TUNEL staining), fibrosis, and oxidative stress (measured by lipid peroxidation, glutathione, and 8-OHdG levels) compared to TAC alone. Moreover, TAC increased the expression of sirtuin proteins (Sirt1, 2, 3, 5, and 7) and phosphorylation of AMPK, both of which are involved in oxidative stress and energy metabolism. This increase in Sirt-1 and -2 protein level was significantly attenuated by αCGRP. In addition, αCGRP markedly reduced phospho-AMPK level in the TAC LV back to control levels. Our results show that αCGRP protects against pressure-induced CHF which may be mediated by the inhibition of myocyte apoptosis and reduction in oxidative stress. Thus, αCGRP is an exciting therapeutic agent in CHF.

Disclosures: A. Kumar: None. **S. Supowit:** None. **J.D. Potts:** None. **D.J. DiPette:** None.

Funding: No

Funding Component:

008

Chronic Central Melanocortin 4 Receptor Activation Attenuates Cardiac Dysfunction After Myocardial Infarction In Rats

Authors: Fabio N. Gava, Alexandre A. da Silva, John E. Hall, **Jussara M do Carmo**, UMMC, Jackson, MS

The melanocortin pathway plays an important role in multiple physiological functions besides its effect on energy homeostasis. In the present study we tested whether activation of the brain melanocortin 4 receptors (MC4R) confers protection against cardiac dysfunction after myocardial infarction (MI). Male Sprague-Dawley rats at 12 weeks of age were implanted with blood pressure 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 3100$^{@}$). After stable baseline measurements for 4 days, the left coronary descending artery was permanently ligated and vehicle (n=6) or the MC4R agonist (MTII, 10 ng/hr, n=7) was infused ICV via osmotic minipump for 28 consecutive days. Chronic MC4R activation significantly decreased cumulative food intake (-121±17 g) and body weight (385±5 to 378±4 g) compared to vehicle treatment (-51±10 g and 358±9 to 427±9 g). Chronic MTII infusion did not significantly alter MAP and HR (113±1 to 115±2 mmHg and 356±3 to 349±9 bpm) whereas MAP and HR were slightly reduced in vehicle-treated rats with MI (109±1 to 105±3 mmHg and HR 357±3 to 324±5). Compared to vehicle, MTII infusion for 4 weeks attenuated cardiac dysfunction caused by MI as evidenced by normalization of global cardiac radial strain (39±4 to 39±3 vs. 37±4 to 25±3 %), cardiac output (117±9 to 117±8 vs. 105±6 to 83±9 ml/min) and improvement of ejection fraction (72±2 to 47±3 vs. 70±2 to 30±2 %). These results suggest that chronic central MC4R activation attenuates the progression of heart failure after MI in rats. (NHLBI-PO1HL51971, NIGMS- P20GM104357 and U54GM115428)

Disclosures: F.N. Gava: None. **A.A. da Silva:** None. **J.E. Hall:** None. **J.M. do Carmo:** None.

Funding: No

Funding Component:

009

Regulatory Role of Endothelial Sirt3 on Blood Pressure and Diastolic Dysfunction in a Female Mice

Authors: Heng Zeng, Rebecca A. Worsham, **Jian Xiong Chen**, Univ Mississippi Medical Ctr, Jackson, MS

Heart failure with preserved ejection fraction (HFpEF) accounts for almost one half of the heart failure population which is characterized by a diastolic dysfunction. HFpEF is strongly associated with advanced age and metabolic comorbidities as well as endothelial dysfunction. Sirt3 emerged as a protein of particular interest to the aging due to its association with long lifespans in humans. SIRT3KO mice have decreased time to development of age-related diseases. Diastolic dysfunction is increasingly prevalent in aged women. Clinical studies have shown that obese and diabetic women are more likely to develop diastolic dysfunction than their male counterparts. Our study showed that ablation of endothelial Sirt3 led to diastolic dysfunction in male mice. However, the role of endothelial SIRT3 deficiency on blood pressure and diastolic function in female mice remains to be investigated.
In the present study, we demonstrate that ablation of endothelial SIRT3 in females led to an increase in blood pressure as compared with control SIRT3 LoxP female mice. Diastolic function measurement also showed that IVRT and MPI were significantly increased whereas E'/A' ratio was reduced in the SIRT3^{EC}KO female mice. To further investigate the regulatory role of endothelial SIRT3 on blood pressure and diastolic dysfunction in metabolic stress, SIRT3^{EC}KO female mice and SIRT3 LoxP female mice were fed with normal diet and high-fat diet (HFD) for 16 weeks. Knockout of

endothelial SIRT3 resulted in an increased blood pressure in female mice fed with HFD. Intriguingly, Sirt3^{EC}KO female mice + HFD exhibited impaired coronary flow reserve (CFR) and more serve diastolic dysfunction as evidence elevated IVRT as compared with control SIRT3 LoxP female mice + HFD. In addition, female SIRT3^{EC}KO mice had higher blood pressure and diastolic dysfunction as compared to male SIRT3^{EC}KO mice. Moreover, female SIRT3^{EC}KO mice + HFD had an impaired CFR and diastolic dysfunction as compared to male SIRT3^{EC}KO mice + HFD. These results implicate a sex different role of endothelial SIRT3 in regulating blood pressure and diastolic function in mice. Deficiency of endothelial SIRT3 may be responsible for a diastolic dysfunction in aging female.

Disclosures: H. Zeng: None. **R.A. Worsham:** None. **J. Chen:** None.

Funding: No

Funding Component:

010

Dual Loss of RGS2 and 5 Exacerbates the Progression of Cardiac Hypertrophy in Mice Chronically Treated With Isoproterenol

Authors: Shelby A Dahlen, Ipsita Mohanty, Ursula Djameh, Alethia Edwards, Jennifer Koch, **Patrick Osei Owusu**, Drexel Univ Coll of Med, Philadelphia, PA

This study determined the effect of chronic isoproterenol (ISO) treatment on cardiac structure and function in male mice without or with dual loss of regulator of G protein signaling (RGS) 2 and 5. RGS2 and 5 act as GTPase-activating proteins (GAPs) that preferentially terminate signaling via $G_{q/11}$- and $G_{i/o}$ class G proteins, by accelerating GTP hydrolysis. Deletion of either RGS2 or 5 increases susceptibility to cardiovascular disease. However, the effects of dual absence of the two RGS proteins on normal physiology and disease are unknown. Using mice concurrently lacking RGS2 and 5 (*Rgs2/5* dbKO) and wild type (WT) cohort, we determined how the dual absence of both RGS proteins affects cardiac response to chronic β-adrenergic receptor stimulation. WT and *Rgs2/5* dbKO mice were infused with saline or 30 μg/g/day of ISO for 3 or 14 days. At baseline, *Rgs2/5* dbKO mice showed cardiac hypertrophy (WT: 9.06 ± 0.34 vs. dbKO: 10.16 ± 0.32 mg/mm tibia length; $p<0.05$) and left ventricular chamber dilation by echocardiography, without tissue fibrosis. ISO infusion for 3 days caused and augmented cardiac hypertrophy in WT (SAL: 9.65 ± 0.38 vs. ISO: 11.68 ± 0.45 mg/mm tibia length; $p<0.05$) and *Rgs2/5* dbKO (SAL: 9.97 ± 0.43 vs. ISO: 12.57 ± 0.69 mg/mm tibia length; $p<0.01$) mice, respectively, as well as interstitial fibrosis and increased expression of hypertrophic and heart failure gene markers, including *Nppa*, *Serca*, *Mybpc3* and *Tnni3*. Sub-chronic ISO infusion also caused a greater decrease in percent fractional shortening by day 3, in *Rgs2/5* dbKO relative to WT mice (WT: -4.22 ± 4.15 vs. dbKO: -7.69 ± 3.86 %; $p<0.05$). In WT mice, cardiac hypertrophy and left ventricular dilation were lower after 14-day compared to 3-day ISO infusion, but similar to saline-treated control levels (ΔSAL to 3d-ISO: 21.04 ± 0.82 vs. ΔSAL to 14d-ISO: 15.05 ± 0.66 %; $p<0.05$). This was accompanied by a robust increase in *Rgs5* but not *Rgs2* mRNA expression in WT mice. In contrast, *Rgs2/5* dbKO mice continued to show abnormal cardiac structure and function after 14-day ISO infusion. Together, these data suggest that increased expression of RGS5 compensates for the lack of change in RGS2 expression and/or function and protects against transition from ISO-induced cardiac hypertrophy to heart failure.

Disclosures: S.A. Dahlen: None. **I. Mohanty:** None. **U. Djameh:** None. **A. Edwards:** None. **J. Koch:** None. **P. Osei Owusu:** None.

Funding: Yes

Funding Component: National Center

011

Regression of Left Ventricle Hypertrophy and Improvement of Ventricular Dysfunction After Renal Revascularization is Dependent of Blood Pressure Response and Etiology of Renal Artery Stenosis

Authors: Luiz Bortolotto, Thiago A Macedo, Luis J Kajita, Heart Inst (InCor), São Paulo, Brazil; Julio C Marino, Heart Inst (InCor), São Paulo, France; Jose J De Lima, Heart Inst (InCor), São Paulo, Brazil

Background: Renovascular hypertension promotes humoral and hemodynamic overload on the cardiovascular system and it is unknown the clinical impact of the correction of renal artery stenosis (RAS) on the structure and function of the heart.We aimed to evaluate the effect of RAS correction on blood pressure (BP) and left ventricle (LV) morphology, function and mass index (LVMI).Methods: Ninety-eight hypertensive patients with significant RAS (> 70%) undergoing percutaneous intervention (26%) or surgical treatment (74%) with a minimum follow-up of 12 months entered the study. We analyzed echocardiographic measurements , including LVMI and ejection fraction (EF) before and after correction of RAS. The BP response after treatment was based on AHA guidelines: cured (BP<140/90 without drugs), improved (BP <140/90 with reduction of number of drugs) and failure. Results: The mean age was 46 ±17 years and 60% were female. The etiology of RAS was atherosclerosis (59%), arteritis (21%) and dysplasia (20%). Before treatment, echocardiography showed LVMI=162 ± 60 g/m2, 73.5% had LV hypertrophy (LVMI > 125 g/m2) and 13 patients had systolic dysfunction (EF<50%). In a mean follow-up of 51 ± 40 months, 62% were considered cured or improved (Benefit group) and 38% as Failure. In the Benefit group, mean LVMI in the follow-up was lower than in Failure group (129 ±44 vs 169 ±42 g/m2, p<.05). The reduction of LVMI was significantly greater in Benefit than in Failure group (23 ±5 vs. 9±9 g/m2, p<.03). The systolic function normalized in 10 from 13 patients with previous dysfunction. The mean LVMI reduction was higher in patients with arteritis than in those with atherosclerosis or dysplasia. Conclusion: Successful renal revascularization promotes significant reduction of LV hypertrophy and normalize previously impaired systolic dysfunction in patients with RAS. The regression of ventricle mass is dependent of BP response after treatment and is more impressive in patients with arteritis.

Disclosures: L. Bortolotto: None. **T.A. Macedo:** None. **L.J. Kajita:** None. **J.C.S. Marino:** None. **J.J.G. De Lima:** None.

Funding: No

Funding Component:

012

Canstatin, a Fragment of Type IV Collagen α2 Chain, Inhibits Isoproterenol-Induced Cardiac Hypertrophy in Rats

Authors: Akira Sugiyama, Muneyoshi Okada, Hideyuki Yamawaki, Sch of Veterinary Med, Kitasato Univ, Towada, Aomori, Japan

Background: Canstatin, a cleaved C-terminal fragment of type IV collagen α2 chain, exerts anti-angiogenic and anti-tumor effects. We previously reported that canstatin is abundantly expressed in normal myocardial tissue of rats and protective against isoproterenol-induced apoptosis in H9c2 rat cardiomyoblasts. In the present study, we investigated the in vivo effects of canstatin on isoproterenol-induced cardiac hypertrophy model rats.
Methods: Male Wistar rats (5-week-old) were subcutaneously treated with isoproterenol (ISO, 5 mg/kg/day) or saline (Cont) for 7 days (n=4). At the same time, recombinant mice canstatin (20 μg/kg/day) or vehicle was intraperitoneally administered (n=4). After the wall thickness was analyzed by an echocardiography, the hearts were isolated and weighed. Histological analysis was performed by an Azan staining. The expression of canstatin in left ventricle was analyzed by an immunohistochemical staining.
Results: ISO significantly increased left ventricular weight (LVW/tail length: TL) (Cont: 30.8±1.7 vs. ISO: 45.2±2.0 mg/cm, P<0.01), which was significantly inhibited by canstatin (38.9±2.1 mg/cm, P<0.05). Echocardiography showed that ISO

significantly increased left ventricular posterior wall at endo-diastole (LVPWd) (Cont: 0.16±0.02 vs. ISO: 0.23±0.01 cm, P<0.05), which was suppressed by canstatin (0.18± 0.01 cm). In histological analysis, ISO significantly increased the diameter of cardiomyocytes (Cont: 17.1±1.5 vs. ISO: 23.0±0.7 μm, P<0.01), which was significantly inhibited by canstatin (17.4±0.3 μm, P<0.01). Immunohistochemical staining showed that the expression of canstatin was decreased in the hypertrophic myocardial tissue (ISO: 76.6±18.2 % relative to Cont). We confirmed that canstatin alone-treatment had not effect on LVW/TL, LVPWd and diameter of cardiomyocytes.
Conclusions: We for the first time revealed that canstatin is preventive against isoproterenol-induced cardiac hypertrophy. Although further studies are needed to clarify the underlying mechanisms, our results indicate canstatin as a novel cardioprotective endogenous molecule.

Disclosures: A. Sugiyama: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; JSPS KAKENHI Grant Number 18J20623. **M. Okada:** None. **H. Yamawaki:** None.

Funding: No

Funding Component:

013

Soluble Guanylate Cyclase Activators Improve Vascular Function and Attenuate Placental Ischemia-Induced Hypertension

Authors: Bhavisha A. Bakrania, Frank T. Spradley, Adam B. Travis, Univ of Mississippi Med. Ctr, Jackson, MS; Peter Sandner, Bayer AG, Wuppertal, Germany; Joey P. Granger, Univ of Mississippi Med. Ctr, Jackson, MS

Preeclampsia (PE) is a pregnancy specific disorder associated with maternal hypertension and endothelial dysfunction caused by the release of anti-angiogenic and pro-inflammatory factors from the ischemic placenta. In addition, PE is associated with depletion of nitric oxide (NO), which, during normal pregnancy, binds to soluble guanylate cyclase (sGC), and synthesizes cGMP, to facilitate vasodilation. A recently developed drug, sGC activators have been shown to bind to the sGC molecule and increase activity independently of NO. However, whether these drugs might have therapeutic potential in PE is not known. We tested the hypothesis that sGC activators attenuates blood pressure in a placental ischemic rat by improving vascular function. Sprague-Dawley rats underwent Sham or RUPP (Reduced Uterine Perfusion Pressure) surgery on gestational day (GD) 14, where silver clips were placed on the abdominal aorta and branches of the ovarian artery to induce placental ischemia. Animals were then placed on placebo (P) or sGC activator (80 ppm, BAY 60-2770) -supplemented (sGC-A) diets, ad libitum, from GD14-19. To determine the effect of sGC activators on vascular function, uterine arteries were isolated from Sham and RUPP operated rats and mounted on a wire myograph. Vessels were pre-constricted and vasodilation was assessed by increasing doses of the sGC activator (Cinaciguat; Sigma, St Louis, MO). On GD19, RUPP surgery had significantly increased mean arterial blood pressure as expected (Sham+P, *n*=6, 100±6 mmHg; RUPP+P, *n*=6, 117±4 mmHg; P<0.01), and was attenuated by treatment with the sGC activator (RUPP+sGC-A, *n*=6, 108±6 mmHg; P=0.02). Interestingly, in the presence of sGC activators (Cinaciguat), uterine arteries isolated from RUPP rats exhibited significantly improved vasodilation at doses of 1μM (Sham, *n*=5, 7±0.5 %; RUPP, *n*=3, 25±0.8 %; P<0.01) and 5μM (Sham, *n*=5, 7±0.5 %; RUPP, *n*=3, 28±1 %; P<0.01) compared to the Sham group. The results of this study demonstrate that activating sGC can reduce blood pressure by improving vascular function in the RUPP rat. In conclusion, these findings suggest there could be a therapeutic potential for treating preeclampsia with sGC activators.

Disclosures: B.A. Bakrania: None. **F.T. Spradley:** None. **A.B. Travis:** None. **P. Sandner:** None. **J.P. Granger:** None.

Funding: No

Funding Component:

014

Betamethasone: A Novel Therapeutic Intervention for Preeclampsia

Authors: Sabrina M Scroggins, Donna A Santillan, Jeremy A Sandgren, Gary L Pierce, Curt D Sigmund, Justin L Grobe, Mark K Santillan, Univ of Iowa, Iowa City, IA

The early pathogenesis of preeclampsia (PE) involves a systemic inflammatory immune response. Recent data demonstrate that increased circulating arginine vasopressin (AVP) in humans is predictive of PE and that infusion of AVP in mouse dams phenocopies the pregnancy-specific cardiovascular and immune alterations observed in human PE. Specifically, AVP suppresses anti-inflammatory cytokines and cells. Betamethasone (BMTZ), commonly given to women at risk for preterm birth, is both an AVP and immune response modulator. We hypothesize that early treatment with BMTZ will prevent the development of AVP-induced PE. C57BL/6J dams were infused with AVP (24 ng/hour) or saline throughout gestation via osmotic minipump. AVP dams received a single subcutaneous injection of BMTZ (100ug) early post-placentation (gestational day (GD) 7). Blood pressure was measured throughout pregnancy. Total protein was measured on 24 hour urine collected on GD 17. Maternal and fetal tissues were collected on GD 18. Cytokine concentrations were determined via commercially available ELISAs and normalized to total protein. BMTZ reversed the hypertension (ANOVA n=11, p=0.007) and proteinuria (ANOVA n=11, p=0.025) induced by AVP. BMTZ reversed the AVP-induced decreases in the maternal and fetal anti-inflammatory responses. In maternal kidney, both anti-inflammatory IL-4 (AVP: 0.034, n=10 vs BTMZ: 0.092, n=5 ug/g, $p<0.05$) and TGFb (AVP: 4.3, n=10 vs BTMZ: 9.2, n=5 ug/g, $p<0.05$) were increased in BMTZ-treated dams. Decreases in fetal kidney IL-4 (AVP: 0.013, n=5 vs BTMZ: 0.043, n=5 ug/g, $p<0.05$), IL-10 (AVP: 1.2, n=5 vs BTMZ: 2.1, n=5 ug/g, p=0.05), and TGFb (AVP: 1.8, n=5 vs BTMZ: 2.9, n=5 ug/g, $p<0.05$) were reversed with BMTZ treatment. Lastly, placental concentrations of IL-4 (AVP: 0.002, n=5 vs BTMZ: 0.005, n=5 ug/g, $p<0.05$) and TGFb (AVP: 0.090, n=5 vs BTMZ: 1.4, n=5 ug/g, $p<0.05$) were also improved following BMTZ in AVP-infused dams. Supportive of our hypothesis, early BMTZ treatment prevented hypertension and reduced proteinuria in AVP-infused dams. BMTZ also reversed AVP-induced inhibition of anti-inflammatory responses, creating a more tolerogenic milieu. These data support the concept for the potential use of BMTZ in early gestation as a novel preventative agent for PE.

Disclosures: S.M. Scroggins: None. **D.A. Santillan:** H. Other; Modest; Patent on Therapeutics and Diagnostics for Preeclampsia. **J.A. Sandgren:** None. **G.L. Pierce:** None. **C.D. Sigmund:** None. **J.L. Grobe:** H. Other; Modest; Patent on Therapeutics and Diagnostics for Preeclampsia. **M.K. Santillan:** H. Other; Modest; Patent on Therapeutics and Diagnostics for Preeclampsia.

Funding: Yes

Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

015

Reduced Placental Expression of Regulator of G Protein Signaling-2 (RGS2) in Preeclampsia: Association, Consequence, and Cause

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

To date, the early-gestational mechanisms driving the pathogenesis of preeclampsia (PreE) remain largely unclear. However, altered G protein signaling has been implicated in PreE, including alterations in GPCR agonists such as vasopressin, endothelin, and angiotensin. Regulator of G protein Signaling 2 (RGS2) is an endogenous terminator of Gαq signaling, and mutations in the *Rgs2* gene are linked to hypertension and increased risk of developing PreE. Therefore, we hypothesized reduced placental RGS2 may increase the risk of developing PreE by disinhibiting Gαq signaling. *In silico*

reanalysis of a publicly-available microarray dataset (GSE75010) revealed a significant reduction in *Rgs2* mRNA in placentas from PreE pregnancies compared to controls (Con n=35, PreE n=49, $p<0.05$). We confirmed this reduction in *Rgs2* mRNA by qPCR using human placental tissue samples (PreE 19% of Con, n=11 vs 9, $p<0.05$). To examine if reduced feto-placental RGS2 was sufficient to induce PreE phenotypes, wildtype C57BL/6J female mice were mated with *Rgs2*-deficient (*Rgs2*-KO) sires or wildtype littermate sires. Dams mated with *Rgs2*-KO sires developed diastolic hypertension (Con 92 ± 2 vs *Rgs2*-KO 98.2 ± 2 [24 hr avg mmHg]; $p<0.05$ vs pregnant control and pre-pregnancy *Rgs2*-KO) and increased proteinuria compared to dams mated with littermate sires (18.2 ± 2.2, n=7 vs 28.4 ± 2.8, n=10 mg/day, $p<0.05$). Preliminary histological analysis of placentas from dams mated with an *Rgs2*-KO sire suggest decreased spiral artery number and diameter (SA Number: Con 7.7 ± 0.2 vs *Rgs2*-KO 5.6 ± 0.4, SA Diameter: Con 128 ± 3 vs *Rgs2*-KO 86 ± 12). Previous studies have outlined a CRE element in the *Rgs2* promoter that is critical for transcriptional regulation of *Rgs2*. Thus, we hypothesized loss of cAMP/CREB regulation may lead to reduced RGS2 in human PreE. Indeed, reduced phosphorylated CREB (p-CREB) binding was observed in PreE placentas (Con 0.146 ± 0.024 vs PreE 0.070 ± 0.021 $p<0.05$). However, loss of p-CREB was not due to decreases in cAMP levels (Con 1.69 ± 0.35 vs PreE 2.18 ± 0.18 nM). These data support a role for reduced placental RGS2 in the pathogenesis of PreE, and demonstrate that changes in p-CREB regulation may explain the loss of RGS2 expression in placental trophoblasts.

Disclosures: **K.J. Perschbacher:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA. **G. Deng:** None. **J.A. Sandgren:** None. **D.A. Santillan:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH. 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; Patents. **E.J. Devor:** None. **G.L. Pierce:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH. **R.A. Fisher:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH. **K.N. Gibson-Corley:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH. **M.K. Santillan:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH. 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; Patents. **J.L. Grobe:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH. 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; Patents.

Funding: Yes

Funding Component: National Center

016

Placental Ischemia Exposure leads to a Pro-Inflammatory Environment in Brains of Embryonic Day 19 Rat Offspring

Authors: Junie P Warrington, Omar C Logue, Gene L Bidwell, Qingmei Shao, Univ Mississippi Medical Ctr, Jackson, MS

Placental ischemia is thought to be the initiating event in the pathophysiology of preeclampsia, a hypertensive disorder of pregnancy. Previous studies have shown that placental ischemia in the rat, induced by surgically reducing uterine perfusion pressure (RUPP), leads to increased circulating pro-inflammatory cytokines and anti-angiogenic factors, similar to the preeclampsia patient. It is not known whether pups exposed to placental ischemia have evidence of neuroinflammation *in utero*; therefore, this study assessed whether placental ischemia contributes to a pro-inflammatory environment in brains of offspring at embryonic day (E)19. Sham or RUPP surgery was performed on

timed-pregnant Sprague Dawley rats (260g) on gestational day 14, and pup brains were extracted on E19. Using a multiplex cytokine/chemokine kit, we found that 7 out of 27 cytokine/ chemokines were significantly increased in brain homogenates from pups exposed to placental ischemia (n=5 pup brains/group). Higher levels of Eotaxin (5.76±0.26 vs. 3.86±0.84 pg/mg protein; p=0.03), Interleukin (IL)-1β (5.17±0.25 vs. 3.70±0.43 pg/mg; p=0.01), IL-6 (72±10.5 vs. 46.3±7.01 pg/mg; p=0.04), LIX (CXCL5; 45.8±2.2 vs. 30.4±4.7; p=0.01), IL-17 (1.12±0.51 vs. 0.27±0.03; p=0.02), IL-18 (330.1±40.9 vs. 226.5±18.3; p=0.02), and macrophage inflammatory protein 2 (MIP-2; 20.8±0.5 vs. 15.7±1.9 pg/mg; p=0.02) were observed in pup brains exposed to placental ischemia. Despite these increases in pro-inflammatory cytokines, brain water content, measured in a different group of pups from same dams, was not different between the two groups (87.77±0.05 vs. 87.73±0.35%, p=0.53). Whether the increase in pro-inflammatory cytokines in brains of rat pups exposed to placental ischemia is a result of increased transport across the placental barrier or a compensatory mechanism to the hypoxic environment is not known. Our findings support the hypothesis that neuroinflammation in the offspring exposed to placental ischemia may contribute to neurodevelopmental disorders and cognitive impairment observed in offspring of preeclampsia-complicated pregnancies.

Disclosures: **J.P. Warrington:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH 4R00HL129192. **O.C. Logue:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; AHA17POST33460172. **G.L. Bidwell:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH R01HL121527. **Q. Shao:** None.

Funding: No

Funding Component:

017

Effect of Sildenafil on Nitric Oxide-Mediated Vasodilation in the Human Placenta: an Ex-Vivo Placental Perfusion Study

Authors: Emilie Hitzerd, Marija Glisic, Katrina M Colafella, René de Vries, Sam Schoenmakers, Daphne Merkus, Irwin K Reiss, A H Danser, Sinno H Simons, Erasmus MC, Rotterdam, Netherlands

Preeclampsia (PE) is a serious pregnancy complication characterized by suboptimal placentation, leading to increased vascular resistance and generalized endothelial dysfunction. A promising treatment is sildenafil, a phosphodiesterase-5 (PDE5) inhibitor that enhances nitric oxide (NO) mediated vasodilation. Although there are currently sildenafil trials ongoing in pregnant women, the effects of sildenafil on the placenta are still unknown. Placentas of healthy (n=17), early-onset (Eo)PE (n=6) and late-onset (Lo)PE (n=5) pregnancies were dually perfused, pre-constricted with serotonin, and subsequently exposed to the NO donor SNP in the absence or presence of 1 µmol/L sildenafil. Two healthy placentas were perfused with sildenafil on the maternal side to study placental transfer. Placental protein expression of PDE5, PDE1A, eNOS and iNOS was assessed using Western blot. Mean baseline pressure ±SEM was significantly lower ($p<0.05$) in EoPE (22.3±2.8 mmHg) and LoPE (22.8±1.7 mmHg) vs. healthy placentas (33.1±2.2 mmHg). There was no effect of sildenafil on baseline pressure or serotonin-induced pre-constriction. Sildenafil tended to enhance vasodilatory response to 10^{-6}M SNP in healthy (mean pressure decrease 4.3±0.8 vs. 8.1±1.9 mmHg, p=0.1) and LoPE placentas (3.0±1.0 vs. 7.3±3.2 mmHg), while no improvement was seen in EoPE placentas (6.0±2.0 vs. 5.0±1.0 mmHg). When sildenafil was added at the maternal side, average fetal-maternal concentration ratio was 0.22 after 3 hours of perfusion, as compared to 0.86 for antipyrine, a freely diffusing molecule. Placental levels of PDE1A tended to be higher in PE (p=0.08), while no differences in expression of PDE5, eNOS and iNOS were observed between PE and healthy placentas. Our study reveals that PE placentas have a significantly lower baseline pressure compared to healthy placentas, possibly due to lower resistance in the feto-placental vasculature as a compensation mechanism for the increased resistance in the spiral arteries. Although sildenafil tended to enhance vasodilation in healthy and LoPE placentas, it did not have those

beneficial effects in EoPE. Possibly PDE1A-selective inhibitors should be applied in PE, or PDE is not the appropriate target for restoring disturbed vasodilation.

Disclosures: E. Hitzerd: None. **M. Glisic:** None. **K.M. Colafella:** None. **R. de Vries:** None. **S. Schoenmakers:** None. **D. Merkus:** None. **I.K.M. Reiss:** None. **A.H.J. Danser:** None. **S.H.P. Simons:** None.

Funding: No

Funding Component:

018

High Stillbirth Rate and Altered Maternal Renal Function and Water Balance are Associated With Spontaneous Gestational Hypertension in Nonhuman Primates

Authors: Chelsea C Weaver, Univ of Kentucky, Lexington, KY; Frances Louard, Biomedical Science Res Group, Lexington, KY; Jeffrey L Osborn, Univ of Kentucky; Biomedical Science Res Group, Lexington, KY

Gestational hypertension (GH) is new-onset hypertension specific to pregnancy (PG) that may precede preeclampsia (PE). While the specific etiology of PE is unclear, defective fluid and electrolyte balances have been implicated. The African Green Monkey (AGM; *Chlorocebus aethiops sabaeus*) is a novel model of spontaneous GH with pathophysiological consequences of PE. Systolic blood pressure (SBP; forearm plethysmography) was measured in AGMs pre-PG, during each trimester, and postpartum (PP). Gestational age was estimated by fetal lengths at 7 weeks/trimester (21 week gestation). Animals were characterized as normotensive (NT; SBP < 120 mmHg in PG and PP) or gestational hypertensive (GH; SBP ≥ 140 mmHg during PG). SBP was unchanged in NT PG (pre-PG 110 ± 5 mmHg; 1st trimester 101± 6 mmHg; 3rd 107 ± 7 mmHg; n = 27). In GH AGMs, SBP increased in the 3rd trimester from 115 ± 6 mmHg (1st) and 117 ± 25 mmHg (2nd) to 152 ± 7 mmHg (3rd; $p<0.05$; n = 16). Plasma osmolality did not change during PG for NT AGMs but declined in GH (NT pre-PG 305 ± 5 mOsm/kg; PG 299 ± 5 mOsm/kg; n = 6; GH pre-PG 313 ± 6; PG 293 ± 4 mOsm/kg; $p<0.05$ ANOVA; n = 15). Maternal body weight increased in GH by the 1st trimester, while NT body weight did not change until the 3rd trimester (NT pre-PG 3.8 ± 0.2 kg; 1st trimester 3.8 ± 0.3 kg; 3rd 4.4 ± 0.2 kg; GH pre-PG 3.7 ± 0.1 kg; 1st trimester 4.4 ± 0.2 kg; 3rd 4.7 ± 0.1 kg; $p<0.05$). Protein excretion was unchanged in NT AGMs but increased with GH compared to pre-PG and NT PG (NT pre-PG 365 ± 45 mg/day; 3rd trimester 466 ± 84 mg/day; GH pre-PG 346 ± 138 mg/day; 3rd trimester 702 ± 69 mg/day; $p<0.05$). Third trimester estimated GFR (eGFR) did not change in NT AGMs but declined in GH (NT 5.0 ± 0.7 ml/min vs GH 2.5 ± 0.5 ml/min; $p<0.05$). Infants from GH pregnancies were smaller (NT 327.4 ± 13.7 g, n = 5, vs GH 290.0 ± 8.5 g n = 15; $p<0.05$) and GH increased fetal stillbirth rate to 40% (6/15). Thus, GH in AGMs has early PG water retention, decreased plasma osmolality and excess weight gain preceding GH. GH AGMs have smaller infants and increased stillbirth rates, showing fetal growth restriction. Elevated SBP during GH may lead to renal dysfunction, proteinuria and reduced eGFR. Thus, the AGM is a novel, translational model of hypertensive pregnancy disorders for the study of acute and long-term effects on fetal development.

Disclosures: C.C. Weaver: None. **F. Louard:** None. **J.L. Osborn:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; Biomedical Science Research Group.

Funding: Yes

Funding Component: Great Rivers Affiliate (Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)

019

A20 in Dendritic Cells Protects Against Hypertension by Inhibiting Dendritic Cell-Mediated T Cell Activation

Authors: Xiaohan Lu, Nathan Rudemiller, Jiandong Zhang, Jamie Privratsky, Gianna Hammer, Joyce Zhang, Yi Wen, Jiafa Ren, Steven D Crowley, Duke Univ, Durham, NC

The ubiquitin-editor protein A20 in dendritic cells (DCs) suppresses NF-κB signaling and inhibits their capacity to activate T cells by limiting DC expression of co-stimulatory molecules. We previously reported that mice with spontaneous DC activation due to heterozygous deletion of A20 in DCs (CD11c-cre+ $A20^{flox/wt}$ = DC ACT) have an exaggerated hypertensive response to low dose (300 ng/kg/min) chronic angiotensin (Ang) II infusion (143±2 vs. 131±4 mmHg; p=0.04) and augmented proportions of effector T cells in the kidney lymph node. Here, we explored the mechanism through which A20 in DCs limits blood pressure elevation. After 7 days of chronic Ang II infusion, flow cytometric analysis of kidney homogenates from wild-type (WT = CD11c-cre^{-} $A20^{flox/wt}$) and DC ACT mice revealed similar numbers of renal DCs in the 2 groups (2832±342 vs. 2531±435; p=NS). By contrast, numbers of $CD11b^{+}$ $Ly6C^{hi}$ inflammatory monocytes (9849±1108 vs. 6019±597; p=0.01) and $CD8^{+}$ T cells (1757±101 vs. 908±155; p<0.03) were increased in the DC ACT kidneys compared to WT controls. Accordingly, the DC ACT kidneys showed upregulated mRNA expression for the pro-hypertensive cytokines TNF-α (1.6±0.2 vs. 1±0.2; p<0.02) and IL-17A (2.3±0.6 vs. 1±0.3; p=0.09) and the mononuclear cell chemokine CCL5 (1.9±0.27 vs. 1±0.11; p=0.01). Both monocytes and T cells have been implicated in the pathogenesis of hypertension. Therefore, to directly test whether enhanced T cell activation in the DC ACT cohort was responsible for their augmented hypertensive response, we chronically infused Ang II into lymphocyte-deficient DC ACT $Rag1^{-/-}$ mice and WT (CD11c-cre- $A20^{flox/wt}$) $Rag1^{-/-}$ controls for 4 wks. In WT $Rag1^{-/-}$ and DC ACT $Rag1^{-/-}$ mice, radiotelemetry blood pressures (128±1 vs. 128±2 mmHg; p=NS) and heart to body weight ratios (6.0±0.1 vs. 6.4±0.2 mg/g body wt) were similar during Ang II, suggesting that T cells are required to mediate the augmented hypertensive response accruing from DC activation due to A20 deficiency. Thus, following stimulation of the renin angiotensin system, A20 suppresses DC activation and thereby mitigates T cell-dependent blood pressure elevation. These studies identify a novel target through which to limit the engagement of adaptive immunity during chronic hypertension.

Disclosures: X. Lu: None. **N. Rudemiller:** None. **J. Zhang:** None. **J. Privratsky:** None. **G. Hammer:** None. **J. Zhang:** None. **Y. Wen:** None. **J. Ren:** None. **S.D. Crowley:** None.

Funding: No

Funding Component:

020

Sex Difference in T Regulatory Cells After Adoptive Transfer From Hypertensive Donors Leads to Protection Against T Cell-Mediated Hypertension in Premenopausal Female Mice

Authors: Dennis P Pollow Jr, Megan A Sylvester, Nathaniel A Husband, Joshua A Uhlorn, Jill Romero-Aleshire, Jennifer Uhrlaub, Janko Nikolich-Zugich, Heddwen L Brooks, Univ of Arizona, Tucson, AZ

Activation of T cell-dependent pro-inflammatory responses are required for Ang II hypertension in male mice. However, females are protected from T cell-mediated hypertension and may suppress hypertension by directly preventing Ang II-induced pro-inflammatory T cell activation. Here we sought to determine whether transferring T cells from hypertensive donor mice eliminates female protection against T cell-mediated hypertension. Splenic $CD3^{+}$ T cells were transferred from normotensive (NT) or Ang II-hypertensive (HT) C57BL/6J male donors to female $Rag\text{-}1^{-/-}$ (NT T cell female-NTF; HT T cell female-HTF) or male $Rag\text{-}1^{-/-}$ (HT T cell male-HTM) recipient mice. Blood pressure was monitored (tail cuff) for 5 weeks post-transfer. Ang II (490ng/kg/min) was infused into recipient mice for 14 days during weeks 4 and 5 post-transfer (NTFA; HTFA; HTMA). Ang II significantly increased MAP in donor male mice (NT 114 vs HT 157 mmHg, p<0.05).

Transfer of T cells from HT donors did not induce HT in female or male recipients. Similarly, T cell donor environment did not affect Ang II-induced blood pressure in female recipients, which remained protected compared to male recipients (MAP: NTF 83 ± 4 mmHg*, HTF 88 ± 6 mmHg*, NTFA 101 ± 5 mmHg*, HTFA 103 ± 5 mmHg*, HTMA 138 ± 3 mmHg, *p<0.05 vs HTMA). Flow cytometry demonstrated similar splenic T cell frequency across all groups (CD3: NTF 18%, NTFA 16%, HTF 17%, HTFA 14%, HTMA 18%, p>0.05). However, regulatory T cells were significantly reduced in male recipients compared to all female groups (Foxp3: NTF 21.6%*, NTFA 22.2%*, HTF 22.8%*, HTFA 22.6%*, HTMA 15.3%, *p<0.05 vs HTMA) Females had significantly less renal T cell infiltration compared to males and infiltration was not impacted by Ang II infusion or T cell donor status (CD3: NTF 12,083*, NTFA 11,317*, HTF 12,656*, HTFA 8,997*, HTMA 22,405, *p<0.05 vs HTMA; CD4: NTF 6,411*, NTFA 4,702*, HTF 5,831*, HTFA 4,579*, HTMA 9,914, *p<0.05 vs HTMA; CD8: NTF 5,397*, NTFA 6,123*, HTF 6,362*, HTFA 3,792*, HTMA 11,727, *p<0.05 vs HTMA). These results demonstrate that female mice prevent T cell-mediated hypertension and renal T cell infiltration regardless of previous T cell exposure to a hypertensive environment, suggesting a direct preventive mechanism in females against pro-hypertensive T cell responses.

Disclosures: D.P. Pollow: None. **M.A. Sylvester:** None. **N.A. Husband:** None. **J.A. Uhlorn:** None. **J. Romero-Aleshire:** None. **J. Uhrlaub:** None. **J. Nikolich-Zugich:** None. **H.L. Brooks:** None.

Funding: No

Funding Component:

021

Interleukin-21 Plays a Critical Role in Hypertension and Vascular Dysfunction

Authors: Bethany L Dale, Vanderbilt Univ, Dept of Molecular Physiology and Biophysics, Nashville, TN; Arvind Pandey, Yuahn Chen, 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, Natalia Barbaro, Justin Van Beusecum, Vanderbilt Univ, Div of Clinical Pharmacology, Dept of Med, Nashville, TN; Alp Ikizler, Aseel Alsouqi, Vanderbilt Univ, Div of Nephrology, Dept of Med, Nashville, TN; Holly M Scott, Vanderbilt Univ, Div of Infectious Diseases, Dept of Med, Nashville, TN; Meena S. Madhur, Vanderbilt Univ, Dept of Molecular Physiology and Biophysics, Nashville, TN

T cell derived cytokines, interleukin 17A (IL17A) and interferon gamma (IFNg), promote angiotensin II (Ang II)-induced hypertension and end-organ damage. Interleukin 21 (IL21), produced primarily by T follicular helper (Tfh) cells, has been shown to induce IL17A and IFNγ production from T effector cells. IL21 is also a potent activator of germinal center (GC) B cells and immunoglobulin (Ig) class switching. We hypothesized that IL21 plays a fundamental role in hypertension and hypertensive end-organ damage through its effects on pro-inflammatory T cell polarization, vascular dysfunction, and enhanced Ig secretion. CD4+ T cells from Ang II infused WT mice exhibit 1.5-fold increased IL21 mRNA expression and 2-fold increased IL-21 production compared to vehicle. We found $IL21^{-/-}$ mice exhibit a 30mmHg reduction in systolic blood pressure (SBP) in response to 4 weeks of Ang II infusion compared to age-matched wild type (WT) mice. Further, IFNg and IL17A production from CD8+ or CD4+ T cells, respectively, was abrogated in $IL21^{-/-}$ mice vs. WT mice infused with Ang II. We also found Tfh cells and GC B cells significantly increased in the aortas and mesenteric vessels of Ang II WT mice but not $IL21^{-/-}$ mice. Ang II induced an increase in percent of lymph node GC B cells and IgG/IgM ratio, consistent with Ig class switching. Ang II-induced increase in renal injury was also blunted in $IL21^{-/-}$ mice vs. WT mice. Mesenteric vessels from $IL21^{-/-}$ mice were protected from Ang II-induced endothelial dysfunction. $IL21^{-/-}$ mice also developed blunted aortic fibrosis and reduced smooth muscle cell hypertrophy after 4 weeks of Ang II vs. WT mice. IL-21 neutralization beginning 2 weeks after Ang II resulted in a 15 mmHg reduction in SBP and reversal of aortic inflammation and vascular endothelial dysfunction compared to isotype control treated animals. Lastly, CD4+ T cell production of IL21 positively correlated with SBP in human volunteers, and in fact, IL-21 production was significantly higher in hypertensive compared to normotensive subjects. Taken together, these studies provide compelling evidence that IL21 may function

as a master cytokine in the pathogenesis of hypertension and suggest that therapies targeting IL21 may reduce hypertension and the associated end-organ damage.

Disclosures: B.L. Dale: None. **A. Pandey:** None. **Y. Chen:** None. **F. Laroumanie:** None. **H. Itani:** None. **L. Xiao:** None. **A. Norlander:** None. **J. Foss:** None. **N. Barbaro:** None. **J. Van Beusecum:** None. **A. Ikizler:** None. **A. Alsouqi:** None. **H.M. Scott:** None. **M.S. Madhur:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

022

Proteasome Inhibition Prevents Renal Inflammation and Hypertension by Abrogation of Dendritic Cell Autoantigen Presentation

Authors: David M Patrick, Liang Xiao, C. Wei Chen, Mingfang Ao, Annet Kirabo, David G Harrison, Vanderbilt Univ Medical Ctr, Nashville, TN

Reactive oxygen species (ROS) have been shown to be important mediators of hypertension. Isolevuglandins (isoLG) are oxidation products of fatty acids that adduct to lysine residues of proteins. Our prior data suggest that isoLG-adducted proteins are presented by dendritic cells (DCs) and activate T-cells resulting in hypertension. Identification of the source and mechanism of presentation of isoLG adducts is critical for the development of therapies targeting this process. Antigens formed intracellularly are processed by the proteasome and presented to $CD8^+$ T cells in MHC class I (MHC-I). We hypothesize that within DCs, isoLG-adducted peptides are processed by the proteasome and presented in MHC-I. Treatment of mouse DCs with the oxidant tert-butyl hydroperoxide (TBHP) increased levels of isoLG-adducts on the DC surface, as detected by flow cytometry, from 1.3×10^3 to $11.4 \times 10^3 / 10^6$ DCs ($N = 9$, $P < 0.01$). Co-treatment of mouse DCs with TBHP and proteasome inhibitors (PIs) bortezomib (BTZ) or MG132 attenuated surface isoLG-adduct levels when compared to TBHP alone (2.7×10^3 or 3.5×10^3 vs $11.4 \times 10^3 / 10^6$ DCs, $N = 9$, $P < 0.001$). Moreover, using flow cytometry-based fluorescence resonance energy transfer, we found that isoLG-adducted peptides and MHC-I interact at the intermolecular level on mouse DCs, suggesting that IsoLG-modified peptides are presented within MHC-I. Co-treatment of these cells with TBHP and BTZ attenuated this interaction compared with TBHP treatment alone (2.9×10^3 vs $32.7 \times 10^3 / 10^6$ DCs, $N = 9$, $P < 0.001$). Next, mice were treated with angiotensin II (ang II) with or without co-treatment with BTZ. Co-treated animals exhibited reduced mean systolic blood pressure (161.7 ± 7.2 vs 122.9 ± 17.9 mmHg, $N = 4$-5, $P < 0.05$) and a reduction in renal macrophages (13.4×10^3 vs 6.57×10^3 DCs/Kidney, $N = 4$-5, $P < 0.001$) and DCs (129.4×10^3 vs 79.9×10^3 DCs/Kidney, $N = 4$-5, $P < 0.05$). These studies provide evidence that the proteasome processes isoLG adducts into peptides that are presented within MHC -I, and that proteasome inhibitors can not only block this process, but in doing so, can reduce renal inflammation and lower blood pressure. Efforts to inhibit processing and presentation of isoLG-modified peptides may therefore have therapeutic benefit in hypertension.

Disclosures: D.M. Patrick: A. Employment; Modest; Vanderbilt University Medical Center. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; 2T32GM007569-42. **L. Xiao:** A. Employment; Modest; Vanderbilt University Medical Center. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; 17SDG33670829. **C.W. Chen:** None. **M. Ao:** None. **A. Kirabo:** A. Employment; Modest; Vanderbilt University Medical Center. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; K01HL130497. **D.G. Harrison:** A. Employment; Modest; Vanderbilt University Medical Center. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; AHA SFRN20420, R01HL125865, R01HL039006.

Funding: No

Funding Component:

023

Redox-Driven T-Lymphocyte Inflammation Sensitizes Mice to Psychological Stress-Mediated Hypertension

Authors: Cassandra M Moshfegh, Christopher W Collins, Allison J Kohl, Safwan Elkhatib, **Adam J Case**, Univ Nebraska Medical Ctr, Omaha, NE

Post-traumatic stress disorder (PTSD) is a debilitating psychological disease that increases sympathoexcitation, norepinephrine (NE) outflow, and the risk of cardiovascular diseases like hypertension by >50%, but the mechanisms remain unclear. Hypertension is regulated in part due to immune system-driven inflammation, and our laboratory has previously reported that exposure to NE can modulate levels of T-lymphocyte pro-inflammatory cytokines by redox signaling mechanisms. Therefore, we hypothesized that psychological stress-induced sympathoexcitation leads to a redox-regulated increase in pro-inflammatory cytokine production from T-lymphocytes, which may impact the development of hypertension. Utilizing a mouse-model of PTSD-like psychological stress (*i.e.* social defeat), increased sympathoexcitation was confirmed by a 2.5 fold increase in splenic NE content and 4.1 fold increase in tyrosine hydroxylase (TH; $p<0.05$). Splenic and peripheral T-lymphocytes demonstrated increased reactive oxygen species (ROS) levels as evidenced by enhanced dihydroethidium and MitoSOX Red oxidation ($p<0.05$). Increases in ROS were correlated with elevated pro-inflammatory cytokines IL-17A, IL-6, and IL-2 in circulation as well as specifically within T-lymphocytes ($p<0.05$). Examination of hemodynamics elucidated a progressive increase in mean arterial pressure (15 mmHg, $p<0.05$) over the stress-induction period, correlating with the elevation of T-lymphocyte driven inflammation. This hypertension was not associated with an increase in circulating renin levels. To investigate the causal contribution of T-lymphocytes in driving this hypertension, recombination activating gene 2 (Rag2) knock-out animals, which are devoid of mature lymphocytes, were utilized. $Rag2^{-/-}$ mice showed no changes in pro-inflammatory cytokines, a 75% decrease in splenic TH levels ($p<0.05$), and decreased blood pressure after stress, suggesting T-lymphocytes may regulate the stress-induced hypertension. Overall, our data suggest the potential for a new paradigm involving redox control of T-lymphocytes in the regulation of psychological stress-driven hypertension.

Disclosures: C.M. Moshfegh: None. **C.W. Collins:** None. **A.J. Kohl:** None. **S. Elkhatib:** None. **A.J. Case:** None.

Funding: No

Funding Component:

024

Genetically Inducing Renal Lymphangiogenesis Prevents Angiotensin II-Induced Hypertension in Mice

Authors: Dakshnapriya Balasubbramanian, Catalina A Lopez-Gelston, Alexandra H Lopez, Geina Iskander, Winter Tate, Emily M Luera, Sunitha Konatham, Joseph M Rutkowski, Brett M Mitchell, Texas A and M Univ HSC, College Station, TX

Angiotensin II (AII)-dependent hypertension (AIIHTN) is associated with renal immune cell infiltration and inflammation. Lymphatic vessels drain interstitial fluid and traffic immune cells to draining lymph nodes; however, the role of renal lymphatics in AIIHTN is unknown. Our hypotheses were that: 1) renal lymphatic vessel density is increased in mice with AIIHTN, and 2) that further augmenting renal lymphatic vessels will prevent AIIHTN. Male and female mice were infused with AII (490 ng/kg/min) or saline for 2 or 3 weeks by subdermal osmotic mini pumps. Male and female mice with AIIHTN had markedly increased renal lymphatic vessel density compared to controls. AIIHTN males had significantly increased renal gene expression of the lymphatic vessel markers *Lyve1, Pdpn,* and *Vegfr3,* while *Pdpn* and the

lymphangiogenic signal *Vegfc* were increased significantly in AIIHTN females. Kidneys of AIIHTN males had significantly increased F4/80+ macrophages at 2 weeks and F4/80+ macrophages and CD3e+ T cells at 3 weeks as determined by flow cytometry. Unlike in males, renal CD11c+ dendritic cells were increased significantly in females. Renal mRNA levels of the pro-inflammatory cyto/chemokines *Tnfa*, *Il1b*, *Mcp1*, and *Cxcl13* were elevated significantly in males at 2 and 3 weeks and in females at 3 weeks. To determine whether augmenting renal lymphatic vessels prior to AII infusion could prevent AIIHTN, 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-specific lymphangiogenesis. Doxycycline initiated 1 week prior to 3-week AII infusion prevented AIIHTN in KidVD+ mice while KidVD- mice still developed AIIHTN (Males SBP: 122±2 vs. 161±3 mmHg; $p<0.05$; Females SBP: 114±1 vs. 131±4 mmHg; $p<0.05$). KidVD+ AIIHTN mice had significantly decreased renal levels of CD11c+ dendritic cells and CD8+ T cells. Renal gene expression of *Tnfa* and *Il1b* were normalized in all KidVD+ mice. These data demonstrate that renal lymphatic vessel density is increased in AIIHTN and that genetically inducing renal lymphangiogenesis prior to AII infusion can prevent AIIHTN by reducing renal immune cells and inflammation.

Disclosures: D. Balasubbramanian: None. **C.A. Lopez-Gelston:** None. **A.H. Lopez:** None. **G. Iskander:** None. **W. Tate:** None. **E.M. Luera:** None. **S. Konatham:** None. **J.M. Rutkowski:** None. **B.M. Mitchell:** None.

Funding: Yes

Funding Component: South Central Affiliate (Arkansas, New Mexico, Oklahoma & Texas)

025

Cellular Mechanisms of Blood-Brain Barrier Disruption in Ang II-Induced Hypertension

Authors: Monica M Santisteban, Giuseppe Faraco, Diane Lane, Gianfranco Racchumi, Steven Segarra, Samantha Schaeffer, Virginia Cipollini, Josef Anrather, Costantino Iadecola, Weill Cornell Medical Coll, New York, NY

The blood-brain barrier (BBB) is critically important for brain health by regulating molecular exchanges between blood and brain. Hypertension (HTN) induces breakdown of the BBB which may contribute to its deleterious effect on the brain, but the cellular bases of the BBB opening remain to be established. We used a model of angiotensin II (AngII) HTN (600ng/kg/min x 2 weeks; n>5/group) to investigate the mechanisms of the BBB opening. BBB permeability was assessed spectrophotometrically in C57BL/6J mice using 3000 MW FITC-dextran as a tracer. HTN increased BBB permeability in Ang II HTN (29.8 ± 0.9 vs 18.1 ± 0.5 ng/g in control, $p<0.01$), an effect partially ameliorated by the angiotensin type 1 receptor (AT1R) antagonist losartan in the drinking water (22.9 ± 0.6 ng/g) but not by hydralazine + hydrochlorothiazide (27.4 ± 0.7 ng/g), suggesting involvement of AT1R and not elevated blood pressure. Next, we sought to identify the mechanisms of the breakdown of the BBB in HTN. First, we used electron microscopy to examine the ultrastructure of endothelial tight junctions (TJ) and assess vesicular transport, key components of the BBB. HTN reduced the length (-25%) and complexity (-11%) of TJ, and increased the number of endothelial vesicles (2.22 vs 1.42 vesicles/endothelial area, $p<0.05$). The TJ remodeling was associated with a reduction in occludin (-17%, $p<0.01$) and claudin-5 (-30%, $p<0.05$) mRNA in microvascular preparations. Additionally, the expression of Mfsd2a, a lipid transporter that also suppresses vesicular transcytosis, was markedly attenuated (-43%, $p<0.01$). Taken together, these data suggest a strong effect of Ang II on cerebral endothelial cells to induce BBB opening. Since perivascular macrophages (PVM) mediate cerebral endothelial dysfunction in HTN (J Clin Invest 2016;126:4674), we tested their involvement in the BBB opening. PVM depletion with icv clodronate or deletion of AT1 receptors in PVM partially attenuated the BBB opening in Ang II HTN ($p<0.05$). Thus, we conclude that AT1R in cerebral endothelial cells and PVM mediate the BBB opening by targeting both paracellular and vesicular transport. Such increase in BBB permeability to circulating agents may contribute to the cerebrovascular and cognitive dysfunction associated with HTN.

Disclosures: **M.M. Santisteban:** None. **G. Faraco:** None. **D. Lane:** None. **G. Racchumi:** None. **S. Segarra:** None. **S. Schaeffer:** None. **V. Cipollini:** None. **J. Anrather:** None. **C. Iadecola:** None.

Funding: Yes

Funding Component: National Center

026

High In-Hospital Systolic Blood Pressure Variability and Poor Functional Outcomes in Primary Intracerebral Hemorrhage Patients

Authors: Jennifer R Meeks, Arvind B Bambhroliya, Ellie G Meyer, Kristen B Slaughter, Christopher J Fraher, Ritvij Bowry, Wamda O Ahmed, Anjail Z Sharrief, Jon E Tyson, Charles C Miller, Univ of Texas McGovern Medical Sch, Houston, TX; Babar Khan, Indiana Univ Sch of Med, Indianapolis, IN; Steven Warach, Dell Medical Sch, Austin, TX; Louise D McCullough, Sean I Savitz, Farhaan S Vahidy, Univ of Texas McGovern Medical Sch, Houston, TX

Introduction: High in-hospital SBP variability (HSBPV) is an emerging marker for poor outcomes among Intracerebral Hemorrhage (ICH) patients. We aimed to determine the risk of severe disability or death (SDD) at day-90 among ICH patients with HSPBV and explore pre-hospital factors associated with HSPBV. **Methods**: Adult, radiologically confirmed primary ICH patients were prospectively enrolled and followed-up until day-90. All routinely collected SBP values were recorded for the inpatient stay. Inter and intra-patient SBPV was quantified using generalized estimating equations. Modified Rankin Scale (mRS) Score of 4 - 6 was defined as SDD. Poisson and logistic regression models were fit to determine the risk of day-90 SDD, and the association of pre-hospital characteristics with HSBPV. **Results**: A total of 566 patients [mean age: 63.5, females 36.6% (207 of 566)] were included. Total in-hospital follow-up period was 4,908 days [median (IQR) per patient = 8.7 (3-11)]. Over 120,500 SBP readings were analyzed. Inter and intra-patient mean SBP standard deviation (SD) was 11.1 and 13.2, respectively. A SD of 13.0 was parameterized as a cut-off for HSBPV. HSBPV patients had a 17% higher adjusted risk of day-90 SDD (Relative Risk, 95% CI: 1.17, 1.02-1.35) (Table). Older age and female sex were independently associated with HSBPV after controlling for hemorrhage volume, pre-morbid mRS, and Glasgow Coma Scale (Figure). **Conclusion**: Quantification of HSBPV is feasible utilizing routinely collected SBP readings. HSBPV is associated with poor outcomes. Elderly and female patients may be more likely to demonstrate HSBPV during hospitalization.

Table: Adjusted Relative Risks for 90-day severe disability or death, among ICH patients with high in-hospital systolic blood pressure variability

	Relative Risk [a]	95% Confidence Interval	P value
High Systolic Blood Pressure Variability	1.17	1.02 – 1.35	0.03
Age > 80 years	1.21	1.05 – 1.39	0.009
Hemorrhage Volume > 30 cc [b,c]	1.11	0.97 – 1.27	0.12
Glasgow Coma Scale Score Category [b]			
13 – 15	Reference Category		
5 – 12	1.54	1.28 – 1.85	< 0.001
3 – 4	1.60	1.33 – 1.91	< 0.001
Premorbid mRS Score > 1	1.25	1.09 – 1.43	0.001

SSD: Severe Disability or Death, ICH: Intracerebral Hemorrhage, mRS: Modified Rankin Scale – model also controls for PTT during hospitalization

[a] Estimates based on modified Poisson regression model. Model selection criteria included statistical and clinical significance of individual variables along with optimal Akaike and Bayesian Information Criteria (AIC / BIC)

[b] Based on admission / presentation characteristics

[c] Included on the basis of clinical importance, and improved AIC / BIC

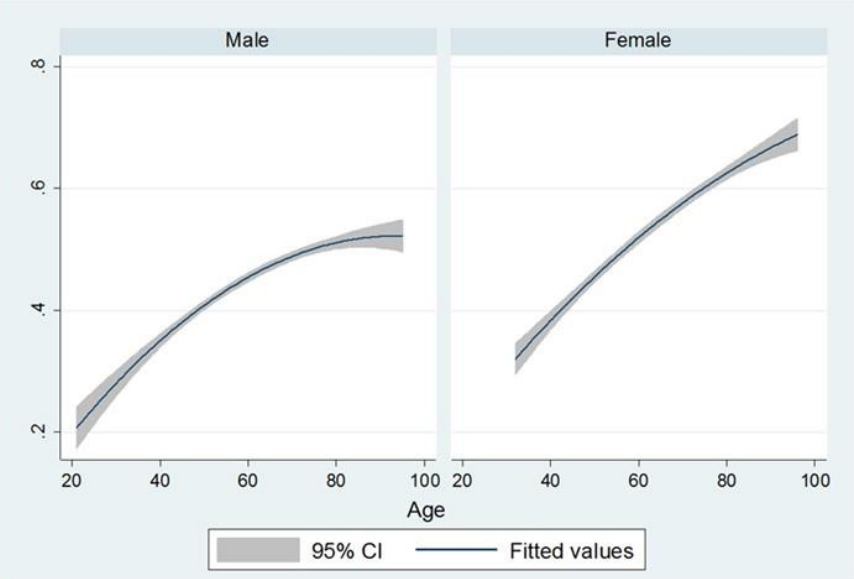


Figure: Predicted probability of in-hospital high systolic blood pressure variability (Y-axis) and increasing age (X-axis) in male and female patients with primary intracerebral hemorrhage

Disclosures: J.R. Meeks: None. **A.B. Bambhroliya:** None. **E.G. Meyer:** None. **K.B. Slaughter:** None. **C.J. Fraher:** None. **R. Bowry:** None. **W.O. Ahmed:** None. **A.Z. Sharrief:** None. **J.E. Tyson:** None. **C.C. Miller:** None. **B. Khan:** None. **S. Warach:** None. **L.D. McCullough:** None. **S.I. Savitz:** None. **F.S. Vahidy:** None.

Funding: No

Funding Component:

027

Hyperglycemia and Oxidative Stress Contribute to Impaired CerebralAutoregulation in Association With Dementia in Diabetes

Authors: Shaoxun Wang, Xiaochen He, George Booz, Richard Roman, Fan Fan, Univ of Mississippi Medical, Jackson, MS

Diabetes mellitus is characterized by hyperglycemia and is well established to associate with oxidative stress. The present study examines whether impaired myogenic response of middle cerebral artery MCA and autoregulation of cerebral blood flow (CBF) in our T2DN diabetic rats is due to hyperglycemia and oxidative stress induced actin cytoskeleton reorganization, and if this contributes to dementia. We identified that both young (4-month) and older (18-month) T2DN diabetic rats exhibit impaired pressure-induced myogenic response in isolated MCAs. Forced dilatation occurred at pressures above 140 mmHg in MCAs isolated from elderly T2DN rats with mild hypertension but not controls. Cortical blood flow measured by laser Doppler flowmetry rose by 137 ± 15% and 36 ± 5%, respectively, in T2DN and SD rats when MAP was increased from 100 to 180 mmHg. Autoregulation of CBF was shifted to lower pressures in elderly T2DN rats and they exhibited breakthrough at pressures above 140 mmHg. The F-Actin distribution area was significantly reduced in the vascular smooth muscle cells (VSMCs) freshly isolated from MCAs of T2DN rats compared with normal Sprague Dawley (SD) rats. The F-actin was disrupted similarly in VSMCs treated with high glucose or H_2O_2. Superoxide production is elevated in fresh isolated middle cerebral arteries (MCA) in T2DN diabetic rats compared to normal SD rats. We also found increased production of superoxide in primary VSMCs isolated from normal SD rats cultured in high glucose condition or freshly isolated from diabetic rats. T2DN rats exhibited neurons death in the hippocampus and cortex. Elderly T2DN rats showed memory disabilities. These results indicate that hyperglycemia induced elevated superoxide production causes actin cytoskeleton reorganization in cerebral VSMCs of diabetic T2DN rats, which may contribute to impaired cerebral vascular function, neurodegeneration and cognitive impairment.

Disclosures: S. Wang: None. **X. He:** None. **G. Booz:** None. **R. Roman:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; P20GM104357, HL36279, DK104184, HL138685. **F. Fan:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AG050049, 16GRNT31200036, P20GM104357.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

028

Susceptibility to Strokes in Spontaneously Hypertensive Rats Due to a Mutation in *Stim1*

Authors: Isha S Dhande, Univ of Texas HSC, Houston, TX; Sterling C Kneedler, Yaming Zhu, Univ of Texas Health Science Ctr at Houston, Houston, TX; M John Hicks, Scott E Wenderfer, Michael C Braun, Baylor Coll of Med, Houston, TX; Peter A Doris, Univ of Texas Health Science Ctr at Houston, Houston, TX

Stroke-prone spontaneously hypertensive rats (SHR-A3/SHRSP) develop cerebrovascular disease as a result of naturally occurring genetic variation. We recently identified a novel truncating mutation in the SHR-A3 line affecting the C-terminus of STIM1, a protein involved in the store-operated Ca^{2+} entry (SOCE) pathway. The SHR-B2 line, which is also hypertensive but resists end organ injury, expresses the 'wild type' *Stim1*. Here, we test whether the emergence of cerebrovascular disease in SHR-A3 is prevented by gene rescue of *Stim1*. We created a *Stim1* congenic line (SHR-A3(*Stim1*-B2)), in which the functional *Stim1* allele was transferred from the SHR-B2 line into the stroke-prone SHR-A3 genome. SHR-A3 and SHR-A3(*Stim1*-B2) rats were salt loaded (1% NaCl in drinking water) for 8 weeks starting at 20 weeks of age to induce strokes. Baseline BP measured by telemetry before salt loading was not different between SHR-

A3 and SHR-A3(*Stim1*-B2) rats (199.5±6.49 vs 196.26±2.431 mmHg, ns). Salt loading resulted in a progressive increase in BP in SHR-A3 rats, but was blunted in SHR-A3(*Stim1*-B2) rats. Compared to SHR-A3 rats, *Stim1*-rescue congenic rats had improved survival (% survival: 100 (9 of 9) vs 22.2% (2 of 9) at the end of 8 weeks) and lower neurological deficit scores (2.45±0.412 vs 1±0.00, p<0.01). Salt loading resulted in significant cerebral edema in SHR-A3 rats but not in the SHR-A3(*Stim1*-B2) rats (% brain wt/body wt: 0.805±0.045 vs 0.617±0.009, p<0.01). Gross morphology of the brain revealed microbleeds and hemorrhages in 5 of 9 SHR-A3 rats. These lesions were absent in SHR-A3(*Stim1*-B2) rats. *Stim1* gene rescue in the congenic line was also associated with decreased susceptibility to renal injury assessed histologically at 40 weeks of age (glomerular injury: 1.915±0.086 vs 1.355±0.071, p<0.001; tubulointerstitial injury: 3.2±0.102 vs 1.69±0.112, p<0.001). Our findings identify *Stim1* as a major candidate gene that promotes susceptibility to strokes as well as renal injury in spontaneously hypertensive rats.

Disclosures: I.S. Dhande: None. **S.C. Kneedler:** None. **Y. Zhu:** None. **M.J. Hicks:** None. **S.E. Wenderfer:** None. **M.C. Braun:** None. **P.A. Doris:** None.

Funding: Yes

Funding Component: South Central Affiliate (Arkansas, New Mexico, Oklahoma & Texas)

029

Epidemiological Study Evaluating Uncontrolled Systolic Blood Pressure in Patients ≥ 50 Years With Hypertension, in Relation to Stroke Risk and Current Antihypertensive Treatment Strategies (systup) - Analysis of India Cohort

Authors: Prof. Rishi Sethi, King George Medical Univ, Lucknow, India; Dr. Ashwani Mehta, Sir Ganga Ram Hosp, New Delhi, India; Dr. Viveka Kumar, Max Super Speciality Hosp, New Delhi, India

Background
Uncontrolled hypertension (HT), a critical risk factor contributing to rising burden of stroke; 57% of all stroke deaths in India. Elevated mean systolic blood pressure (MSBP) accounts for most cases of uncontrolled HT.
Aim
To assess systolic blood pressure (SBP) in patients ≥ 50 years with uncontrolled SBP, to illustrate correlation of MSBP with stroke risk (using Stroke Riskometer^{TM}), and the management of patients with uncontrolled SBP.
Methods
SYSTUP, a non-interventional, observational study, recruited patients with age ≥50 years, with uncontrolled SBP (≥ 140 mm Hg), from 176 centers across India in real-world clinical setting. Descriptive statistics and Logistic regression analysis were used for risk calculation.
Results
3791 patients (men 60 %) - mean age 62 years, mean BMI 27 kg/m^2 and mean BP 157/90 mmHg. A significant positive correlation was found between MSBP and stroke risk. The likelihood of 5 year and 10 year stroke risk increased by approximately 4% with 1 mmHg increase in MSBP (P <.0001). MDBP did not exhibit any significant correlation with 5 year (P=0.242) and 10 year (P=0.8038) stroke risk. Comorbid diabetes was the main risk factor of 5 year stroke risk with odds ratio of 1.6.
Majority of the patients were receiving angiotensin receptor blocker (ARB), irrespective of the risk categories - low, moderate, high, very high risk (Table).
Conclusion: The SYSTUP India results show that elevated MSBP increases stroke risk in patients ≥ 50 years with uncontrolled HT mainly treated with ARB. These real-world clinical findings call for strengthening BP control in primary care to prevent stroke.

Table: Stroke risk as per therapeutic class of treatment used at the time of screening

Projection years	Therapeutic class	Stroke risk (N=3791)			
		Low	Moderate	High	Very high
5 year	Total	2504 (66.1)	769 (20.3)	255(6.7)	263(6.9)
	ACE inhibitor	424 (16.9)	118 (15.3)	62 (24.3)	54 (20.5)
	Angiotensin receptor blocker	916 (36.6)	337 (43.8)	123 (48.2)	111(42.2)
	Beta-blocker	507 (20.2)	198 (25.7)	59 (23.1)	76 (28.9)
	Calcium channel blocker	845 (33.7)	256 (33.3)	87 (34.1)	88 (33.5)
	Diuretic	643 (25.7)	241 (31.3)	98 (38.4)	95 (36.1)
	Alpha-blocker or centrally acting agent	28 (1.1)	14 (1.8)	4 (1.6)	6 (2.3)
10 year	Total	1119 (29.5)	1176 (31.0)	583 (15.4)	913 (24.1)
	ACE inhibitor	154 (13.8)	224 (19.0)	99 (17.0)	181 (19.8)
	Angiotensin receptor blocker	412 (36.8)	427 (36.3)	242 (41.5)	406 (44.5)
	Beta-blocker	212 (18.9)	239 (20.3)	144 (24.7)	245 (26.8)
	Calcium channel blocker	371 (33.2)	402 (34.2)	207 (35.5)	296 (32.4)
	Diuretic	275 (24.6)	320 (27.2)	159 (27.3)	323 (35.4)
	Alpha-blocker or centrally acting agent	11 (1.0)	15 (1.3)	9 (1.5)	17 (1.9)

Data presented are n (%). The % of therapeutic classes are related to within stroke risk class.

Disclosures: P. Sethi: None. **D. Mehta:** None. **D. Kumar:** None.

Funding: No

Funding Component:

030

Stem Cell-Derived Extracellular Vesicles Attenuates Aging-Associated Arterial Stiffness and Hypertension

Authors: Rui Feng, Chongqing Medical Univ, Chongqing, China; Mujib Ullah, Kai Chen, Lin Yi, Univ Of Tennessee Hsc, Memphis, TN; Han Lei, Chongqing Medical Univ, Chongqing, China; **Zhongjie SUN**, Univ Of Tennessee Hsc, Memphis, TN

Objective. Stem cell-derived extracellular vesicles are decreased with aging. Aging is a major risk factor for the development of arterial stiffness and hypertension. This study was designed to assess the effect of inducible pluripotent stem cells (iPSC)-derived extracellular vesicles (EVs) on the aging-related arterial stiffness and hypertension.
Methods and Results. Adult (6 months) and aged (20 months) mice were used. Pulse wave velocity (PWV), an index of arterial stiffness and blood pressure were measure twice a week. PWV and blood pressure were increased significantly in aged mice, indicating arterial stiffness and hypertension. One of the exciting findings is that intravenous administration of iPSC-derived EVs significantly decreased aging-related arterial stiffness and hypertension. Treatment

with EVs also enhanced endothelium-dependent vascular relaxation and arterial compliance in aged mice. It was noticed that elastin degradation and collagen I deposition (fibrosis) were increased in aortas of aged mice, formulating a structural basis of arterial stiffness. The aging-associated structural remodeling was nearly eliminated by EVs. Mechanistically, EVs abolished downregulation of Sirt1 and eNOS protein expression in aortas of aged mice. In cultured human aortic endothelial cells, we found that EVs promoted the expression and phosphorylation of Sirt1, AMPKα, and eNOS. In addition, EVs attenuated aging-associated renal glomerulosclerosis and tubulointerstitial fibrosis.
Conclusion. This study demonstrates for the first time that iPSC-derived EVs improved aging-associated vascular endothelial dysfunction, arterial stiffening, renal remodeling and hypertension likely *via* regulating the Sirt1-AMPKα-eNOS pathway. A decrease in stem cell-derived EVs plays an important role in aging-associated arterial stiffening and hypertension. This finding sheds light on the therapeutic potential of EVs for arterial stiffness and hypertension associated with aging.

Disclosures: R. Feng: None. **M. Ullah:** None. **K. Chen:** None. **L. Yi:** None. **H. Lei:** None. **Z. Sun:** None.

Funding: No

Funding Component:

031

Thresholds for Defining Home Blood Pressure Elevation Among US adults

Authors: Hamza Lodhi, Colby Ayers, Sandeep Das, Jarett Berry, Amit Khera, Univ of Texas Southwestern Medical Ctr, DALLAS, TX; Ronald Victor, Cedar Sinai Medical Inst/Hypertension Ctr, Los Angeles, CA; Feng-Chang Li, Univ of North Carolina at Chapel Hill, Chapel Hill, NC; Anthony Viera, Duke Univ, Durham, NC; Yuichiro Yano, Univ of Mississippi, Jackson, MS; James de Lemos, Wanpen Vongpatanasin, Univ of Texas Southwestern Medical Ctr, DALLAS, TX

Background: Most hypertension guidelines have recommended lower home than clinic BP thresholds based on epidemiological studies conducted in non-US populations. However, diagnostic thresholds to define home BP elevation have never been determined in the general population in the United States.
Methods: We analyzed data from 2 US cohorts with home and clinic BP data in the same participants, the Dallas Heart Study (DHS, n=5,768) and the North Carolina Masked Hypertension study (NCMH, n = 420). Home BP thresholds for stage 1 (BP ≥ 130/80 mmHg) and stage 2 hypertension (BP ≥ 140/90 mmHg) were identified using a regression-based approach in both cohorts and an outcome-derived approach in the DHS cohort. The composite of all-cause mortality or a cardiovascular disease (CVD) event was used in the outcome-derived approach. For this approach, BP thresholds were identified only for systolic BP (SBP) as clinic diastolic BP (DBP) was not associated with the outcome.
Results: Among untreated participants in DHS, the regression-derived thresholds for home BP corresponding to clinic BP for stage 1 and stage 2 hypertension were 130/81 and 137/88 mmHg in blacks and 127/79 and 135/86 mmHg in nonblacks, respectively. The results are virtually identical in the NCMH cohort. The 11-year composite CVD and mortality events corresponding to stage 1 (clinic SBP ≥ 130) and stage 2 (clinic SBP ≥ 140 mmHg) were higher in untreated blacks than untreated non-blacks [13.3 (11.06-15.54) and 17.59 (14.8-20.37)% vs. 6.78 (5.17-8.39) and 9.86 (6.94-12.77)%, respectively]. Using a race/ethnicity-specific composite outcome, the outcome-derived thresholds from the DHS for home SBP corresponding to stage 1 and stage 2
hypertension were 131 and 140 mmHg in untreated blacks and 130 and 139 mmHg in untreated nonblacks, respectively.
Conclusions: Based on the regression-derived approach, we found home BP thresholds for stage 1 and 2 hypertension from both cohorts to be similar to the current ACC/AHA guidelines. The outcome-derived approach also identified similar home SBP thresholds for stage 1 hypertension but slightly higher home SBP thresholds for stage 2 hypertension than recommended guidelines.

Disclosures: H. Lodhi: None. **C. Ayers:** None. **S. Das:** None. **J. Berry:** None. **A. Khera:** None. **R. Victor:** None. **F. Li:** None. **A. Viera:** None. **Y. Yano:** None. **J. de Lemos:** None. **W. Vongpatanasin:** None.

Funding: No

Funding Component:

032

Reconstitution of Autophagy Ameliorates Endothelium-Dependent Relaxation, Vascular Smooth Muscle Calcium Sensitization, & Arterial Stiffening in Spontaneously Hypertensive Rats

Authors: Cameron G. McCarthy, Camilla F. Wenceslau, Fabiano B. Calmasini, Nicole S. Klee, R. Clinton Webb, Augusta Univ, Augusta, GA

Insufficient autophagy has been proposed as a mechanism of cellular aging, as this leads to the accumulation of dysfunctional macromolecules and organelles. Premature vascular aging occurs in hypertension. In fact, many factors that contribute to the deterioration of vascular function as we age are accelerated in clinical and experimental hypertension. Previously, we have reported decreased autophagic activity in arteries from spontaneously hypertensive rats (SHR); however, the effects of restoring autophagic activity on blood pressure and vascular function are currently unknown. We hypothesized that reconstitution of arterial autophagy in SHR would decrease blood pressure and improve endothelium-dependent relaxation. We treated 13-18 week old Wistar and SHR with autophagy activator trehalose (2% in drinking water) for 28 days. Blood pressure was measured by telemetry and vascular function was performed on isolated mesenteric resistance arteries using wire and pressure myographs. Treatment with trehalose had no effect on mean arterial pressure in SHR ($p>0.05$). Trehalose treatment in SHR improved the relaxation to acetylcholine (ACh) [%Relaxation (ACh 1 μM), vehicle: 59.6±8.4 vs. trehalose: 82.4±4.2; $p<0.05$], and this was not different from arteries incubated with cyclooxygenase inhibitor indomethacin and reactive oxygen species scavenger tempol [%Relaxation (ACh 1 μM), trehalose: 82.4±4.2 vs. vehicle+indomethacin: 92.7±3.9 and vehicle+tempol: 89.3±5.4; both $p>0.05$]. Trehalose treatment in SHR also prevented the relaxation to Rho kinase inhibition [%Relaxation (Y-27632 0.3 μM), vehicle: 30.4±4.3 vs. trehalose: 8.4±3.5; $p<0.05$]. Finally, trehalose treatment in SHR decreased arterial stiffness as indicated by the slope of the stress-strain curve [β (stress-strain), vehicle: 8.3±0.6 vs. trehalose: 5.5±0.2; $p<0.05$]. Overall these data indicate that reconstitution of arterial autophagy in SHR improves endothelial and vascular smooth muscle function, which could synergize to prevent arterial stiffening. As a result, restoration of arterial autophagic activity could be a novel therapeutic for premature vascular aging in hypertension, a recently adopted clinical guideline for cardiovascular disease prevention.

Disclosures: C.G. McCarthy: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; 18POST34060003. **C.F. Wenceslau:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; K99GM118885. **F.B. Calmasini:** None. **N.S. Klee:** None. **R. Webb:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; P01HL134604.

Funding: Yes

Funding Component: National Center

033

Identification of s Primary Renal AT_2 Receptor Defect in Spontaneously Hypertensive Rats (SHR)

Authors: Nancy L. Howell, Brandon Ambrose Kemp, Susanna R. Keller, John J. Gildea, Univ of Virginia, Charlottesville, VA; Weijian Shao, Tulane Univ, New Orleans, LA; L. Gabriel Navar, Tulane Univ, New Orleans, VA; Robert M. Carey, Univ of Virginia, Charlottesville, VA

Angiotensin III (Ang III) [des-aspartyl[1]-Ang II] is the predominant endogenous agonist for angiotensin type-2 receptor (AT_2R)-induced natriuresis in rats. Hypertensive 12 wk-old SHR lack natriuretic responses to Ang III, and renal interstitial (RI) Ang III administration induces natriuresis in 4 wk-old Wistar-Kyoto rats (WKY), but not in 4 wk-old pre-hypertensive SHR. The defect in natriuresis could be explained by the concurrent observation that in WKY, but not SHR, Ang III induced AT_2R translocation to renal proximal tubule apical plasma membranes and internalization/inactivation of Na^+-transporters Na^+- H^+ exchanger-3 (NHE-3) and Na^+/K^+ATPase (NKA). These findings suggest an Ang III/AT_2R signaling defect in pre-hypertensive SHR. The present study sought to determine the cause of the defect: (1) accelerated intrarenal Ang III metabolism or (2) a primary AT_2R (or post-receptor) signaling defect. Selective AT_2R agonist Compound-21 (C-21) was infused interstitially [20, 40 and 60 ng/kg/min, each dose for 30 min] into experimental (left) kidneys of 4 wk-old WKY and SHR (N=7) pretreated for 24h with systemic infusion of Ang type-1 receptor (AT_1R) antagonist candesartan (0.01 mg/kg/min by osmotic minipump); vehicle was infused into control (right) kidneys. Pre-infusion urinary Na^+ excretion ($U_{Na}V$) was 0.022 ± 0.004 µmol/min in WKY and 0.024 ± 0.005 µmol/min in SHR (P=NS). In WKY, C-21 dose-dependently increased $U_{Na}V$ from 0.022 ± 0.004 to 0.058 ± 0.017, 0.078 ± 0.014 and 0.079 ± 0.079 µmol/min (P=0.01 from pre-infusion and time control kidneys). In contrast, SHR did not increase $U_{Na}V$ in response to C-21 [highest $U_{Na}V$ 0.033 ± 0.007 µmol/min (P=NS from pre-infusion and time control kidneys)]. Mean arterial pressure (MAP) was 64.6 ± 1.80 mmHg in WKY and 76.4 ± 2.2 mmHg (P<0.01) in SHR and did not change with C-21 or vehicle in WKY and SHR. In a separate set of 4 wk-old WKY and SHR (N=8), RI Ang II and III concentrations were measured by HPLC and ELISA assay. RI Ang II and Ang III levels, respectively, in WKY were 55 ± 20 and 59 ± 17 pg/ml (P=NS) and in SHR were 37 ± 7 and 181± 44 pg/ml (P=0.01 vs SHR Ang II; P<0.025 vs WKY Ang III). These findings exclude accelerated Ang III metabolism as a cause and identify a primary renal AT_2R defect as responsible for increased Na^+ reabsorption in SHR.

Disclosures: N.L. Howell: None. **B.A. Kemp:** None. **S.R. Keller:** None. **J.J. Gildea:** None. **W. Shao:** None. **L.G. Navar:** None. **R.M. Carey:** None.

Funding: No

Funding Component:

034

Resilience of Isolated, Perfused Cerebral Penetrating Microarterioles to Angiotensin II (Ang II) Contractions Depends on Local Generation of Prostaglandin D_2 (PGD_2)

Authors: Lingli Li, EnYin Lai, Zaiming Luo, Georgetown Univ Div of Nephrology, Washington, DC; Robin Davisson, Cornell Univ, Ithica, NY; William Welch, Christopher S Wilcox, Georgetown Univ Div of Nephrology, Washington, DC

The brain depends on a continuous supply of blood for its oxygenation, whereas the kidney is over-perfused for its metabolic needs in order to provide sufficient plasma to form a glomerular filtrate. Thus, the brain requires resilience to vasoconstriction to prevent ischemia and vascular cognitive impairment, but the mechanisms are unclear. *Methods and results:* Single penetrating cerebral microarterioles (CMAs, 12-18µM) were dissected from the frontal cortex and single renal afferent arterioles (RAAs, 8-12µM) from the kidney cortex to investigate the hypothesis that CMAs deploy unique mechanisms to provide resilience to Ang II vasoconstriction. Individual arteriolar genes were assessed by RNAseq or RT-PCR of endothelial cells (ECs). The mRNA for lipocalin type PGD_2 synthase (LPGDS) and the PGD 1 receptor (PD1R) were >

3,000-fold higher in CMAs than RAAs whereas RAAs expressed 3-fold more mRNA for thromboxane A_2 synthase. Both microarterioles had similar expression of AT1Rs. Endothelial cells cultured from these vessels had similar patterns of gene expression. Single isolated perfused RAAs contracted strongly with Ang II (at 10^{-6} mol·l^{-1}; -47 ± 2%; P<0.005) whereas CMAs were totally resistant to Ang II (0.1 ± 0.1%; NS), yet both contracted similarly to endothelin I or perfusion pressure (n = 6 per group). However, CMAs from COX 1 -/- (vs +/+) mice did contract with Ang II (-15 ± 2 vs 0.1 ± 0.1%; P<0.01) and contracted with Ang II after incubation with parecoxib (vs vehicle) to block COX2 (-7 ± 3 vs 0.1 ± 0.1%; P<0.01) or after dual blockade of COX1 + 2 (-20 ± 2%; P<0.01) or after incubation with AT-56 (vs vehicle) to block LPGDS (-20 ± 3 vs 0.1 ± 0.1%; P<0.01). During LPGDS blockade, incubation of CMAs with BW245c (stable PD1R agonist) reduced Ang II contraction > 65% (-8 ± 2%; P<0.01). In contrast, COX blockade reduced Ang II contractions of RAAs, indicating opposing effects of PGs on cerebral and renal vessels. Measurements of cerebral and renal blood flow and MAP in anesthetized mice confirmed selective renal vasoconstriction with Ang II, yet selective cerebral vasodilation with BW245c. *Perspective:* Resilience against Ang II vasoconstriction in cerebral arterioles depends on the generation of PGD_2 and could be a therapeutic target for vascular dementia and stroke.

Disclosures: **L. Li:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Co Investigator. **E. Lai:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Co Investigator. **Z. Luo:** None. **R. Davisson:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Investigator. **W. Welch:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Co Investigator. **C.S. Wilcox:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PI.

Funding: No

Funding Component:

035

Increasing Renal AcSDKP by Eliminating the ACE N-Domain Blocks Renal Inflammation and Sodium Retention During Diabetic Nephropathy

Authors: Jorge F Giani, Masahiro Eriguchi, Ellen A. Bernstein, Luciana C. Veiras, Zakir Khan, Duo Y. Cao, Cedars-Sinai Medical Ctr, Los Angeles, CA; Sebastien Fuchs, Western Univ of Health Sciences, Pomona, CA; Alicia A. McDonough, Keck Sch of Med of USC, Los Angeles, CA; Jorge E. Toblli, Hosp Aleman, Buenos Aires, Argentina; Romer A. Gonzalez-Villalobos, Kenneth E. Bernstein, Cedars-Sinai Medical Ctr, Los Angeles, CA

Angiotensin-converting enzyme (ACE) plays a key role in renal inflammation and sodium retention associated with diabetic nephropathy. Although most effects of ACE have been classically related to angiotensin (Ang) II synthesis, studies highlight an Ang II-independent role of ACE in inflammation. Indeed, ACE has two catalytic domains, the N- and C-domains, that can process a wide diversity of substrates besides Ang I. Here, we study the relative contributions of ACE domains to renal inflammation and sodium retention during diabetic nephropathy. Diabetes was induced with streptozotocin in wild-type (WT) mice and mice lacking either a functional ACE N-domain (NKO) or C-domain (CKO) (n=5-8). After 6 months of diabetes, we evaluated the natriuretic response to volume expansion. For this, mice were injected with 0.9% NaCl equivalent to 10% of their body weight. After 5 hours, diabetic NKO mice excreted 30% more urinary sodium in response to the saline challenge compared to diabetic WT or CKO mice (P<0.05). This enhanced natriuretic response was associated with a 47% reduction (P<0.05) of renal epithelial sodium channel (ENaC) cleaved (active) α and γ subunits. Further, diabetic NKO displayed lower levels of renal fibrosis (46% reduction, P<0.05), IL-1β (56% reduction, P<0.05), TNFα (50% reduction, P<0.05) and albuminuria (53% reduction, P<0.01) compared to WT and CKO diabetic mice. This protective phenotype was not associated with changes in renal Ang II levels (WT: 237 ± 73; NKO: 283 ± 53 CKO: 245 ± 39 fmol/g kidney, P=NS). We next evaluated whether the anti-inflammatory tetrapeptide N-acetyl-seryl-

asparyl-lysyl-proline (AcSDKP), an ACE N-domain specific substrate, mediates the protective phenotype of NKO. For this, diabetic mice were treated with the prolyl oligopeptidase inhibitor, S17092 (10 mg/kg, IP), that prevents the synthesis of AcSDKP. In diabetic NKO mice receiving S17092, sodium excretion in response to a saline challenge, ENaC α and γ subunit cleavage, renal inflammation and renal injury were indistinguishable from equally treated diabetic WT mice. In summary, these data indicate that increasing AcSDKP by blocking the ACE N-domain improves sodium handling and ameliorates diabetic kidney disease independently of intrarenal Ang II regulation.

Disclosures: J.F. Giani: None. **M. Eriguchi:** None. **E.A. Bernstein:** None. **L.C. Veiras:** None. **Z. Khan:** None. **D.Y. Cao:** None. **S. Fuchs:** None. **A.A. McDonough:** None. **J.E. Toblli:** None. **R.A. Gonzalez-Villalobos:** None. **K.E. Bernstein:** None.

Funding: Yes

Funding Component: Western States Affiliate (California, Nevada & Utah)

036

Interference With PPARγ in the Endothelium Produces Endothelial Dysfunction in the Cerebral Circulation in Response to Activation of the Endogenous Renin-Angiotensin System

Authors: Anand R Nair, Larry N. Agbor, Masashi Mukohda, Xuebo Liu, Chunyan Hu, Jing Wu, Curt D. Sigmund, Univ of Iowa, Iowa City, IA

Low salt diet (LSD) is beneficial in salt-sensitive hypertension but may provoke cardiovascular risk in patients with heart failure, diabetes, or other cardiovascular abnormalities because of renin-angiotensin system (RAS) activation. PPARγ is a transcription factor which promotes an anti-oxidant pathway in the endothelium. We studied transgenic mice expressing a dominant-negative mutation in PPARγ selectively in the endothelium (E-DN) to test the hypothesis that endothelial PPARγ plays a protective role in response to LSD-mediated RAS activation. Plasma renin and angiotensin were significantly and equally increased in all mice fed LSD for 6-weeks (Renin - NT: 39±7 vs 20±1 ng/ml; E-DN: 34±1 vs 16±4 ng/ml; Ang - NT: 257±54 vs 47±6 pg/ml; E-DN: 294±69 vs 63±14 pg/ml $p<0.05$, n=5). Vasorelaxation to acetylcholine was not affected in basilar artery from E-DN at baseline, but was significantly and selectively impaired in E-DN after LSD (33±5 vs 69±2%, $p<0.05$, n=6). Unlike basilar artery, LSD was not sufficient to induce vascular dysfunction in carotid artery (carotid artery: 86±4 vs 92±3%, n=5). Endothelial dysfunction in the basilar artery from E-DN mice fed LSD was attenuated by scavengers of superoxide (improved from 29±5% to 55±7%, n=6), inhibitors of NADPH oxidase (improved from 23±3% to 54±7%, $p<0.05$, n=6), or blockade of the angiotensin-II AT1 receptor (improved from 31±5% to 64±9%, $p<0.05$, n=5). Gene expression levels of Nox2 was elevated (2.1±0.3 vs 0.4±0.1, $p<0.05$, n=7) while those of antioxidant enzymes catalase and SOD3 were blunted in cerebral vessels of E-DN mice on a LSD (catalase: 0.5±0.1 vs 2.5±0.2; SOD3: 0.2±0.1 vs 1.1±0.1, $p<0.05$, n=7). Simultaneous AT1 and AT2 receptor blockade revealed the restoration of endothelial function after AT1 receptor blockade was not a consequence of AT2 receptor activation (59±10 vs 48±2, $p<0.05$, n=4). 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, mediated at least in part, through AT1 receptor activation and perturbed redox homeostasis. Moreover, our data suggest that the cerebral circulation may be particularly sensitive to inhibition of PPARγ activity and RAS activation.

Disclosures: A.R. Nair: None. **L.N. Agbor:** None. **M. Mukohda:** None. **X. Liu:** None. **C. Hu:** None. **J. Wu:** None. **C.D. Sigmund:** None.

Funding: Yes

Funding Component: National Center

037

Global ACE2 Over-Expression is Protective Against a Dysfunctional Brain-Gut-Lung Axis in Hypoxia-Induced Pulmonary Hypertension

Authors: Ravindra K Sharma, Aline C Oliveira, Tao Yang, Victor Aquino, Gilberto O Lobaton, Andrew J Bryant, Eric G Krause, Annette D de Kloet, Elaine M Richards, Mohan K Raizada, Univ of Florida, Gainesville, FL

Background and Objectives: Angiotensin converting enzyme 2 (ACE2), key enzyme of the vasoprotective axis of the renin angiotensin system, has been implicated in many pulmonary diseases including pulmonary hypertension (PH). Plasma levels of ACE2 in group-1 pulmonary hypertensive patients are decreased and activation of this enzyme by small molecule activators, overexpression or administration of recombinant ACE2, all have beneficial outcomes on PH. Furthermore, involvement of neuroinflammation and impaired brain-gut-lung axis have been proposed in PH. These observations have led us to propose that beneficial effect of ACE2 are, at least in part, due to attenuation of neuroinflammation and rebalancing of gut microbiota and improvement in pathology. Methods: ACE2 knock-in (ACE2KI) and wild-type mice (WT; C57BL/6) were subjected to hypoxia (10% FIO2) or room air for 4 weeks (n=8-10/group). Right ventricular systolic pressure (RVSP), and electrocardiography were performed, tissues examined by standard histological techniques, and stool collected for 16S rRNA analysis at 4 weeks. Results: Hypoxia-induced significantly increased RVSP in WT but not ACE2KI mice (20±1.6 vs 32±1.9, $p<0.001$ and 24.3±1.5 vs 21±1.3mmHg, respectively). This was accompanied in WT, but not ACEKI, by ~5-fold increase in sympathetic activation (LF/HF; $p<0.001$) and ~2-fold increase ($p<0.01$) in microglia activation in the paraventricular nucleus (PVN) of the hypothalamus. There was increased fibrotic area ($p<0.01$) and muscularis layer thickening ($p<0.001$) and decreased villi length ($p<0.01$) and goblet cells ($p<0.001$) in the small intestine of WT but not ACE2KI mice following hypoxia. Finally, beta diversity of gut microbiome of WT, but not ACE2KI, mice was significantly altered by hypoxia (ANOSIM P=0.001). Conclusions: Microglial activation in PVN, sympathetic activation, gut pathology and altered gut microbiome are associated with hypoxia-induced PH. Global overexpression of ACE2 prevents all of these parameters. The involvement of organs other than lungs, and ACE2, present novel therapeutic potentials for PH.

Disclosures: R.K. Sharma: None. **A.C. Oliveira:** None. **T. Yang:** None. **V. Aquino:** None. **G.O. Lobaton:** None. **A.J. Bryant:** None. **E.G. Krause:** None. **A.D. de Kloet:** None. **E.M. Richards:** None. **M.K. Raizada:** None.

Funding: No

Funding Component:

038

The Dual Role of B-Cell Lymphoma/Leukemia 10 in Angiotensin II-Induced Renal Damage

Authors: Lajos Markó, Experimental Clinical Rsrch Ctr&Dzhk (German Ctr For Cardiovascular Res)&Berlin Inst Of Health (Bih)&Charité, Berlin, Germany; Joon-Keun Park, Hannover Medical Sch, Hannover, Germany; Norbert Henke, Helios Clinic Damp, Berlin, Germany; Song Rong, Transplantation Ctr, Zunyi Medical Coll&Nephrology, Hannover Medical Sch, Zunyi, China; András Balogh, Experimental Clinical Rsrch Ctr&Dzhk (German Ctr For Cardiovascular Res)&Berlin Inst Of Health (Bih)&Charité, Berlin, Germany; Samuel Klamer, Experimental Clinical Rsrch Ctr, Berlin, Germany; Nicola Wilck, Experimental Clinical Rsrch Ctr&Dzhk (German Ctr For Cardiovascular Res)&Berlin Inst Of Health (Bih)&Charité, Berlin, Germany; Jürgen Ruland, Inst for Clinical Chemistry and Biochemistry, Klinikum rechts der Isar, Technical Univ of Munich, Munich, Germany; Friedrich C Luft, Experimental Clinical Rsrch Ctr&Max Delbruck Ctr For Molecular Med, Berlin, Germany; Ralf Dechend, Experimental Clinical Rsrch Ctr&Dzhk (German Ctr For Cardiovascular Res)&Berlin Inst Of Health (Bih)&Charité&10Helios Clinic Berlin-Buch, Berlin, Germany; Dominik N Müller, Experimental Clinical Rsrch Ctr&Dzhk (German Ctr For Cardiovascular Res)&Berlin Inst Of Health (Bih)&Charité&Max Delbruck Centr For Molecular Med, Berlin, Germany

Angiotensin (Ang) II activates NF-κB and thereby induces organ damage. B-cell Lymphoma/Leukemia 10 (Bcl10) is a member of the CARMA-Bcl10-MALT1 signalosome that links Ang II and antigen-dependent activation of immune cells to NF-κB signaling. We have shown earlier that Bcl10 plays a significant role in the generation of Ang II–induced cardiac fibrosis and electric remodeling in a blood pressure independent fashion. In our present study we investigated the role of Bcl10 in Ang II–induced renal damage.
Bcl10 knockout mice (Bcl10 KO) and wild-type (WT) controls were given 1% NaCl in the drinking water and Ang II (1.44 mg/kg/d) for 14 days. Additionally, Bcl10 KO or WT kidneys were transplanted onto WT mice and were challenged by the same protocol for 7 days. Kidneys of Bcl10 KO mice developed less fibrosis and showed reduced number of infiltrating macrophages and T cells. Nevertheless, renal expression of neutrophil gelatinase-associated lipocalin (Ngal) and kidney injury molecule 1 expression was higher after Ang II treatment in Bcl10 KO mice compared to WT mice indicating exacerbated tubular damage (4.94±1.56 vs 1.44±0.44 and 1.14±0.22 vs 2.87±1.05 AU respectively). In addition, albuminuria was significantly higher in Ang II-treated Bcl10 KO mice than in controls (1194±238 vs. 377±112 µg/day) accompanied by reduced glomerular nephrin expression and reduced podocyte number (5.9±0.4 vs. 8.2±0.5 Wilms' tumor-1 protein (WT1) positive cells/glomerulus). Ang II-treated WT mice transplanted with Bcl10 KO kidney showed significantly higher albuminuria (6003±1988 vs. 1779±831 µg/day) and renal Ngal expression (1.75±4.08 vs 0.71±0.57 AU) compared to WT->WT kidney transplanted mice, as well as lower podocyte number (5.4±0.3 vs. 4.9±0.2 WT1 positive cells/glomerulus) but similar extent of fibrosis and cell infiltration. Lacking Bcl10 in the kidney only was protective from Ang II-induced cardiac hypertrophy.
Bcl10 has a multi-faceted action in hypertensive renal damage. While global Bcl10 deficiency ameliorates renal fibrosis and cell infiltration, the lack of renal Bcl10 aggravates albuminuria and podocyte damage. These data indicate that Bcl10 plays a crucial role in maintaining podocyte integrity and renal function.

Disclosures: L. Markó: None. **J. Park:** None. **N. Henke:** None. **S. Rong:** None. **A. Balogh:** None. **S. Klamer:** None. **N. Wilck:** None. **J. Ruland:** None. **F.C. Luft:** None. **R. Dechend:** None. **D.N. Müller:** None.

Funding: Yes

Funding Component:

039

Differential Expression of miR-146b-5p Modulates Sex-Specific Cardiac Phenotypes in a Rat Model of Chronic Kidney Disease

Authors: Mark Paterson, Alison J. Kriegel, Medical Coll Wisconsin, Milwaukee, WI

Type 4 cardiorenal syndrome is a complex disorder in which primary chronic kidney disease (CKD) contributes to secondary cardiovascular disease (CVD). Although the pathological link between the kidney and heart are well-characterized, the molecular switches which drive cardiac pathology are not well understood. miRNAs have the potential to be master regulators of cellular signaling networks through modulation of RNA and protein expression profiles and have been shown to be important in both cardiovascular health and disease. Previous work in our laboratory identified a significant increase of miR-146b-5p expression in both the heart and the kidney in a 5/6 nephrectomy (5/6Nx) model of CKD in the Sprague-Dawley rat. To evaluate how miR-146b-5p is involved with pathological changes in the heart following 5/6Nx, echocardiography, blood pressure and left ventricle (LV) pressure-volume analyses were performed on male and female wild-type (WT) and miR-146b-5p null mutant ($146b^{-/-}$) rats for seven weeks following 5/6Nx or sham surgery. Structural analysis of the heart revealed that male and female rats exhibited significant hypertrophy of the LV wall in both the WT (+21% [1.50 vs. 1.24g] and +24% [1.00 vs. 0.81g] vs. sham, respectively; $p<0.001$) and $146b^{-/-}$ (+17% [1.36 vs. 1.17g] and +26% [1.03 vs. 0.82g] vs. sham, respectively; $p<0.001$) genotypes. Furthermore, dilation of the LV chamber was observed in WT male (+9% LVIDd [9.45 vs. 8.67mm] vs. sham; $p=0.032$) and female (+29% LVIDd [9.39 vs. 7.30mm] and +36% LVIDs [5.80 vs. 4.86mm] vs. sham; $p<0.005$) rats 7 weeks post-5/6Nx. However, LV dilation was

attenuated in 146b$^{-/-}$ females (-18% LVIDd [8.18 vs. 9.39mm] and -26% LVIDs [4.88 vs. 5.80mm] vs. WT; p=0.07) but not in 146b$^{-/-}$ males. Functionally, LV pressure-volume analysis revealed 146b$^{-/-}$ females exhibit an increase in stroke work (+59% vs. sham; p=0.063) and a significantly elevated mean arterial blood pressure (+23mmHg vs. sham; p=0.02). These alterations in cardiovascular function are not observed in miR-146b$^{-/-}$ males. Taken together, these data suggest 146b$^{-/-}$ female rats may be resistant to eccentric dilation despite increased afterload and highlight the sex-specific modulation of cardiorenal phenotypes by miR-146b-5p following 5/6Nx.

Disclosures: M. Paterson: None. **A.J. Kriegel:** None.

Funding: Yes

Funding Component: National Center

040

Vascular Dysfunction and Androgen Deficiency - Insights From Children With Hypospadias

Authors: Angela K Lucas-Herald, Rheure Alves-Lopes, Laura Haddow, Inst for Cardiovascular and Medical Sciences, BHF Ctr for Res Excellence, Univ of Glasgow, Glasgow, United Kingdom; Stuart O'Toole, Syed Basith Amjad, Martyn Flett, Mairi Steven, Boma Lee, Dept of Paediatric Surgery, Royal Hosp for Children, Glasgow, United Kingdom; Augusto C Montezano, Inst for Cardiovascular and Medical Sciences, BHF Ctr for Res Excellence, Univ of Glasgow, Glasgow, United Kingdom; Syed Faisal Ahmed, Developmental Endocrinology Res Group, Sch of Med, Dentistry and Nursing, Univ of Glasgow, Glasgow, United Kingdom; Rhian M Touyz, Inst for Cardiovascular and Medical Sciences, BHF Ctr for Res Excellence, Univ of Glasgow, Glasgow, United Kingdom

Background: Hypospadias in boys may be associated with a lack of androgen exposure during the masculinisation programming window. As testosterone has effects on the vasculature, we assessed whether boys with hypospadias show any evidence of vascular dysfunction. Methods: Excess foreskin tissue was obtained from boys undergoing hypospadias repair (cases) or circumcision (controls) and small arteries dissected from this tissue. Vascular contractility was assessed by wire myography in response to U46619 (thromboxane A2 analogue). Vascular smooth muscle cells (VSMCs) were cultured and generation of reactive oxygen species (ROS) was measured by amplex red and chemiluminescence. NADPH oxidase (Nox) mRNA expression was measured by qPCR. Results: 19 cases and 22 age-matched controls were enrolled in this study (median age 1.9 (range 1.3,12.2) years). There were no differences in clinical cardiometabolic or biochemical parameters between the cases and controls. Arteries from cases demonstrated increased constriction to U46619 compared to controls (Emax 175.6 vs 66.3 $p<0.001$), an effect inhibited by the ROS scavenger N-acetylcysteine (NAC). VSMC superoxide anion (5.3 fold) production and H_20_2 (3.3 fold) levels were increased in cases compared to controls ($p<0.05$). Expression of Nox5, a major ROS-generating oxidase in vascular cells, was increased in cases (2.6 fold,$p<0.05$). Exposure of vessels to testosterone increased vasoconstriction to U46619 (Emax 66.3 to 124.6 $p<0.001$) in controls, but not in cases. Incubation with NAC abolished the testosterone-induced vascular effects. Vascular hypercontractility in boys with hypospadias was associated with reduced endothelium-dependent and -independent vasorelaxation, compared with controls. Conclusions: These novel data, from a unique cohort of patients, demonstrate that small arteries from boys with hypospadias exhibit increased vascular contractility and decreased vasorelaxation with associated increased Nox-derived ROS generation. The functional significance of vascular dysfunction in these boys is unclear but may play a role in immediate surgical outcome as well as altered long-term cardiovascular risk.

Disclosures: A.K. Lucas-Herald: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; British Heart Foundation Centre of Research Excellence RE/13/5/30177. **R. Alves-Lopes:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; British Heart Foundation Centre of Research Excellence RE/13/5/30177. **L. Haddow:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending

grants as well as grants already received); Significant; British Heart Foundation Centre of Research Excellence RE/13/5/30177. **S. O'Toole:** None. **S. Amjad:** None. **M. Flett:** None. **M. Steven:** None. **B. Lee:** None. **A.C. Montezano:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; British Heart Foundation Centre of Research Excellence RE/13/5/30177. **S. Ahmed:** None. **R.M. Touyz:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; British Heart Foundation Centre of Research Excellence RE/13/5/30177.

Funding: No

Funding Component:

041

Brain Cytochrome P450 1B1-Testosterone Generated Metabolite 6Beta-Hydroxytestosterone Contributes to Angiotensin II-Induced Increased Sympathetic Outflow and Blood Pressure

Authors: Purnima Singh, SaeRam Oh, Shubha Ranjan Dutta, Chi Young Song, Kafait U. Malik, Univ of Tennessee, Memphis, TN

Cytochrome P450 1B1 (CYP1B1)-generated testosterone (T) metabolite 6β-hydroxytestosterone (6β-OHT) contributes to angiotensin (Ang) II-induced hypertension in the male mice. However, the site of action of CYP1B1-testosterone metabolite 6β-OHT in Ang II-induced hypertension is unknown. The demonstration that Ang II increases blood pressure by its action in the subfornical organ and the presence of CYP1B1 in the brain led us to hypothesize that T contributes to Ang II-induced hypertension via its metabolism to 6β-OHT in the brain. To test this hypothesis, we examined the effect of intracerebroventricularly (ICV) administered T in castrated (Cas) wild-type (*Cyp1b1*$^{+/+}$) and Cas *Cyp1b1*$^{-/-}$ mice on the action of systemic Ang II (700 ng/kg/min) for 14 days. T (3 µg/2µL/injection/every 2nd day) or its vehicle (2-Hydroxypropyl-β-cyclodextrin dissolved in artificial CSF) was injected via a cannula implanted in the brain. Mean arterial blood pressure (MAP, mmHg) was measured by radiotelemetry (n=4-5). Ang II increased MAP in T but not its vehicle-injected Cas *Cyp1b1*$^{+/+}$ mice on Day 12 (159±2 vs. 98±2, P<0.05). In Cas *Cyp1b1*$^{-/-}$ mice Ang II increased MAP in ICV injected 6β-OHT (1.5 µg/2µL/injection every 2nd day) but not T (151±3 vs. 123±5, P<0.05). Power spectral analysis of the data on day 12 showed that Ang II increased the low to high-frequency ratio of heart rate variability, index of sympathetic outflow modulation in ICV T injected Cas *Cyp1b1*$^{+/+}$ but not Cas *Cyp1b1*$^{-/-}$ mice (3.8±0.0.4 vs. 1.9±0.0); whereas ICV 6β-OHT in the later mice increased this ratio (4.7±0.6). Ganglionic blocker hexamethonium (30 mg/Kg, ip) on day 14 of Ang II infusion resulted in greater reduction in MAP (P<0.05) in centrally 6β-OHT injected Cas *Cyp1b1*$^{-/-}$, and T-injected Cas *Cyp1b1*$^{+/+}$ (Δ81±11 and Δ87±10) than in T injected Cas *Cyp1b1*$^{-/-}$ mice (Δ50±8). Furthermore, in intact *Cyp1b1*$^{-/-}$, but not Cas *Cyp1b1*$^{-/-}$ mice transduction with adenovirus (Ad) of CYP1B1-DNA (ICV 2µL of 1.0 X 10^{12} particles/mL) increased Ang II-induced systolic blood pressure (P<0.05, n=5/group) measured by tail-cuff on Day12 (176±11 vs. 120±2, mm Hg). These data suggest that T contributes to Ang II-induced hypertension most likely via generation by the brain CYP1B1 to 6β-OHT, which increases sympathetic outflow in male mice.

Disclosures: P. Singh: None. **S. Oh:** None. **S. Dutta:** None. **C. Song:** None. **K.U. Malik:** None.

Funding: No

Funding Component:

042

Gender Differences in Response to Chronic Angiotensin-(1-7) Treatment in Obesity

Authors: Melissa White, Amanda J Miller, Justin Loloi, Sarah Bingaman, Amy Christine Arnold, Penn State Coll of Med, Hershey, PA

Angiotensin (Ang)-(1-7) is a vasodilatory hormone of the renin-angiotensin system (RAS) that has recently been shown to play a role in glucose homeostasis. Our previous study showed that obese male mice are deficient in circulating Ang-(1-7), and that chronic Ang-(1-7) restoration improves insulin sensitivity independent of blood pressure. Obese female mice also develop insulin resistance and glucose intolerance; however, the ability of Ang-(1-7) to improve metabolic function in females has not been explored. We hypothesized that Ang-(1-7) would equally improve glucose homeostasis in obese female and male mice. Adult male and female C57Bl/6J mice were placed on standard chow or 60% high fat diet (HFD) for 11 weeks, with Ang-(1-7) [400ng/kg/min] or saline given subcutaneously during the last 3 weeks of diet (n=8-12/group). During the last week of treatment, we assessed body composition and performed intraperitoneal insulin tolerance (ITT) and glucose tolerance (GTT) tests. While females had lower body mass throughout the study, both genders similarly increased adiposity in response to HFD (females: 7±1 chow vs 22±1% of body mass HFD; p=0.001; males: 6±1 vs 21±2% of body mass HFD, p=0.001). Ang-(1-7) had no effect on body composition in obese males, but reduced body mass (35±1 vs 31±2 g; p=0.028) and adiposity (22±1 vs 17±2% of body mass; p=0.024) and improved lean mass (p=0.019) in obese females. Ang-(1-7) improved insulin sensitivity in both obese male and female mice (p<0.001), with no gender differences (p=0.429). Ang-(1-7) improved glucose tolerance in obese female mice [area under the curve (AUC) for change in blood glucose levels during GTT: 20748±3156 chow vs 37582±2219 HFD vs 22884±5610 HFD+Ang-(1-7); p=0.044], with no effect in obese male mice (p=0.894). There was no effect of Ang-(1-7) on any outcome in chow fed male or female mice. Our data suggests that obese female mice are more responsive to chronic Ang-(1-7) therapy in terms of metabolic outcomes compared with obese male mice. These findings may have implications for understanding gender differences in RAS mechanisms involved in obesity and related metabolic complications.

Disclosures: M. White: None. **A.J. Miller:** None. **J. Loloi:** None. **S. Bingaman:** None. **A.C. Arnold:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; HL122507.

Funding: No

Funding Component:

043

Targeting the Angiotensin Type 2 Receptor With Relaxin Confers Protection Against Renal Fibrosis and Vascular Dysfunction in Female Stroke Prone-Spontaneously Hypertensive Rats

Authors: Giannie Barsha, Edmund Kwok, Katrina M Mirabito Colafella, Lucinda M Hilliard, Tracey Gaspari, Robert E Widdop, Chrishan S Samuel, Katherine M Denton, Monash Univ, Melbourne, Australia

The angiotensin type 2 receptor (AT_2R) mediates cardiovascular and renal protection in females of reproductive age, a mechanism that is lost with advancing age. Recently, we demonstrated that the nitric oxide-promoting and transforming growth factor-β1 inhibitory actions of relaxin (RLX) involve an interaction between the relaxin-insulin like family peptide receptor 1 (RXFP1) and the AT_2R. In the present study, we aimed to determine whether RLX induces cardio-renal protection and slows the progression of end-organ damage, via an AT_2R-dependent mechanism. In female stroke prone-spontaneously hypertensive rats (SP-SHR) at 6 (young) and 14-15 (aged reproductively senescent) months of age arterial pressure, cardiac function, glomerular filtration rate (GFR) and proteinuria were measured before and after 4 weeks of treatment with vehicle (sodium acetate s.c.), RLX (serelaxin; 0.5mg/kg/day s.c.) or RLX+PD123319 (AT_2R antagonist;

3mg/kg/day s.c). In addition, aortic endothelium-dependent relaxation in response to acetylcholine was assessed and cardiac and renal tissues were histologically analyzed for fibrosis. An age-related decline in GFR and increases in arterial pressure, proteinuria and cardiac and renal fibrosis were observed (all $P<0.05$; n=4-8). RLX treatment had no significant effect on these variables in the aged group. However, in aged animals, RLX markedly improved endothelial function (~30% increase in maximal vasodilation as compared to vehicle counterparts, $P=0.0002$; n=4-6). This effect was partially reversed by co-infusion with PD123319, relative to RLX alone (~14% reduced maximal vasodilation, $P=0.03$; n=6). In young females, RLX+PD123319 as compared to RLX treatment alone, exacerbated glomerular (11.6±1.2% vs 8.5±0.7%, respectively, $P=0.04$) and tubulointerstitial (3.3±0.2% vs 1.6±0.2%, respectively, $P=0.0002$) fibrosis. In conclusion, the cardio-renal protective actions of relaxin are dependent in part, upon an interaction with the AT_2R. Thus, targeting the RXFP1-AT_2R axis may be a therapeutic option for the treatment of cardiovascular disease and associated end-organ damage in hypertensive women.

Disclosures: G. Barsha: None. **E. Kwok:** None. **K.M. Mirabito Colafella:** None. **L.M. Hilliard:** None. **T. Gaspari:** None. **R.E. Widdop:** None. **C.S. Samuel:** C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; Relaxin supplied by Novartis. **K.M. Denton:** C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; Relaxin supplied by Novartis.

Funding: No

Funding Component:

044

Purinoceptor-Dependent Regulation of Sodium Excretion is Sexually Dimorphic

Authors: Eman Y Gohar, Malgorzata Kasztan, Shali Zhang, Edward W. Inscho, David M. Pollock, Univ of Alabama at Birmingham, Birmingham, AL

Premenopausal women have a lower risk of hypertension compared to age-matched men. Recently, prominent roles have been assigned to $P2Y_2$ and $P2Y_4$ purinoceptor subtypes in promoting Na^+ excretion, implicating dysfunction of these receptors as potential contributors to hypertension. We recently reported that activation of $P2Y_2$ and $P2Y_4$ receptors in the renal medulla by UTP promotes Na^+ excretion in male rats. In intact females, UTP did not stimulate Na^+ excretion while ovariectomy unmasked UTP-induced natriuresis. These observations led us to hypothesize that intact females have higher basal renal medullary activity of $P2Y_2$ and $P2Y_4$ receptors in regulating Na^+ excretion compared to male and ovariectomized (OVX) rats. To test that, we determined (i) $P2Y_2$ and $P2Y_4$ mRNA and protein expression in the inner medulla from male, intact female and OVX Sprague Dawley rats and (ii) the effect of inhibiting medullary purinoceptors (P2 receptors) on Na^+ excretion in those rats. We found that $P2Y_2$ and $P2Y_4$ mRNA expression was higher in the inner medulla from females compared to males (1.00±0.09 vs. 0.70±0.05 and 1.00±0.22 vs. 0.29±0.05, respectively, $P<0.5$, n=5-10). These sex differences in $P2Y_2$ and $P2Y_4$ mRNA expression were eliminated by ovariectomy (0.60±0.06 and 0.29±0.04, respectively, $p<0.5$,n=5,8). Consistently, Western blots on inner medullary lysates showed that intact females have higher expression of $P2Y_2$ receptor, compared to males. In anesthetized rats, medullary P2 receptor inhibition by suramin (P2 receptor antagonist, 750 µg/kg/min) significantly attenuated Na^+ excretion in intact females (0.4±0.1 vs. 0.9±0.2 µmol/min, $P<0.5$, n=7), but not in male or OVX rats.To test whether estradiol (E_2) increases the expression of $P2Y_2$ and $P2Y_4$ receptors, we subjected cultured mouse inner medullary collecting duct cells (mIMCD3) to different concentrations of E_2 (0, 10, 100 and 1000 nM). We found that E_2 dose-dependently increased the expression of $P2Y_2$ and $P2Y_4$ mRNA in mIMCD3. These data suggest that females have enhanced $P2Y_2$ and $P2Y_4$-dependent regulation of Na^+ excretion in the renal medulla, compared to male and OVX rats, at least partially via an E_2-dependent mechanism. This pathway may contribute to facilitated renal Na^+ handling in premenopausal females.

Disclosures: E.Y. Gohar: None. **M. Kasztan:** None. **S. Zhang:** None. **E.W. Inscho:** None. **D.M. Pollock:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

045

Sox6 Has Protective Role During Renal Artery StenosisSInduced Hypertension

Authors: Mohammad Saleem, Liang Xiao, Kandi Horton, Vanderbilt university medical cente, Nashville, TN; Conrad P Hodgkinson, Dept of Med. Duke Univ Medical Ctr, Durham, NC; Jose A Gomez, Vanderbilt university medical cente, Nashville, TN

Introduction: Renal artery stenosis is a common condition in patients with atherosclerosis. Narrowing of the renal arteries stimulate increases in renin production and release resulting in hypertension. Hypertension causes kidney injury and end organ damage. As such, there is much interest in developing novel treatments for the hypertension induced by renal artery stenosis. In renal artery stenosis, renin is a key disease driver. The objective of our study is to determine if the transcription factor Sox6 plays a role in renin induction of hypertension during renal artery stenosis.
Methods: A novel transgenic mouse model ($Ren1d^{Cre}/Sox6^{fl/fl}$-Sox6KO) was used to determine the impact of specific Sox6 ablation in renin expression after different stimuli. Ten days of low sodium diet (0.01% Na) and furosemide (0.1 mg/g body weight) was used to induce JG cell expansion. 2 Kidney 1 Clip (2K1C) Goldblatt model was used as renal artery stenosis model. Blood pressure was measured by tail-cuff method.
Results: In wild-type mice renin and Sox6 expression increased during JG cell expansion. However, specific Sox6 ablation in renin expressing cells halted the increase in the number of JG cells (8.75 fold decrease, n= 6 to 9, P= 0.001). Furthermore, Sox6 KO mice were protected from developing hypertension and kidney damage after renal artery stenosis. Systolic blood pressure (116±2.13 and 132.9±3.3, n=9-10) and mean arterial pressure (103.3±1.9 and 121.5±1.01, n=9-10) were significantly lower in Sox6 KO compared to wild-type mice. When stenosed kidneys were compared, renin expression was higher in wild-type compared to Sox6 KO group as measured by immunoblotting (0.49 ± 0.05898, and 0.2266±0.02716; *P*=0.0358, n=2-3). Creatinine levels in urine were higher in wild-type group compared to Sox6 KO group (2382 ± 399 and 1804 ± 177.8, *P*=0.0358, n=9).
Conclusion: Our data indicates that Sox6 plays a novel role in modulating renal renin expression during pathological conditions. These results suggest that Sox6 is a potential drug target for therapies which seek to control blood pressure in hypertensive patients.

Disclosures: M. Saleem: None. **L. Xiao:** None. **K. Horton:** None. **C.P. Hodgkinson:** None. **J.A. Gomez:** None.

Funding: No

Funding Component:

046

Cardiac Mitochondria are Impaired After Transient Neonatal High Oxygen Exposure in a Rat Model of Prematurity-Related Condition

Authors: Daniela Ravizzoni Dartora, Ying He, Muhammad Oneeb Rehman Mian, Alyson Deprez, Camille Girard-Bock, Anik Cloutier, Dept of Pediatrics, Univ de Montréal and CHU Sainte-Justine Res Ctr, Montréal, QC, Canada; Gael Cagnone, Perrine Gaub, Jean-Sebastien Joyal, Dept of Pharmacology and Physiology, Univ de Montréal and CHU Sainte-Justine Res Ctr, Montréal, QC, Canada; Thuy Mai Luu, Dept of Pediatrics, Univ de Montréal and CHU Sainte-Justine Res Ctr, Montréal, QC, Canada; Anne Monique Nuyt, Dept of Pediatrics, Dept of Pharmacology and Physiology, Univ de Montréal and CHU Sainte-Justine Res Ctr, Montréal, QC, Canada

The heart relies on adequate mitochondrial (mito) ATP production to match myocardial demand, mostly through oxidative phosphorylation. Preterm birth (PT) results in ex utero development of an immature myocardium and PT-born individuals represent a newly recognized group at high risk of cardiovascular diseases. Our group has shown that newborn rats exposed to high oxygen (O_2), mimicking PT-related conditions, develop O_2-induced cardiomyopathy (OIC) and dysfunction later in life. However, whether mito impairments prevail in the programmed left ventricle (LV) changes associated with PT is unknown. We ***aimed*** to determine whether OIC is associated with altered LV mito characteristics in 4 wks-old rats. Male rat pups were kept with their mother in 80% O_2 (OIC) or room air (Ctrl) from days 3 to 10 of life. Results are mean±SEM; OIC vs. Ctrl are compared using t-test (n=4/group.P<0.05). At 4 wks, extracellular flux analysis of isolated LV cardiomyocytes revealed significantly decreased O_2 consumption rate in OIC (12.14±5.74 vs. 67.39±13.61 pmoles/min/ug). OIC rats show reduced LV mito copy number determined by the ratio between mito and genomic DNA (26.7±6.30 vs. 52.7±7.32), biogenesis markers Pgc1α (0.59±0.14 vs. 1.18±0.25), and citrate synthase (0.20±0.16 vs. 1.60±0.56) mRNA, and reduced Complex IV (0.66±0.15 vs. 1.26±0.09) protein expression. Gene expression (RT-PCR) of key glycolytic enzymes, hexokinase (3.34±0.59 vs. 0.88±0.39) and glucose-6-phosphate dehydrogenase (2.85±0.48 vs. 0.58±0.08) is significantly increased, indicating a shift toward glycolysis. OIC also were found to have higher LV mtROS production (7581±51 vs. 5191±99, fluorescence arbitrary units) and decreased antioxidant SOD2 protein expression (0.84±0.09 vs. 1.04±0.05). Taken together our results indicate that neonatal hyperoxia exposure leads to decreased LV mito biogenesis and function with impaired oxidative phosphorylation capacity in juvenile animals. Further studies are needed to determine the role of long-term mito dysfunction in the increased susceptibility to heart failure observed after deleterious neonatal conditions.

Disclosures: D. Ravizzoni Dartora: None. **Y. He:** None. **M. Mian:** None. **A. Deprez:** None. **C. Girard-Bock:** None. **A. Cloutier:** None. **G. Cagnone:** None. **P. Gaub:** None. **J. Joyal:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; CIHR grant. **T. Luu:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; CIHR and Heart and Stroke Foundation of Canada grants. **A. Nuyt:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; CIHR and Heart and Stroke Foundation of Canada grants.

Funding: No

Funding Component:

047

Sorting Nexin 19: A Novel & Key Player in Renal Dopamine D_1R Regulation

Authors: Andrew C Tiu, Albert Einstein Medical Ctr, Philadelphia, PA; Jian Yang, Laureano D Asico, Prasad Konkalmatt, Xiaoyan Wang, Pedro A Jose, Van Anthony M Villar, George Washington Univ, Washington, DC

The cellular localization and signal transduction of a ligand-occupied G protein-coupled receptor (GPCR) are tightly regulated to generate an appropriate response in terms of specificity, magnitude, and duration through the interplay of key proteins. We recently identified sorting nexin 19 (SNX19) as a binding partner for the renal dopamine D_1 receptor (D_1R) and demonstrated its role in receptor lipid raft distribution and activity. We have shown that SNX19 and D_1R co-immunoprecipitated (co-IP'd) and colocalized at the plasma membrane of human renal proximal tubule cells (hRPTCs) and at the brush border of RPTs of C57Bl/6 mice. Treatment with the D_1R/D_5R agonist fenoldopam promoted the colocalization to the cytoplasm of RPTCs. SNX19 silencing in hRPTCs decreased the D_1R expression (-60±4% of basal, P<0.05, n=4) and impaired D_1R recruitment and endocytosis following agonist stimulation, as observed via live cell imaging. SNX19 silencing markedly decreased the fenoldopam-mediated increase in cAMP production in hRPTCs (+115±30% *vs*. +45±12% in mock siRNA-treated control cells, P<0.05, n=4), and abrogated the fenoldopam-mediated inhibition of Na^+,K^+-ATPase activity in polarized hRPTCs (+21±1.2% intracellular Na^+ *vs*. +2±2% in control cells, P<0.05, n=4). Kidney-restricted *Snx19* silencing in C57Bl/6 mice increased the systolic blood pressure (124±3 mm Hg *vs*. 101±2

mm Hg in control mice, P>0.05, n=5), thus demonstrating the importance of SNX19 in renal D_1R activity and blood pressure control. SNX19 also co-IP'd with GRK4 and GODZ finger protein, an enzyme involved in GPCR palmitoylation, which is a process that promotes lipid raft partitioning. Mutation of the canonical PX domain of SNX19, which prevented its targeting to the plasma membrane, and the heterologous expression of these mutants in hRPTCs decreased the abundance of D_1R, which became exclusively distributed in non-lipid rafts. The disruption of lipid rafts in the kidneys of mice resulted in hypertension (120±3 mm Hg *vs*. 101±2 mm Hg in control mice, n=4). Our results highlight the crucial role of SNX19 in the proper trafficking and functioning of renal D_1R through its ability to promote the localization of D_1R in lipid rafts for an effective signal transduction and appropriate cellular response.

Disclosures: A.C. Tiu: None. **J. Yang:** None. **L.D. Asico:** None. **P. Konkalmatt:** None. **X. Wang:** None. **P.A. Jose:** None. **V.M. Villar:** None.

Funding: No

Funding Component:

048

Impact of New Clinical Practice Guidelines on Cardiovascular Disease Risk Assessment in an Obese Pediatric Population

Authors: Edem Binka, Philip Spevak, Lauren Dibert, Tammy McLoughlin Brady, Johns Hopkins Hosp, Baltimore, MD

Objectives: Cardiovascular disease (CVD) risk factor prevalence has increased in children. The new clinical practice guidelines (CPG) revised hypertension (HTN) and left ventricular hypertrophy (LVH) definitions. We investigated the impact of the CPG on CVD risk assessment in obese youth. **Methods:** Cross-sectional study of youth evaluated for high blood pressure at an obesity HTN clinic. Characteristics were compared across historic and current LVH definitions (table 1) using ANOVA. Logistic regression evaluated odds of LVH by HTN status and BMI. **Results:** Of 54 youth, aged 6-21 yrs, 78% (42 of 54) had class III obesity, 57% (31 of 54) had HTN and 17-74% (9-40 of 54) had LVH. Youth with LVH using CPG definitions were older and more obese (table 1). BMI, not HTN, was predictive of LVH using LVMI > 51g/$m^{2.7}$ (table 2). **Conclusions:** In this at-risk population, LVH was highly prevalent. Adiposity, not HTN, was predictive of LVH when using the adult definition recommended in the CPG. This may have future implications for recommended CVD risk assessment of youth with severe obesity.

Table 1: Comparison of patient characteristics based on definition of LVH

	Older Definitions		Current CPG Definitions		
Characteristic mean (SD) or % (n)	LVMI>38.6 g/m^2.7 74% (n=40)	LVMI>95th %ile 69% (n=37)	LVMI>51 g/m^2.7 30% (n=16)	LV mass/BSA >115 g/BSA for boys >95 g/BSA for girls 17% (n=9)	p-value
Age (years)	13.7 (4.0)	13.9 (4.0)	16.3 (2.8)	16.9 (2.3)	0.009
Male Sex	55 (22)	54 (20)	63 (10)	78 (7)	0.6
African American Race	90 (36)	89 (33)	94 (15)	89 (8)	0.08
BMI (kg/m^2)	38.7 (9.9)	39.3 (10.0)	45.7 (9.2)	44.5 (8.3)	0.0005
BMI z-score	2.6 (0.4)	2.6 (0.4)	2.8 (0.3)	2.8 (0.3)	0.04
Waist z-score	2.6 (0.6)	2.6 (0.6)	2.9 (0.5)	2.8 (0.4)	0.2
Waist-hip z-score	1.8 (1.3)	1.7 (1.3)	2.3 (1.2)	2.4 (1.2)	0.2
SBP z-score	1.2 (1.2)	1.2 (1.2)	1.4 (1.2)	1.8 (1.4)	0.5
DBP z-score	-0.1 (1.0)	-0.1 (1.0)	0.1 (1.1)	0.4 (1.3)	0.4
HTN	60 (24)	62 (23)	69 (11)	67 (6)	0.8
Ejection Fraction	64.0 (6.2)	64.5 (5.9)	63.1 (5.8)	64.3 (3.0)	0.5

Table 2: Adjusted and unadjusted odds of LVH based on HTN diagnosis and BMI

	LVMI>38.6 g/m^2.7 OR (95% CI)	LVMI>95th %ile OR (95% CI)	LVMI>51 g/m^2.7 OR (95% CI)	LVM/BSA >115 g/BSA for boys >95 g/BSA for girls OR (95% CI)
HTN				
– unadjusted	1.50 (0.44, 5.10)	1.85 (0.58, 5.90)	1.98 (0.58, 6.80)	1.60 (0.36, 7.21)
– adjusted for age/sex/race	1.24 (0.29, 5.30)	1.68 (0.45, 6.35)	0.80 (0.17, 3.72)	0.46 (0.07, 3.08)
– adjusted for age/sex/race AND BMI	1.26 (0.29, 5.47)	1.75 (0.45, 6.81)	0.80 (0.13, 4.91)	0.40 (0.05, 2.99)
BMI				
– unadjusted	1.03 (0.96, 1.11)	1.05 (0.98, 1.14)	1.19 (1.08, 1.33)	1.09 (1.01, 1.18)
– adjusted for age/sex/race	1.05 (0.94, 1.17)	1.07 (0.96, 1.19)	1.21 (1.04, 1.41)	1.08 (0.98, 1.20)
– adjusted for age/sex/race AND HTN	1.05 (0.94, 1.17)	1.07 (0.96, 1.19)	1.21 (1.04, 1.40)	1.09 (0.98, 1.21)

Disclosures: **E. Binka:** None. **P. Spevak:** None. **L. Dibert:** None. **T. McLoughlin Brady:** None.

Funding: No

Funding Component:

049

Endothelial-Derived Microparticles as Biomarkers and Mediators of Endothelial Cell Injury in VEGF Inhibitor-Treated Cancer Patients: Implications in Hypertension

Authors: Karla B Neves, Francisco J Rios, Univ of Glasgow, Glasgow, United Kingdom; Martin McLeod, Experimental Cancer Med Ctr, Glasgow, United Kingdom; Judith Dixon Hughes, Cancer Res UK Glasgow Clinical Trials Unit, Glasgow, United Kingdom; Robert Jones, Jeff Evans, Augusto Montezano, Rhian M Touyz, Univ of Glasgow, Glasgow, United Kingdom

Vascular endothelial growth factor receptor inhibitors (VEGFRi), used as anti-angiogenic drugs to treat cancer, induce severe hypertension although the underlying molecular mechanisms are still unclear. Endothelial microparticles (MPs) are biomarkers of endothelial injury and are also functionally active since they influence downstream target cell signalling and function. We questioned whether MP status is altered in cancer patients treated with VEGFRi and whether they influence endothelial cell function associated with vascular dysfunction in hypertension. Plasma MPs were isolated from cancer patients before and after treatment with VEGFRi. Human aortic endothelial cells (HAEC) were stimulated with human plasma-isolated MPs (10^6 MPs/mL). MP characterization was assessed by flow cytometry; protein and gene expression by immunoblotting and qPCR; ROS and NO production by lucigenin and immunofluorescence. Patients treated with VEGFRi had significantly increased in endothelial cell-derived MPs (EMP) (0.19±0.02 Pre vs. 0.31±0.04 Post-treatment). HAEC exposed to post-treatment MPs increased pre-pro-ET-1 mRNA (4.10±0.97 vs. 10.57±3.54), corroborating the raise in ET-1 levels (µg/mL: 631.9±46.1 vs. 780.2±37.2) observed in HAEC stimulated with vatalanib (VEGFRi). Post-treatment MPs increased ROS generation in HAEC (100.0 vs. 5 min- 123.4±7.7, 30 min- 162.4±22.5, 60 min- 183.9±44.7), effects that were attenuated by ET_A and ET_B receptor blockers. VEGFRi post-treatment MPs increased phosphorylation of the inhibitory site of eNOS (Thr^{495}) (100.0 vs. 181.3±18.7) and decreased NO levels in HAEC (100.0 vs. 72.7±9.9) which was inhibited by ET_B receptor blockade (eNOS: 109.8±14.9; NO: 142.1±25.9). Gene expression of proinflammatory mediators was increased in HAEC exposed to post-treatment MPs (1.0 vs. TNF-α: 5.4±1.8, MCP-1: 2.1±0.3, iNOS: 3.1±0.8, COX2: 2.4±0.4, ICAM1: 2.7±0.6), effects inhibited by BQ123 and BQ788. In conclusion, our data identify EMPs as biomarkers of VEGFi-induced endothelial injury and important mediators of ET-1-sensitive redox-regulated endothelial cell signalling. These molecular processes may play a role in vascular dysfunction associated with hypertension in VEGFRi-treated cancer patients.

Disclosures: **K.B. Neves:** None. **F.J. Rios:** None. **M. McLeod:** None. **J.D. Hughes:** None. **R. Jones:** None. **J. Evans:** None. **A. Montezano:** None. **R.M. Touyz:** None.

Funding: No

Funding Component:

050

Hypertensive Urgency: An Emergency Department Pipeline to Primary Care Pilot Study

Authors: Antonio Giaimo, Stephen Huot, Angela Kang, Yale Univ, New Haven, CT

Introduction: The incidence of HTN related ED visits is high and increasing. The optimal management of hypertensive urgency in the ED is not well established but the 2017 ACC/AHA HTN guidelines recommend treatment initiation and outpatient follow up within 1 week. There is a low incidence of poor short-term outcomes associated with hypertensive urgency and high cost but low yield for admitting such patients to the hospital. Our health system lacked an effective referral process for these patients, resulting in poor follow up, unnecessary hospital admission, and recurrent ED utilization. The objectives of our pilot study were to 1) create a process for referring and connecting these patients with outpatient HTN management in a primary care setting and 2) measure the impact of this pipeline referral process on BP control and ED utilization.
Methods: Patients presenting to the Yale New Haven Hospital ED with hypertensive urgency (SBP ≥180 mm Hg and/or DBP ≥110 mm Hg without end organ damage) who were current patients of the resident physician primary care center or who do not have a PCP were included. Patients meeting inclusion criteria were contacted by a referral coordinator who scheduled their initial appointment to occur within 10 days. Patients were seen by a resident physician supervised by an attending with expertise in HTN. The resident physician leading the visit became their PCP.
Results: Data are reported for the first 24 referred patients seen in the clinic. Average time to first clinic visit was 7.5 days. Mean age was 54 years (range 32-71), 85% (20/24) were African-American and mean pooled 10 year ASCVD risk was 19%. Mean BP was reduced from 193/111 mmHg at time of ED visit to 131/77 by six weeks after first visit and was sustained at 90 days. Total ED visits by these 24 patients decreased from 30 in the 90 days prior to referral, to 11 in the 90 days after the first clinic visit.
Discussion: Our results show that ED patients with hypertensive urgency are a high ASCVD risk population. A coordinated referral collaboration between the ED and primary care can provide safe, timely care for this at-risk population that leads to significant reductions in BP and ED utilization. Reduced ED utilization is most likely due to connecting patients with comprehensive primary care.

Disclosures: A. Giaimo: None. **S. Huot:** None. **A. Kang:** None.

Funding: No

Funding Component:

051

High Salt Enhances ROS and Ang II Contractions of Glomerular Afferent Arterioles From Mice With Reduced Renal Mass

Authors: Lingli Li, EnYin Lai, Zaiming Luo, Glenn Solis, Margarida Mendonca, Georgetown Univ Div of Nephrology, Washington, DC; Kathy Griendling, Emory Univ, Atlanta, GA; Anton Wellstein, William Welch, Christopher S WILCOX, Georgetown Univ Div of Nephrology, Washington, DC

High salt intake, angiotensin II (Ang II), and reactive oxygen species (ROS) enhance progression of chronic kidney disease (CKD). We reported that myogenic contractions of renal afferent arterioles (RAAs) were enhanced by superoxide ($O_2^{\cdot-}$) generated from $p47^{phox}$/NOX2 but inhibited by H_2O_2 generated from POLDIP2/NOX4. We tested the hypothesis that feeding a high salt diet to mice with the reduced renal mass (RRM) model of CKD generates specific ROS in their RAAs that enhances Ang II contractions. *Methods and results:* C57BL/6 mice received surgical RRM or sham operations and 6% or 0.4% NaCl salt for 3 months. Ang II contractions were measured in RAAs perfused at 45 mmHg and superoxide ($O_2^{\cdot-}$) and H_2O_2 by fluorescence microscopy. RRM enhanced the gene expression in RAAs for $p47^{phox}$ and NOX2 and high salt intake in mice with RRM enhanced the gene expression for AT1Rs, POLDIP2 and NOX4 and reduced the gene expression for catalase. Mice with RRM fed a normal salt diet had contractions to 10^{-6} mol·l^{-1} Ang II similar to sham (-56 ± 5 vs -52 ± 5 %; NS). However, RRM mice fed a high salt diet had an enhanced $O_2^{\cdot-}$ and H_2O_2 generation ($P<0.005$) with Ang II in RAAs and enhanced Ang II maximal contractions (-72 ± 2 vs -45 ± 2%; $P<0.005$) that were dependent on $O_2^{\cdot-}$ from NOX2 since they were prevented in $p47^{phox}$ -/- mice and on H_2O_2 from NOX4 since they were prevented in mice with transgenic

smooth muscle cell expression of catalase ($tg^{CAT\text{-}SMC}$), and in POLDIP2 +/- mice. However, RAA contractions to lower concentrations of Ang II (10^{-8} to 10^{-11} mol·l^{-1}) were paradoxically enhanced in tg $^{CAT\text{-}SMC}$ vs Wt mice (-17 ± 2 vs -1 ± 1%; P<0.01) and in POLDIP2 +/- vs +/+ mice (-22 ± 3 vs -5 ± 3; P<0.01). Tempol normalized the ROS and Ang II contractions in RAAs from mice with RRM. In conclusion, both $O_2^{\cdot-}$ from $p47^{phox}$/NOX2 and H_2O_2 from NOX4/POLDIP2 enhance maximal Ang II contractions of RAAs from mice with RRM fed a high salt diet but H_2O_2 from NOX4/POLDIP2 reduces the sensitivity to lower concentrations of Ang II by >100-fold and tempol prevents all of these changes Thus, although a high salt intake reduces circulating Ang II, blockade of angiotensin receptors or ROS may prove beneficial for patients with CKD unable to restrict salt.

Disclosures: L. Li: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Co Investigator. **E. Lai:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Co Investigator. **Z. Luo:** None. **G. Solis:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Investigator. **M. Mendonca:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Investigator. **K. Griendling:** None. **A. Wellstein:** None. **W. Welch:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Co Investigator. **C.S. Wilcox:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PI.

Funding: No

Funding Component:

052

Role of Dopamine D2 Receptors in The Regulation of Cell Proliferation in Renal Injury

Authors: Megha Kumar, George Washington Univerisity, Washington, DC; Laureano Asico, Prasad Konkalmatt, George Washington Univ, Washington, DC; Zach Freyberg, Univ of Pittsburgh, Washington, PA; Pedro A Jose, Ines Armando, George Washington Univ, Washington, DC

The dopamine D2 receptor (D2R) in the kidney has a direct and significant role in blood pressure (BP) control and in negatively regulating the mechanisms involved in the development of inflammation and injury. Impaired D2R function results in renal inflammation and end organ damage. Injury to the kidney triggers a cell proliferation response that can be adaptive, leading to repair of the tubular epithelium, or maladaptive, leading to fibrosis and chronic kidney disease. We investigated the role of renal D2Rs in the cell proliferation response in models of renal injury in mice and human renal proximal tubule cells (hRPTCs). Renal selective silencing of the D2R in mice resulted in an increase in the mRNA expression of the proliferation marker Ki-67 (2.8±0.8 vs 1.0±0.1 fold; qRT-PCR; P<0.05; n=4-5) along with an increase in the number of proliferating cells in renal cortical slices determined by nuclear Ki-67 staining (36±3 vs 6±1; positive cells/ field; P<0.05) in comparison with mice with no silencing. Rescue of D2R function in these mice by the ureteral infusion of AAV carrying a D2R vector reduced the expression of cortical Ki-67 mRNA (42±3 vs 100±5 %; P<0.05; n=4) and the number of positive cells/field (16±2 vs 32±5; P<0.05) in comparison with mice treated with a control AAV. In mice subjected to renal ischemia reperfusion KI-67 mRNA expression was higher (1.5±0.03 vs 1.0±0.05 fold; P<0.05; n=3-4) than in mice with sham operation. Treatment with AAV carrying D2R after ischemic injury reduced Ki-67 expression (0.65±0.05 vs 1±0.1; P<0.05) and the number of Ki-67 positive cells in kidney sections. Rescue of function or D2R overexpression reduced tissue damage, fibrosis and the increase in BP in these models. Treatment of hRPTCs with the nephrotoxic, aristolochic acid (5 µg/ml, 24h), increased mRNA Ki-67 expression (2.8±0.2 fold; P<0.05) in comparison with cells treated with vehicle while the presence in the medium of a D2R agonist, quinpirole (1µM, 24h), reduced the increase by 42%. The D2R agonist also reduced the increase in fibronectin 1 and collagen 1a1 elicited by aristolochic

acid. Our data suggest that regulation of cell proliferation is one of the mechanisms involved in the protective effect of D2R function on renal inflammation and injury.

Disclosures: M. Kumar: None. **L. Asico:** None. **P. Konkalmatt:** None. **Z. Freyberg:** None. **P.A.A. Jose:** None. **I. Armando:** None.

Funding: No

Funding Component:

053

Dietary Sodium Restriction versus Diuretics for Salt-Sensitive Hypertension in Chronic Kidney Disease

Authors: Dominique M Bovée, Alexander H Danser, Robert Zietse, Ewout J Hoorn, Erasmus Medical Ctr, Rotterdam, Netherlands

Fluid overload and salt-sensitive hypertension are hallmarks of advanced chronic kidney disease (CKD) and associated with worse outcomes. Dietary sodium (Na^+) restriction is an accepted intervention, but long-term adherence remains a challenge. Distal diuretics could provide an alternative approach, but they are considered less effective in advanced CKD. Here, we compared both approaches head-to-head. **Methods:** Twenty-six patients with CKD stage 3 or 4 and hypertension were included in this single-center, open-label, randomized cross-over trial (baseline eGFR 39 ± 13 ml/min/1.73 m^2). Renin-angiotensin inhibitors and diuretics were discontinued 2 weeks prior to interventions and during study period. Subsequently, we compared dietary Na^+ restriction (60 mmol/day) versus amiloride/hydrochlorothiazide (5/50 mg once daily). Both interventions lasted for two weeks and were separated by a 2-week wash-out period. The primary endpoint was 24h systolic blood pressure (SBP). **Results:** Urinary Na^+ excretion was successfully lowered with dietary Na^+ restriction (160 ± 66 to 64 ± 37 mmol/day, $p < 0.01$), and remained similar with diuretics (154 ± 47 to 153 ± 63 mmol/day, $p = 0.95$). Dietary Na^+ restriction lowered 24-hour SBP (134 ± 12 to 129 ± 14 mmHg, $p < 0.05$), while diuretics had a greater effect (138 ± 12 to 124 ± 13 mmHg, $p < 0.01$ for within and between interventions). Both maneuvers significantly lowered indices of fluid overload, including body weight (-1.6 ± 1.1 kg with dietary Na^+ restriction and -1.9 ± 1.5 kg with diuretics), NT-pro-BNP (median -10 and -7 pmol/L), and overhydration as assessed by bioimpedance (-0.6 ± 0.6 and -1.3 ± 0.7 L). Both interventions also lowered eGFR (-2 ± 4 and -5 ± 5 ml/min/1.73 m^2, $p<0.05$ for both) and showed a trend towards albuminuria reduction (median -5 mg/day and -20 mg/day). The reduction in overhydration and eGFR was greater with diuretics than with dietary Na^+ restriction ($p < 0.05$). **Conclusions:** Distal diuretics and dietary Na^+ restriction effectively lower blood pressure in CKD 3 and 4 in the absence of renin-angiotensin inhibitors. Both interventions also lower indices of fluid overload. Diuretics produce greater effects than dietary Na^+ restriction. These beneficial effects may outweigh the (hemodynamic) reduction in eGFR.

Disclosures: D.M. Bovée: None. **A.H.J. Danser:** None. **R. Zietse:** None. **E.J. Hoorn:** None.

Funding: No

Funding Component:

054

Tissue-Specific Effects of Targeted Mutation of *Mir29b1* in Rats

Authors: Hong Xue, Fudan Univ, Shanghai, China; Guangyuan Zhang, Aron M Geurts, Kristie Usa, David M Jensen, Yong Liu, Mingyu Liang, Medical Coll of Wisconsin, Milwaukee, WI

miR-29 is a master regulator of extracellular matrix genes and is generally considered anti-fibrotic, and it improves nitric oxide (NO) production in human and rat arterioles by targeting Lypla1.Targeted mutation in the *Mir29b1* gene exacerbates hypertension in a rat model derived from the Dahl salt-sensitive rat. The kidney, particularly the renal outer medulla, plays a key role in the development of salt-sensitive hypertension. In the present study, we examined the effect of the targeted *Mir29b1* mutationon tissue fibrosis and NO levels with a focus on kidney regions. The abundance of miR-29b and the co-transcribed miR-29a was substantially lower in the renal cortex, renal outer medulla, heart and liver in mutant rats. In mutant rats on a 0.4% NaCl diet and prior to the development of overt hypertension, tissue fibrosis was significantly increased in the renal outer medulla, but not in the renal cortex, heart or liver, compared with wild-type littermates. Levels of NO metabolites were significantly lower, and Lypla1 protein abundance significantly higher, in the renal outer medulla, but not in the renal cortex. After 14 days of a 4% NaCl diet, tissue fibrosis remained significantly higher in the renal outer medulla and became higher in the heart of mutant rats compared with wild-type littermates, but not in the renal cortex. 24h urine volume was significantly lower in mutant rats on either the 0.4% or 4% NaCl diet. Microalbuminuria was exacerbated by the 4% NaCl diet, but was not significantly different between mutant rats and wild-type littermates. These findings indicate the effects of miR-29 are tissue-specific. The renal outer medulla might be particularly susceptible to the injurious effects of a miR-29 insufficiency, which might contribute to the development of hypertension in *Mir29b1* mutant rats.

Disclosures: H. Xue: None. **G. Zhang:** None. **A.M. Geurts:** None. **K. Usa:** None. **D.M. Jensen:** None. **Y. Liu:** None. **M. Liang:** None.

Funding: Yes

Funding Component: National Center

055

ND-13, a DJ-1-Derived Peptide, Protects Against the Renal Fibrosis and Inflammation Associated With Unilateral Ureter Obstruction

Authors: Carmen De Miguel, Univ of Alabama at Birmingham, Birmingham, AL; Mitchell Saludes, Laureano D Asico, Patricia S. Latham, The George Washington Univ Sch of Med and Health Sciences, Washington, DC; Daniel Offen, The Felsenstein Medical Res Ctr, Tel Aviv, Israel; Pedro A Jose, The George Washington Univ Sch of Med and Health Sciences, Washington, DC; Santiago Cuevas, Children's Natl Health System, Washington, DC

Oxidative stress and inflammation are important players in the pathogenesis of cardiovascular and renal diseases. DJ-1 is a redox-sensitive chaperone that regulates the expression of several antioxidant genes. Activation of the DJ-1/Nrf2 pathway in the kidney inhibits the development and progression of several renal diseases. The 20 aa peptide ND-13 consists of 13 highly conserved aa from the DJ-1 sequence and a TAT- derived 7 aa sequence to help in cell penetration. ND-13 prevents neuronal degeneration in mice; however, its effects on kidney damage remain unknown. We hypothesized that treatment with ND-13 would prevent the renal damage and inflammation associated with unilateral ureter obstruction (UUO). C57Bl/6 mice and *DJ1*$^{-/-}$ mice underwent UUO and were divided in 3 groups: control (no UUO), UUO+scrambled peptide (SP) or UUO+ND-13 (3 mg/kg, s.c. daily). After 14 days of treatment, urine and kidneys were collected for analysis of renal damage. ND-13 treatment prevented the development of fibrosis in C57Bl/6 mice (UUO+SP: 702±189% of control, UUO+ND-13: 264±8% of control, n=2-4/group, $p<0.05$), suggesting that ND-13 is protective against connective tissue deposition in the kidney. Treatment with ND-13 decreased renal mRNA expression of *TNF-* α *(*fold change from control: 101±46 in UUO+SP; 18±7 in UUO+ND-13, n=4-5/group, $p<0.05$), *IL-6* (6.7±2 in UUO+SP; 1.54±0.3 in UUO+ND-13, $p<0.05$), *TGF-*β *(*3.3±1.12 UUO+SP; 1.4±0.03 UUO+ND-13, $p<0.05$) and *Colagen1*α*1* (79±16 UUO+SP; 33±3.8 in UUO +ND-13, $p<0.05$) in C57Bl/6 mice. In DJ1$^{-/-}$ mice, treatment with ND-13 similarly decreased expression of TNF-α, IL-6 and TGF-β, but, in contrast, failed to prevent renal fibrosis or kidney expression of *col1*α*1*. UUO also led to elevated urinary NGAL, marker of proximal tubular injury, in *DJ-1*$^{-/-}$ mice and ND-13 treatment

prevented that increase (71±17% UUO vs control: -18±21% UUO+ND-13 vs control, n=5/group). Our results suggest that ND-13 has protective effects on renal injury, fibrosis and inflammation, crucial mechanisms in the pathogenesis of renal disease. Thus, ND-13 treatment may be a new therapeutic approach for the prevention of renal injury, fibrosis and inflammation in renal diseases. Funded by T32DK007545 to CDM and 5P01 HL074940-10, 7R01 DK039308-31 to PAJ.

Disclosures: C. De Miguel: None. **M. Saludes:** None. **L.D. Asico:** None. **P.S. Latham:** None. **D. Offen:** None. **P.A. Jose:** None. **S. Cuevas:** None.

Funding: No

Funding Component:

056

Knockout of Dual-Specificity Protein Phosphatase 5 Protects Against Hypertension Induced Chronic Kidney Disease

Authors: Chao Zhang, Xiaochen He, Shaoxun Wang, Richard J Roman, George Booz, Fan Fan, Univ of MS Medical Ctr, Jackson, MS

Dual-specificity protein phosphatase 5 (Dusp5) is a member of serine-threonine phosphatase family that regulates intracellular signal transduction by dephosphorylating ERK1/2. We previously generated Dusp5 zinc-finger knockout (KO) rats and found that KO of Dusp5 enhances myogenic response of middle cerebral artery and autoregulation of cerebral blood flow. This study investigated whether Dusp5 KO also improves renal hemodynamics and protects against hypertension-induced chronic kidney disease (CKD). Renal tissue expression of Dusp5 was decreased and levels of phosphorylated ERK1/2 (p-ERK1/2) and p-PKC enhanced in KO rats. Blood pressure increased similarly to 180 mmHg in both WT and Dusp5 KO rats treated with DOCA/salt. KO of Dusp5 enhanced autoregulation of renal blood flow (RBF increased by 12.1 ± 4.1% vs. 32.7 ± 4.7% when MAP increased from 100 to 180 mmHg, $p<0.05$) and reduced renal vascular remodeling compared with WT rats (0.64±0.03 vs. 0.94±0.06 wall-to-lumen ratio, $p<0.05$). Improvement in renal hemodynamics in hypertensive Dusp5 KO vs. WT rats was associated with decreased proteinuria (226.71 ± 23.82 vs. 337.62 ± 30.41 mg/day, $p<0.05$) and protein cast formation in the renal corticomedullary region (1.01 ± 0.17% vs. 2.4 ± 0.51%, $p<0.05$). The degree of glomerular injury was reduced (2.22 ± 0.04 vs. 3.12 ± 0.04, $p<0.05$) and nephrin expression was greater in hypertensive Dusp5 KO rats vs. WT rats. Hypertension-induced renal fibrosis was attenuated in Dusp5 KO rats (5.32 ± 0.6% vs. 9.90 ± 0.7%, $p<0.01$) in association with decreased MCP-1 expression, reduced ED-1^+ macrophage infiltration, down-regulated TGF-β1 expression, and attenuated fibrosis-related epithelial-mesenchymal transition (EMT) in the kidney (decreased expression of α-SMA and vimentin, and increased expression of E-cadherin). In conclusion, these results indicate that KO of Dusp5 protects against hypertension-induced proteinuria and renal fibrosis by enhancing renal hemodynamics and reducing vascular remodeling, glomerular injury, macrophage infiltration, TGF-β1 expression and EMT in the kidney. Thus, targeting Dusp5 may offer a novel approach for treating or preventing hypertension-induced CKD.

Disclosures: C. Zhang: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 201606100208 from China Scholarship Council. **X. He:** None. **S. Wang:** None. **R.J. Roman:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; P20GM104357, HL36279, DK104184 and HL138685 from National Institutes of Health. **G. Booz:** None. **F. Fan:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AG050049 from National Institutes of health and 16GRNT31200036 from American Heart Association.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

057

BBSome in Smooth Muscle is Required for the Regulation of Vascular Function

Authors: John J Reho, Deng-Fu Guo, Donald Morgan, Kamal Rahmouni, Univ Iowa, Iowa City, IA

The BBSome is a protein complex composed of 8 Bardet-Biedl syndrome (BBS) proteins, including *Bbs1*, involved in the regulation of various cellular processes including trafficking of receptors to cilia and the plasma membrane. This protein complex has emerged as a key regulator of cardiovascular function and blood pressure. We tested the hypothesis that the BBSome in vascular smooth muscle contributes to the regulation of vascular function. We selectively disrupted the BBSome in smooth muscle cells by deleting the *Bbs1* gene. We crossed mice harboring floxed alleles of the *Bbs1* gene ($Bbs1^{F/F}$) with mice expressing tamoxifen-inducible Cre in smooth muscle cells ($smMHC^{CreERT2}$). Validation of *Bbs1* gene deletion in smooth muscle was confirmed via decreased *Bbs1* mRNA levels in the aorta (1.0±0.2 vs 0.4±0.1 AU). We used wire myography to assess isolated aortic and mesenteric arterial ring function. *Bbs1* gene deletion from smooth muscle resulted in a significant decrease in the relaxation responses evoked by acetylcholine (Max relaxation: 55±7 vs 25±12%) and sodium nitroprusside (Max relaxation: 85±3 vs 61±8%) in aortic rings indicating dysfunction in both the endothelium and smooth muscle. Interestingly, aortic rings from $SM^{Cre}/Bbs1^{F/F}$ mice exhibited enhanced contractility to endothelin-1 compared to controls (Max contraction: 0.1±0.01 vs 0.06±0.01g). Additionally, resistance mesenteric arterial rings of $SM^{Cre}/Bbs1^{F/F}$ mice displayed enhanced contractile responses evoked by U-46619, phenylephrine, endothelin-1 and KCl-induced depolarization relative to controls ($p<0.05$). Western blot analysis revealed reduced expression of eNOS (1.0±0.1 vs 0.4±0.1 AU) and PKCα (1.0±0.2 vs 0.3±0.2 AU) in aortic lysates of $SM^{Cre}/Bbs1^{F/F}$ mice compared to controls. Radiotelemetric mean arterial pressure was not different in $SM^{Cre}/Bbs1^{F/F}$ mice at 4 weeks post-tamoxifen (96±4 mmHg) relative to controls (99±1 mmHg). Finally, arterial stiffness, measured using pulse wave velocity 4 weeks post-tamoxifen, showed a trend toward increased aortic stiffness in $SM^{Cre}/Bbs1^{F/F}$ mice (2.6±0.1 m/s) compared to controls (2.3±0.1 m/s, $p=0.06$). These data point to the vascular smooth muscle BBSome as a novel regulator of vascular function in a manner independent of blood pressure.

Disclosures: J.J. Reho: None. **D. Guo:** None. **D. Morgan:** None. **K. Rahmouni:** None.

Funding: Yes

Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

058

Novel Mechanisms at Immune-Vascular Interfaces Regulate Myogenic Tone of Resistance Arteries and Induce Hypertension in Mice

Authors: Daniela Carnevale, Sapienza Univ of Rome and Irccs Neuromed, Pozzilli, Italy; Daniele Iodice, Iolanda Vinciguerra, IRCCS Neuromed, Pozzilli, Italy; Marialuisa Perrotta, Sapienza Univof Rome, Pozzilli, Italy; Giuseppe Lembo, Sapienza Univ of Rome and Irccs Neuromed, Pozzilli, Italy

Multiple reports recognized that immunity participates in hypertension (HTN), although direct mechanisms that orchestrate the immune-vascular interaction are still unknown. Mice infused with AngiotensinII (AngII) typically increase blood pressure (BP), concomitantly recruiting immune cells in the vasculature, with CD8 T cells playing a crucial role. To investigate the molecular regulators of vascular-immune interface, we established a system of vessel organ culture, enabling the study of physiological properties of resistance arteries via a modified pressure myograph which keeps

vessels alive and functional for several days, while co-culturing immune cells of interest. We tested potential direct effects of CD8 T cells isolated from AngII-HTN or vehicle mice on resistance arteries from naïve mice. After co-incubation in the organ culture, CD8 T cells from HTN mice significantly increased myogenic tone (MT) of arteries, while no effect was induced by CD8 T of vehicle mice (%MT=$CD8^{AngII}$:27±2; $CD8^{veh}$:16±2, p<0.01). Our previous studies showed that an intracellular signaling involved in the acquisition of CD8 effectors functions, phosphatidylinositol-3-kinase-γ (PI3Kγ), was also involved in AngII-induced HTN, being its inhibition protective toward increase in BP and in vascular resistance. Here we took advantage of a mouse model expressing constitutively active PI3Kγ ($PI3K\gamma^{CX}$), to test immune functions of PI3Kγ in HTN. $PI3K\gamma^{CX}$ showed a spontaneous hypertensive phenotype (SBP_{mmHg}:129±1 vs WT:104±2, p<0.001) accompanied by infiltration of activated CD8 T cells in renal vasculature. Then, CD8 T cells isolated from $PI3K\gamma^{CX}$ mice and placed in co-culture with WT arteries increased their MT. Conversely, the CD8 T cells from WT mice had no effect when cultured with vessels (%MT=$CD8^{CX}$:31±1; $CD8^{WT}$:19±1, p<0.001). To test the in vivo relevance, we performed an adoptive transfer of $CD8^{CX}$ in WT mice, finding that they developed spontaneous HTN, while no effect was induced by $CD8^{WT}$ (SBP_{mmHg}: $CD8^{CX}$128±2 vs $CD8^{WT}$103±2, p<0.001). Taken together these data show that CD8 T cells from AngII-HTN mice are able to directly increase MT of resistance arteries and that PI3Kγ signaling is a crucial modulator of this effector function, likely contributing to BP increase.

Disclosures: D. Carnevale: None. **D. Iodice:** None. **I. Vinciguerra:** None. **M. Perrotta:** None. **G. Lembo:** None.

Funding: No

Funding Component:

059

Local Endothelial and Smooth Muscle Genomic Instability Reproduce Specific Features of Cardiovascular Aging

Authors: Paula Katherine Bautista-Nino, Eliana Portilla-Fernandez, ErasmusMC, Rotterdam, Netherlands; Alexandra Santu, Catherine Shanahan, King's Coll, London, United Kingdom; Anton Roks, ErasmusMC, Rotterdam, Netherlands

Nuclear and mitochondrial DNA damage contribute to aging and related cardiovascular disease. To explore if the aging features are cell autonomous we compared cardiovascular function of mice with a specific DNA repair system knockout in vascular endothelial cells (EC-KO) to that of mice with specific knockout in smooth muscle cells (SMC-KO). We evaluated cardiac function by echocardiography, blood pressure by the tail cuff method and *ex vivo* vascular function in aorta, coronary and carotid arteries using wire myographs organ baths setups. EC-KO showed macrovascular and microvascular vasodilator dysfunction due to specific loss of endothelium-dependent nitric oxide (NO) signaling (maximal vasorelaxation response to acetylcholine in aorta: 59.0% in EC-KO vs 75.0% in WT mice; p-value lt 0.0001). The reduction of vasodilator response was associated with a temporary systolic blood pressure increase at 3 months (138 mm Hg in EC-KO vs 125 mm Hg in WT mice; p-value= 0.03). In addition, EC-KO mice showed a severely compromised microvascular barrier function in the kidney, leading to papillary necrosis. Cardiac output was slightly affected at 5 months of age (16 ml/min in EC-KO vs 18 ml/min in WT mice; p-value= 0.13) and aortic distensibility was reduced (0.2 mm in EC-KO vs 0.3 mm in WT mice; p-value =0.05), suggesting decreased cardiac contractility and increased vascular stiffness. In sharp contrast, SMC-KO mice showed a specific decrease of endothelium-independent NO-mediated relaxation (maximal vasorelaxation response to the NO donor sodium nitroprusside: 83.4% in SMC-KO vs 98.6% in WT mice; p-value lt 0.001). Furthermore, SMC-KO showed increased carotid artery stiffness (Mediastress at intraluminal pressure of 120 mm Hg: $1.44*10^6$ in SMC-KO vs $1.82*10^6$ dyness/cm^2 in WT mice; p-value lt 0.001), displayed aortic root dilation and regurgitation. We conclude that DNA damage in EC and VSMC each lead to specific pathological changes that are found also in human cardiovascular aging. In complement, the changes reproduce the vascular phenotype of whole body DNA repair knockout mice (*Ercc1*$^{d/-}$) previously found by us. Therefore, the cardiovascular aging effects of DNA damage are at least partly cell autonomic, and represent an important treatment target.

Disclosures: P. Bautista-Nino: None. **E. Portilla-Fernandez:** None. **A. Santu:** None. **C. Shanahan:** None. **A. Roks:** None.

Funding: No

Funding Component:

060

Mitochondrial Deacetylase Sirt3 in Endothelial and Smooth Muscle Cells Protects From Vascular Dysfunction and Attenuates Hypertension

Authors: Anna Dikalova, Arvind Pandey, Liang Xiao, Hana Itani, Tatiana Sidorova, Vanderbilt Univ Medical Ctr, Nashville, TN; Eric Verdin, Gladstone Insts, Univ of California, San Francisco, CA; Johan Auwerx, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; David G Harrison, Sergey I Dikalov, Vanderbilt Univ Medical Ctr, Nashville, TN

Vascular dysfunction plays a key role in the development of hypertension and heart disease which causes one-third of deaths worldwide. Cardiovascular disease risk factors reduce mitochondrial deacetylase Sirt3 and Sirt3 declines with age paralleling the increased incidence of cardiovascular disease and hypertension; however, the role of Sirt3 was largely ignored. We hypothesized that Sirt3 plays critical role in vascular dysfunction and hypertension. To test this hypothesis we have developed novel tamoxifen-inducible endothelium specific Sirt3 knockout ($Ec^{Sirt3KO}$), endothelial Sirt3 overexpressing ($Ec^{Sirt3OX}$), smooth muscle Sirt3 knockout ($Smc^{Sirt3KO}$), smooth muscle Sirt3 overexpressing ($Smc^{Sirt3OX}$) and global Sirt3 overexpressing (Sirt3OX) mice on C57Bl/6J background, and examined the effect of Sirt3 expression on vascular dysfunction and angiotensin II-induced hypertension. It was found that cell-specific Sirt3 depletion in endothelial and smooth muscle in $Ec^{Sirt3KO}$ and $Smc^{Sirt3KO}$ mice increases vascular permeability by 2-fold, raises T cell and monocyte vascular accumulation, diminishes endothelial NO and increases blood pressure by 10 mm Hg compared with wild-type Sham mice. Angiotensin II infusion in $Ec^{Sirt3KO}$ and $Smc^{Sirt3KO}$ mice caused vascular hyperpermeability, increased vascular hypertrophy, exacerbated endothelial dysfunction and hypertension. Meanwhile, Sirt3 overexpression reduced vascular permeability and diminished vascular inflammation compared to Sham wild-type mice. Sirt3 overexpression in endothelial and smooth muscle prevents angiotensin II-induced vascular hyperpermeability, inhibits vascular oxidative stress, preserves endothelial-dependent relaxation, diminishes vascular hypertrophy and attenuates hypertension by 20 mm Hg in angiotensin II-infused $Ec^{Sirt3OX}$ and $Smc^{Sirt3OX}$ mice compared with the wild-type littermates. Interestingly, Sirt3 depletion in human aortic endothelial cells and human vascular smooth muscle cells reduces expression of differentiation markers. We suggest that Sirt3 is central in redox and metabolic regulations of smooth muscle and endothelial cells. Our data support a therapeutic potential of targeting Sirt3 in vascular dysfunction and hypertension.

Disclosures: A. Dikalova: None. **A. Pandey:** None. **L. Xiao:** None. **H. Itani:** None. **T. Sidorova:** None. **E. Verdin:** None. **J. Auwerx:** None. **D.G. Harrison:** None. **S.I. Dikalov:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

061

Mitochondrial Fission Mediates Hypertensive Vascular Remodeling and Aneurysm Development

Authors: Hannah A Cooper, Temple Univ, Philadelphia, PA; Tatsuo Kawai, Temple university, Philadelphia, PA; Steven Forrester, Emory Univ, Atlanta, GA; Kathy J Elliott, Temple university, Philadelphia, PA; Kyle Preston, Rosario Scalia, Victor Rizzo, Satoru Eguchi, Temple Univ, Philadelphia, PA

The prevalence of hypertension is growing steadily with mortality reaching 30,221. While effective blood pressure reduction therapy exists, the underlying pathophysiology leading to vascular remodeling remains poorly understood. Hypertension is a risk factor for abdominal aortic aneurysms (AAA) which when ruptured has 80% mortality. Surgery is the only treatment due to insufficient understanding of the disease process. Mitochondrial dysfunction has been implicated in various cardiovascular diseases but the role of mitochondrial dynamics, a mechanism regulating mitochondrial homeostasis, is under-investigated in hypertension and aneurysm. Our data shows that enhancement of mitochondrial fission via a GTPase, Drp1, in vascular smooth muscle cells (VSMCs) is involved in hypertensive vascular remodeling and aneurysm. In vitro, AngII induced transient mitochondrial fission (2-4 h) and enhanced mitochondrial ROS production in rat aortic VSMCs. Mitochondrial fission, mito-ROS generation, total cell protein, cell volume and extracellular collagen increased by 100 nM AngII were all attenuated in VSMCs by pretreatment with adenovirus encoding Drp1 siRNA/control non-silencing RNA or mdivi1, a Drp1 inhibitor. In vivo, male C57BL/6 mice were infused with AngII (1000ng/kg/min) for 2 weeks (hypertensive remodeling model) +/- mdivi1 (25 mg/kg ip every other day) or infused with AngII for 4 weeks with beta-aminopropionitrile in the drinking water (AAA model) +/- mdivi1 (25 mg/kg ip 3x per week). In the 2-week AngII model, mdivi1 suppressed left ventricular hypertrophy, vascular hypertrophy and perivascular fibrosis induced by AngII in aorta, heart and kidney, independent of blood pressure. In the AAA model, mdivi1 attenuated aneurysm development (External AA diameter (mm) Mean±SEM: 2.15±0.13 vs 1.49±0.07 (, $p<0.01$). Reduced KDEL and nitro-tyrosine staining in aorta (4w model), coronary and renal arteries (2w) in mdivi1 treated mice, suggests attenuation of ER stress and oxidative stress, respectively. These data suggest that inhibition of mitochondrial fission prevents AngII-induced cardiovascular remodeling and aneurysm development independently of hypertension via ER stress/mito-ROS mechanisms.

Disclosures: H.A. Cooper: None. **T. Kawai:** None. **S. Forrester:** None. **K.J. Elliott:** None. **K. Preston:** None. **R. Scalia:** None. **V. Rizzo:** None. **S. Eguchi:** None.

Funding: No

Funding Component:

062

Renal Hydrogen Peroxide Prevents Salt-Sensitive Hypertension

Authors: Santiago Cuevas, Laureano D Asico, Pedro A Jose, **Prasad R Konkalmatt**, The George Washington Univ, Washington, DC

C57Bl/6 and BALB/c are different in the sensitivity of their blood pressure (BP) to NaCl. High NaCl diet increases BP in C57Bl/6J but not BALB/c mice. However, C57Bl/6J mice are less prone to hypertension-induced renal injury than other mouse strains. The goal of this study was to determine the role of H_2O_2 in salt-sensitive hypertension. Basal renal levels of reactive oxygen species, including H_2O_2, were higher in BALB/c renal proximal tubule cells (RPTC-BALB/c) than RPTCs from C57Bl/6J mice (RPTC-C57Bl/6J) (60.8 ± 5 % vs 41.8 ± 12 %, n=8, $P<0.03$). Incubation media with 170 mM NaCl (HS) increased H_2O_2 production in RPTC-BALB/c but not in RPTC-C57Bl/6J, compared with RPTCs incubated in low (90 mM) or normal (145 mM) NaCl (+68±43%, 90 vs 170 mM, n=7, $P<0.05$). H_2O_2 (10 μM) treatment of the basolateral side of RPTC-BALB/c in Transwells increased intracellular Na^+ 1.62-fold that of vehicle-treated cells (n=4, $P<0.05$). Over-expression of catalase, abrogated the H_2O_2-induced increase in intracellular Na^+ in RPTC-BALB/c. Over-expression of catalase in the kidneys of BALB/c mice on normal (0.9%) NaCl diet did not alter their SBP. However, on high (4%) NaCl diet, SBP was increased in catalase over-expressing mice, relative to vehicle-treated controls (98±1.1 vs 112±1.4 mm Hg, n=3, $P<0.05$). Decreasing H_2O_2 by overexpression of catalase predisposes BALB/c mice to salt-sensitive hypertension, suggesting that high salt-induced H_2O_2 negatively regulates renal sodium transport and provides resistance to salt-induced hypertension.

Disclosures: S. Cuevas: None. **L.D. Asico:** None. **P.A. Jose:** None. **P.R. Konkalmatt:** None.

Funding: No

Funding Component:

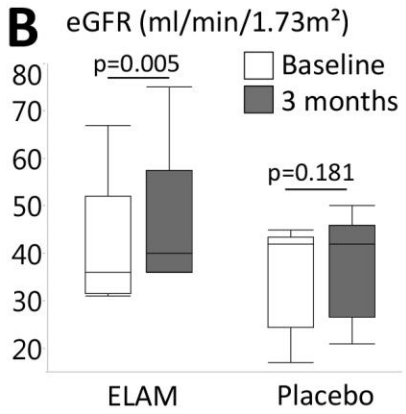
063

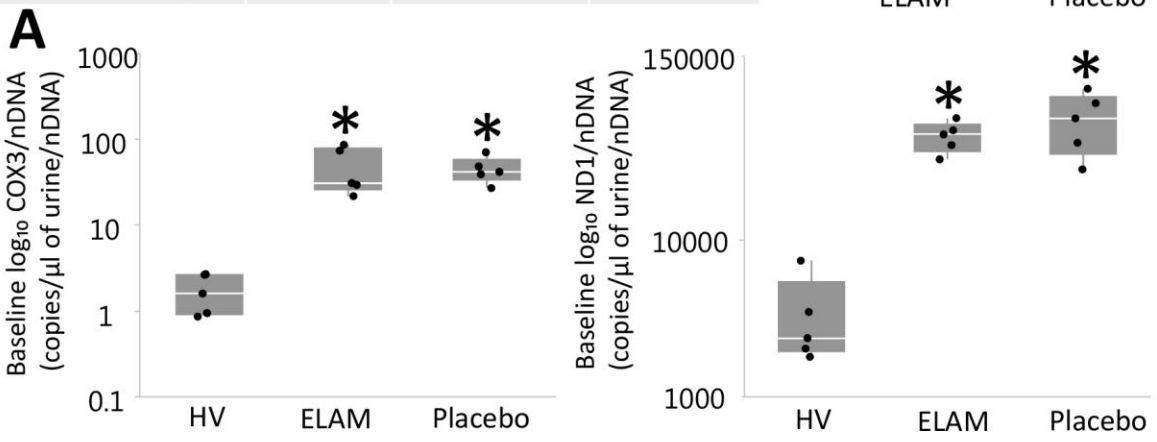
Urinary Mitochondrial DNA Copy Number Identifies Renal Mitochondrial Injury in Renovascular Hypertensive Patients Undergoing Renal Revascularization

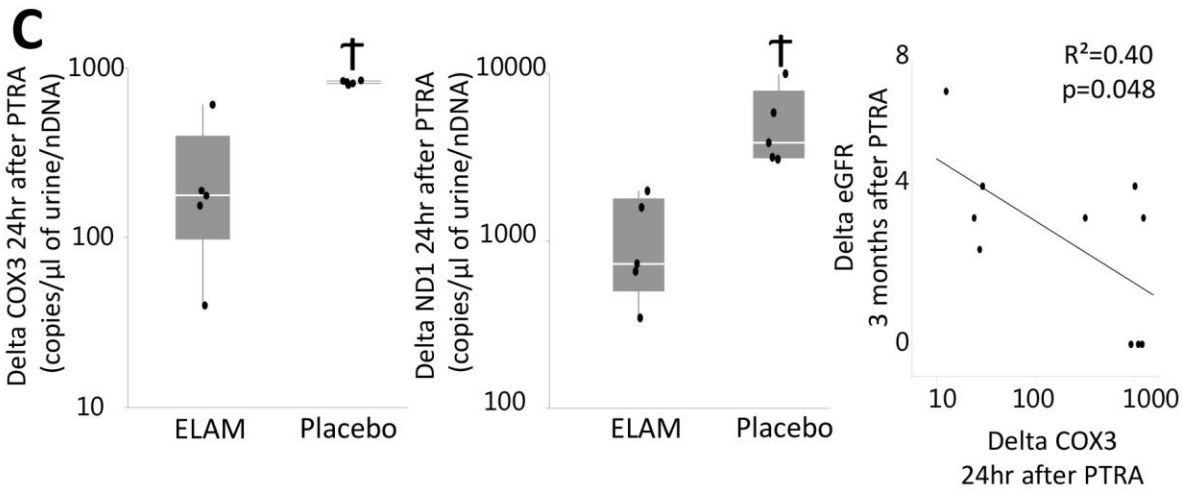
Authors: Alfonso Eirin, Sandra M Herrmann, Ahmed Saad, Hui Tang, Amir Lerman, Lilach O Lerman, Mayo Clinic, Rochester, MN

Background: We have previously shown in patients with renovascular hypertension (RVH) that elevated urinary mtDNA copy numbers represent surrogate markers of renal mitochondrial injury. Revascularization with percutaneous transluminal renal angioplasty (PTRA) can lead to acute renal reperfusion injury. However, whether PTRA induces renal mitochondrial injury remains unknown. Methods: We prospectively measured urinary copy number of the mtDNA genes cytochrome-c oxidase-3 (COX3) and NADH dehydrogenase subunit-1 (ND1) by qPCR in 5 RVH patients before and 24hrs after PTRA. Five additional patients were treated before and during PTRA with the mitoprotective drug elamipretide (ELAM, 0.05 mg/kg/hr IV infusion). Healthy volunteers (HV) served as controls (n=5). Results: Baseline blood pressure was similarly elevated in both RVH groups, and eGFR lower than HV (Table). Baseline urinary COX3 and ND1 levels were similarly higher in both RVH groups compared with HV (Fig. A), and directly correlated with serum creatinine levels (R^2=0.74, p=0.001 and R^2=0.45, p=0.033, respectively). Over 3 months, eGFR increased only in ELAM-treated patients (Fig. B). Furthermore, the rise in urinary mtDNA 24hrs after PTRA was blunted in PTRA+ELAM versus PTRA+Placebo, and inversely correlated with the change in eGFR of 3 months after PTRA (Fig. C). Conclusion: PTRA induces an acute rise in urinary mtDNA levels, likely reflecting renal mitochondrial injury due to reperfusion injury that inhibits renal recovery. Mitoprotection in this cohort limited PTRA-associated mitochondrial injury and improved renal outcomes after revascularization.

	HV	ELAM	Placebo
Age (years)	65.0±8.3	66.2±7.7	70.0±9.1
Gender (M/F)	2/3	2/3	2/3
BMI	30.4±2.5	34.17±13.6	33.14±6.0
MAP (mmHg)	81.4±9.1	105.1±8.9*	98.7±10.5*
Creatinine (mg/dl)	0.9±0.2	1.6±0.4*	1.8±0.68*
eGFR (ml/min/1.73m²)	78.3±9.0	40.6±6.7*	35.6±5.1*







A: Demographic data of HV and RVH patients treated with PTRA+ELAM or PTRA+Placebo. **B:** Urinary COX3 and ND1 in study groups. **C:** Delta (24hrs after PTRA–baseline) change in urinary COX3 and ND1 were lower in RVH patients treated with PTRA+ELAM compared with those treated with PTRA+Placebo, and correlated with the change in eGFR of 3 months after PTRA . **p<0.05 vs. HV; †p<0.05 vs. ELAM*

Disclosures: **A. Eirin:** None. **S.M.S. Herrmann:** None. **A. Saad:** None. **H. Tang:** None. **A. Lerman:** None. **L.O. Lerman:** None.

Funding: No

Funding Component:

064

Purinergic P2X1 and P2X7 Receptors Activation Attenuate Angiotensin AT1 Receptors Dominance in Regulating the Preglomerular Renal Microcirculation in Angiotensin II Dependent Hypertension

Authors: Supaporn Kulthinee, Weijian Shao, Tulane Univ, New Orleans, LA; Martha Franco, Insto Nacional de Cardiologia Ignacio Chavez, Mexico City, Mexico; L. Gabriel Navar, Tulane Univ, New Orleans, LA

Two important mechanisms regulating afferent arterioles (AA) in angiotensin II (ANG II) hypertension are the purinergic receptors (P2R) and the angiotensin AT1 receptors (AT1R); however, the nature of the interactions between the respective receptors has not been established. Accordingly, studies were performed in rats subjected to 2 weeks of ANG II infusion (80 ng/min via osmotic minipumps) which has been shown to increase interstitial ATP and ANG II concentrations. Experiments using the in vitro isolated juxtamedullary nephron preparation allowed direct visualization of the AA. To determine the interaction between P2XR and AT1R on AA at hypertensive renal perfusion pressure, afferent arteriolar inside diameters (AAD) were measured in response to increases in renal perfusion pressure (RPP) from 100 mmHg to 140 mmHg followed by superfusion with the inhibitors (i). P2X1Ri (NF-449) 1 μM, P2X7Ri (A-438079) 1 μM and P2X1Ri 1 μM plus P2X7Ri 1 μM followed by superfusion with AT1Ri (SML-1394) 1 μM. Increases in RPP to 140 mmHg decreased AAD from 14.75±0.19 μm to 11.69±0.23 μm (n=15, P<0.05) demonstrating autoregulation. Treatment with P2X1Ri significantly increased AAD from 11.94±0.54 μm to 14.33±0.15 μm (n=5, P<0.05) and increased further to 15.33±0.33 μm by treatment with the AT1Ri to values similar to those at RPP 100 mmHg (14.88±0.39 μm). Treatment with the P2X7Ri also vasodilated afferent arteriolar but to a lesser extent (13.34±0.06 μm vs. 10.94±0.21 μm, n=5, P<0.05); and further treated by AT1Ri, AAD returned to values similar to those at RPP 100 mmHg (14.05±0.20 μm vs. 14.16 ± 0.19 μm). Treatment with P2X1Ri plus P2X7Ri significantly increased AAD from 12.2±0.13 μm to 14.76±0.12 μm (n=5, P<0.05) a value not significantly different from the value at RPP 100 mmHg (15.22±0.17 μm). Further treatment with AT1Ri after treatment with the P2X1Ri plus P2X7Ri significantly increased AAD to values higher than the value observed at treatment with P2X1Ri plus P2X7Ri (15.43±0.23 μm vs. 14.76 ± 0.12 μm, P<0.05). The results indicate that renal P2X1R and P2X7R exert dominant roles in the regulation of AAD during elevation in arterial pressure and attenuate most of the AT1R influence on AAD in kidneys from ANG II hypertensive rats.

Disclosures: S. Kulthinee: None. **W. Shao:** None. **M. Franco:** None. **L. Navar:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIGMS; CoBRE P30GM103337, NIH NHLBI, Carol Lavin Bernick Faculty Grant Program. 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; Abbott, Merck, Pfizer, AstraZeneca, Baxter, Abbvie, Bectron Dickerson, Cardinal Health, Life Sciences, Hospira, Medtronics. G. Consultant/Advisory Board; Modest; Merck, University of Mississippi.

Funding: No

Funding Component:

065

Enhanced Glomerular Capillary Pressure and Tubuloglomerular Feedback (tgf) in Obese Alms1 (alstrom Syndrome 1) Knock Out Rats

Authors: Sumit R Monu, Keyona King-Medina, Pablo Ortiz, Henry Ford Health System, Detroit, MI

The ALMS1 gene has been associated to decreased renal function (lower GFR) and hypertension in genome-wide studies humans. The role of ALMS1 in renal dysfunction is unclear. Our lab has identified the ALMS1 protein as an interacting partner with the Na/K/2Cl cotransporter NKCC2, which mediates NaCl absorption by the thick ascending limb. We also

found that rats with ALMS1 deletion exhibited increased NKCC2 activity, hypertension and progressive obesity. NKCC2 also mediates the tubuloglomerular feedback (TGF) mechanism. TGF is initiated by an increase in NaCl transport via NKCC2 in the macula densa, which causes subsequent afferent arteriolar constriction and decreases GFR. We hypothesized that ALMS1 deletion enhances TGF and reduces GFR in rats. To test this we studied homozygous ALMS1 knockout (KO) and littermates wild-type Dahl SS (WT) rats. We measured TGF using in vivo renal micropuncture technique. TGF was calculated as a reduction in stop flow pressure (P_{SF}) during simultaneous perfusion of late proximal tubule incrementally from 0 to 20 and then 40 nl/min. P_{SF} is an index of glomerular capillary pressure (P_{GC}) and increased P_{GC} is linked to renal dysfunction. Increasing the perfusion rate from 0 to 20nl/min decreased P_{SF} in ALMS1 KO rats but not in WT rats, indicating enhanced TGF sensitivity (ALMS1: 45.5±2.9 to 41.4±3.0 mmHg, $p<0.05$ vs. WT: 34.2±1.4 to 31.4±1.9 mmHg, N.S). Baseline P_{GC} was elevated (ALMS1: 46.9±3.3 vs. WT: 34.9±1.5 mmHg, $p<0.05$) while renal interstitial hydrostatic pressure (RIHP) was reduced in ALMS1 KO (ALMS1: 2.6±0.9 vs. WT: 5.2±0.5 mmHg, $p<0.05$). We then measured GFR (calculated from inulin clearance) and found it was reduced in ALMS1 KO rats (ALMS1: 0.5±0.1 vs. WT: 1.6±0.3 ml/min/100gBW, $p<0.05$). Mean arterial pressure was higher in ALMS1 KO rats (ALMS1: 146±2 vs. WT: 113±3 mmHg, $p<0.05$). We concluded that ALMS1 is involved in the control of glomerular hemodynamics. Deletion of ALMS1 enhanced TGF sensitivity and this may be in part responsible for decreased GFR. It is likely that increased TGF-sensitivity, enhanced glomerular capillary pressure and hypertension combine to reduce GFR in ALMS1 KO rats. These are the first data supporting a role of ALMS1 in glomerular hemodynamics.

Disclosures: S.R. Monu: None. **K. King-Medina:** None. **P. Ortiz:** None.

Funding: No

Funding Component:

066

NO Regulates Trafficking of Claudin-19 in the Thick Ascending Limb via c-GMP

Authors: Casandra M Monzon, Jeffrey L Garvin, Case Western Reserve Univ, Cleveland, OH

Claudins are a family of tight junction proteins that provide size and charge selectivity to solutes traversing this route. Claudin function can be regulated by posttranslational modifications that affect their localization. Claudin-19 is expressed in TALs and has been implicated in the regulation of Na permeability in this segment. We previously showed that nitric oxide (NO) via cGMP decreases paracellular Na reabsorption in TALs, but the mechanism by which this occurs has not been elucidated. We hypothesize that NO/cGMP regulate claudin-19 trafficking to the plasma membrane. To test this we measured the effects of a NO donor or cGMP on dilution potentials in perfused isolated TALs from Sprague Dawley rats with and without antibodies against an extracellular domain of claudin-19 and Tamm-Horsfall protein (control). Dilution potentials were generated by reducing bath NaCl from 141 to 32 mM. During the control period, the dilution potential was -18.2 ± 1.8 mV. After the NO donor spermine NONOate (SPM; 200 µM) was added, it fell to -14.7 ± 2 mV ($n = 8$; $p < 0.04$). In the presence of the claudin-19 antibody, SPM had no significant effect on dilution potentials (claudin-19 antibody alone: -12.7 ± 2.1 mV vs claudin-19 antibody + SPM: -12.9 ± 2.4 mV; $n = 6$). The claudin-19 antibody alone had no effect on dilution potentials. In the presence of the Tamm-Horsfall protein antibody, SPM reduced the dilution potential from -9.7 ± 1.0 mV to -6.3 ± 1.1 mV ($p<0.006$, $n = 6$). Dibutyryl cGMP (500 uM) reduced the dilution potential -19.6 ± 2.6 mV to -17.2 ± 2.3 mV ($n = 6$; $p < 0.02$). Dibutyryl-cGMP increased surface expression of claudin-19 from 29.9 ± 3.8% to 65.9 ± 10.1 % ($n = 6$; $p < 0.02$) while reducing total expression from 13.3 ± 1.9 to 9.5 ± 1.5 arbitrary units ($p< 0.003$). We conclude that NO via cGMP reduces Na reabsorption via the paracellular pathway by increasing the amount of claudin-19 in the plasma membrane. We speculate that NO increases both insertion of claudin-19 into and retrieval from the plasma membrane, and the latter leads to enhanced degradation.

Disclosures: C.M. Monzon: None. **J.L. Garvin:** None.

Funding: No

Funding Component:

067

Mechanism of Action of 8-Aminoguanine on Renal Excretory Function

Authors: Edwin K Jackson, Zaichuan Mi, Thomas R Kleyman, Dongmei Cheng, Univ Pittsburgh Sch Med, Pittsburgh, PA

The endogenous purines 8-aminoguanosine and 8-aminoguanine (8-AG) are K^+-sparing natriuretics that increase glucose excretion and attenuate salt-induced hypertension. Most effects of 8-aminoguanosine are not direct, but require conversion in the systemic circulation to 8-AG (i.e., 8-aminoguanosine is a prodrug/prohormone). However, the mechanism of action by which 8-AG affects renal excretory function is unknown and is the subject of this investigation. Because 8-AG has structural similarities with inhibitors of epithelial Na^+ channels (ENaC), Na^+/H^+ exchangers (NHE), and adenosine A_1 receptors, we examined the effects of 8-AG on amiloride-sensitive ENaC activity in mouse collecting duct cells, on intracellular pH of human renal proximal tubule epithelial cells, and on in vivo (in rats) pharmacological responses to 2-chloro-N^6-cyclopentyladenosine (A_1-receptor agonist). 8-AG did not block ENaC, NHE, or A_1 receptors. Because Rac1 enhances activity of mineralocorticoid receptors and some guanosine analogues inhibit Rac1, we examined the effects of 8-AG on Rac1 activity in mouse collecting duct cells. Rac1 activity was significantly (*P*=0.024) inhibited by 8-AG (30 µM; from 2.5 ± 0.7 to 1.7 ± 0.7 arbitrary units). Because 8-AG is an inhibitor of purine nucleoside phosphorylase (PNPase; metabolizes inosine to hypoxanthine and guanosine to guanine), we compared the renal effects (in rats) of approximately equipotent intravenous doses of 8-AG (33.5 µmol/kg) vs. 9-deazaguanine (9-DG; 67 µmol/kg; PNPase inhibitor). 8-AG and 9-DG induced similar increases in urinary Na^+ (µmol/30 min; 8-AG, from 4.4 ± 2.0 to 38 ± 17; 9-DG, from 5.6 ± 3.3 to 34 ± 8.6) and glucose (µg/30 min; 8-AG, from 31 ± 17 to 225 ± 77; 9-DG, from 6.9 ± 3.9 to 144 ± 81) excretion. Intravenous 8-AG increased urinary excretion of guanosine and inosine (PNPase substrates), yet decreased excretion of guanine and hypoxanthine (PNPase products). 8-AG reduced K^+ excretion (µmol/30 min; 8-AG, from 29 ± 6.6 to 5.4 ± 1.7) whereas 9-DG did not. However, the Rac1 inhibitor Nsc23766 (9.4 µmol/kg) mimicked the effects of 8-AG on K^+ excretion (µmol/30 min; from 40 ± 7.1 to 18 ± 6.5). Conclusion: Likely 8-AG increases Na^+ and glucose excretion by blocking PNPase and decreases K^+ excretion by inhibiting Rac1.

Disclosures: E.K. Jackson: None. **Z. Mi:** None. **T.R. Kleyman:** None. **D. Cheng:** None.

Funding: No

Funding Component:

068

Kelch-Like 3 Dephosphorylation by Calcineurin as a Mechanism Regulating Na-cl Cotransporter

Authors: Qin Wang, Ken-ichi Ishizawa, Osamu Yamazaki, Yoshifuru Tamura, Yoshihide Fujigaki, Shunya Uchida, Shigeru Shibata, Teikyo Univ, Tokyo, Japan

Tacrolimus, an inhibitor of the phosphatase calcineurin, is widely used as an immunosuppressive agent. However, its use is associated with hypertension and hyperkalemia. Although previous studies indicated that the activation of thiazide-sensitive, Na-Cl cotransporter (NCC) plays a key role in the pathogenesis, the identity of the direct target of calcineurin in kidney remained unclear. Kelch-like 3 (KLHL3) is a component of an E3 ubiquitin ligase that critically regulates NCC function. In pseudophyoaldoseronism type 2, the disease-causing mutations in KLHL3 impair binding and degradation of with-no-lysine 1 (WNK1) and WNK4, resulting in hypertension and hyperkalemia that are correctable by thiazide. Previously, we reported that phosphorylation of KLHL3 at serine 433 ($KLHL3^{S433\text{-}P}$) by protein kinase C critically regulates

KLHL3 function by abrogating the substrate-binding ability. However, phosphatases responsible for KLHL3^{S433-P}regulation were unknown. We here show that calcineurin acts as a phosphatase for KLHL3^{S433-P}. In in vitro phosphatase assay, we found that calcineurin suppressed KLHL3^{S433-P}levels (65% reduction from baseline). Consistently, the activation of calcineurin by ionomycin significantly reduced KLHL3^{S433-P}abundance in distal convoluted tubule cells and in HEK cells expressing KLHL3, an effect that was almost completely prevented by calcineurin knockdown. Moreover, we found that tacrolimus increased KLHL3^{S433-P}levels by 1.8 fold in mouse kidney, which was associated with the WNK accumulation and increased NCC activity. Finally, we found that tacrolimus attenuated KLHL3-mediated WNK4 ubiquitination and degradation, and this effect was abolished by non-phosphorylatable S433A substitution in KLHL3. These data identify Kelch-like 3 as a direct target of calcineurin, and provide evidence that KLHL3 is involved in the pathophysiology of tacrolimus-induced hypertension.

Disclosures: Q. Wang: None. **K. Ishizawa:** None. **O. Yamazaki:** None. **Y. Tamura:** None. **Y. Fujigaki:** None. **S. Uchida:** None. **S. Shibata:** None.

Funding: No

Funding Component:

069

Antihypertensive Medication Treatment Regimens in SPRINT

Authors: Jordan B King, Kaiser Permanente Colorado, Aurora, CO; Andrew Moran, The Columbia Hypertension Ctr, Columbia Univ Medical Ctr, Columbia Univ Medical Ctr, New York, NY; Catherine Derington, Kaiser Permanente Colorado, Aurora, CO; Jennifer Herrick, Dept of Population Health Sciences, Sch of Med, Univ of Utah, Salt Lake City, UT; Ian Kronish, The Columbia Hypertension Ctr, Columbia Univ Medical Ctr, New York, NY; Barry Stults, Dept of Internal Med, Sch of Med, Univ of Utah, Salt Lake City, UT; Daichi Shimbo, The Columbia Hypertension Ctr, Columbia Univ Medical Ctr, New York, NY; Paul Muntner, Dept of Epidemiology, Univ of Alabama at Birmingham, Birmingham, AL; Tom Greene, Dept of Population Health Sciences, Sch of Med, Univ of Utah, Salt Lake City, UT; William Cushman, Preventive Med Section, Veterans Affairs Medical Ctr; Dept of Preventive Med, Univ of Tennessee Health Science Ctr, Memphis, TN; William Weintraub, MedStar Washington Hosp Ctr, Washington, DC; Adam Bress, Dept of Population Health Sciences, Sch of Med, Univ of Utah, Salt Lake City, UT

Background: Details of antihypertensive medication regimens used to achieve intensive systolic blood pressure (SBP) goals have not been described. **Objective:** Determine the distribution and longitudinal changes in antihypertensive medication regimens in the Systolic Blood Pressure Intervention Trial (SPRINT). **Methods:** We used antihypertensive medication data collected by pill bottle review at each visit to categorize antihypertensive regimens by medication class. Free text string variables of medication names were independently reviewed by two clinical pharmacists to create standardized generic medication names and classes. **Results:** Figure 1 illustrates longitudinal changes in class combinations and number of drugs at the randomization, 6, 12, and 18-month visits. Fifty-six percent of participants modified their initial regimen by the 6-month visits; 43% of participants made additional modifications to their regimens from the 6-month to the 18-month visit. The most common initial regimens, and least likely regimens to be changed over time, were combinations with an ACEI/ARB and diuretics ± other classes (42% of initial regimens). Participants in the intensive arm added a mean (standard deviation) of 0.6 (0.9) medications to their initial regimens in the first 18-months compared to -0.1 (0.9) in the standard arm. **Conclusion:** Intensive blood pressure treatment requires more medication complexity in terms of class and dose. Further study of distinct regimens may reveal if certain class and dose combinations provide better SBP control, safety, or patient satisfaction.

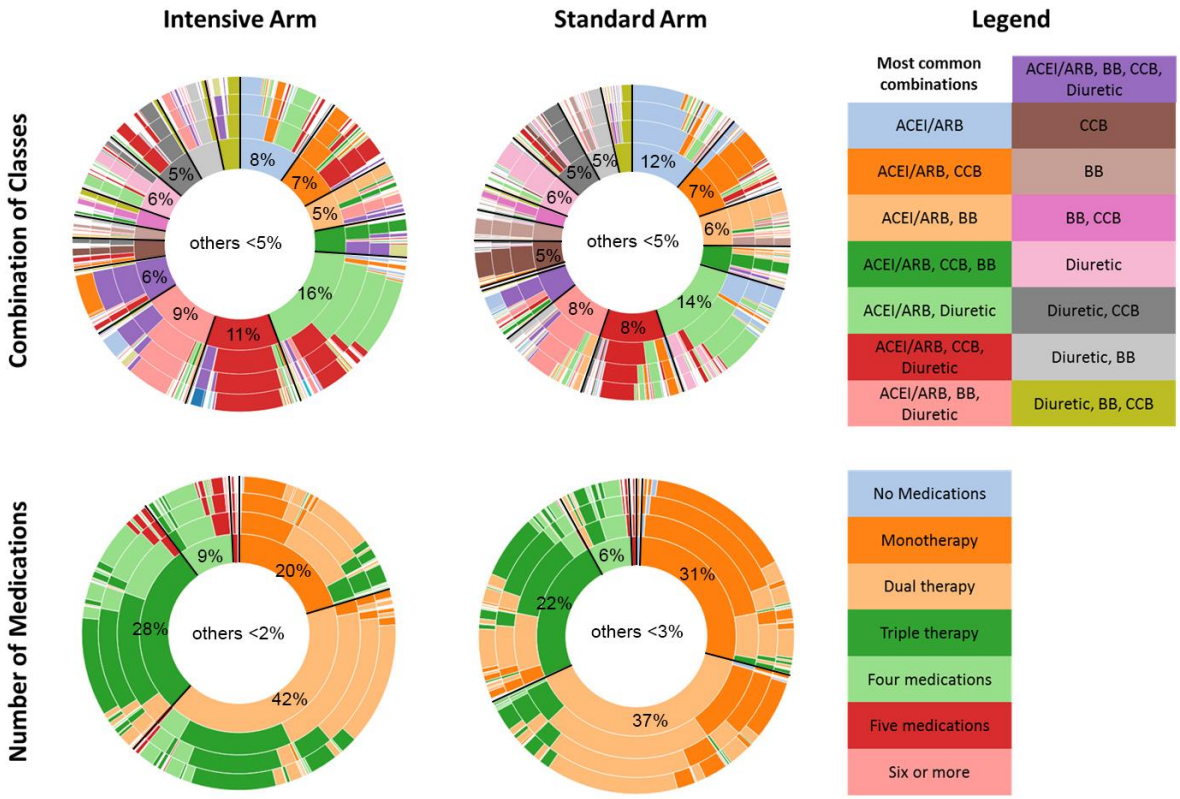


Figure 1. Sunburst plots of the antihypertensive medication regimens used during SPRINT. The upper panels represent distinct combinations of antihypertensive medication classes and the bottom panels represent the number of antihypertensive medications used. The Sunburst plot presents a hierarchical relationship with each circle representing a time point in the trial: inner ring, randomization visit; 2nd from inner ring, 6-month visit; 3rd from inner ring, 12-month visit; and outer ring, 18-month visit. Study participants remain within the same hierarchical ring as the ring extends outward.

Disclosures: **J.B. King:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Consultant on unrelated investigator-initiated Novartis grant. **A. Moran:** None. **C. Derington:** None. **J. Herrick:** None. **I. Kronish:** None. **B. Stults:** None. **D. Shimbo:** None. **P. Muntner:** None. **T. Greene:** None. **W. Cushman:** None. **W. Weintraub:** None. **A. Bress:** None.

Funding: No

Funding Component:

070

Adherence to Antihypertensive Drugs: Insights from the SPYRAL HTN Trials and Implications for Hypertension Trial Design

Authors: Michael A Weber, SUNY Downstate Coll of Med, New York, NY; David Kandzari, Piedmont Heart Inst, Atlanta, GA; Michael Böhm, Felix Mahfoud, Klinik für Innere Med III, Homburg/Saar, Germany; Kazuomi Kario, Jichi Medical Univ Sch of Med, Tochigi, Japan; Tony Walton, Ingrid Hopper, The Alfred Hosp, Melbourne, Australia; Manesh Patel, Crystal Tyson, Duke Univ Medical Ctr, Durham, NC; Chandan M Devireddy, Janice P Lea, Emory Univ Sch of Med, Atlanta, GA; Joachim Weil, Tolga Agdirlioglu, Sana Cardiomed Heart Ctr, Luebeck, Germany; Markus Meyer, Experimental and Clinical Toxicology, Saarland Univ, Saarland, Germany; Martin Fahy, Medtronic PLC, Santa Rosa, CA; Raymond Townsend, Sch of Med, Univ of Pennsylvania, Philadelphia, PA

Background: Variable adherence to prescribed antihypertensive drugs may have confounded prior renal denervation trials. Adherence to prescribed drugs was measured in the SPYRAL HTN-OFF MED and ON MED trials of renal denervation for uncontrolled hypertension when no drugs, and when up to 3 anti-hypertensive drug classes were

prescribed, respectively.

Methods: Patients were enrolled with uncontrolled hypertension defined as office systolic blood pressure (SBP) ≥150 and <180 mmHg with diastolic blood pressure >90 mmHg and 24-hour mean SBP ≥140 and <170 mmHg. Patients were randomized 1:1 to renal denervation or sham control. OFF MED trial patients were either drug naïve or had antihypertensive drugs discontinued at enrollment. ON MED trial patients had to maintain baseline prescribed antihypertensive medications. Both groups were informed on enrollment that urine and blood samples would be obtained for drug adherence testing. This testing utilized tandem HPLC and mass spectrometry. Following enrollment, both groups were followed for 4-8 weeks prior to randomization.

Results: In OFF MED, antihypertensive drug or drug metabolites were detected in 10% of patients just prior to randomization, 11.2% at 3 months following randomization, and in 15% of patients at either time point, despite protocol-required absence of all antihypertensive medications following enrollment. In ON MED, 62.5% were fully adherent to prescribed medications (i.e., all prescribed medications identified) at baseline, 55% at 3 months and 62.5% at 6 months. Drug adherence was not consistent: 24% of baseline adherent patients were non-adherent at 3 months, and 16% of patients adherent at 3 months were no longer adherent at 6 months.

Conclusions: Despite being aware that drug adherence testing was being conducted, almost half did not take all medication when they were prescribed and a minority took anti-hypertensive medications when instructed to abstain. Furthermore, non-adherence was inconsistent, with individual behavior at one time point not predictive of drug adherence at other time points. Attention to protocol adherence in device-based clinical hypertension trials is critical to determine non-drug treatment effects.

Disclosures: **M.A. Weber:** D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Menarini. G. Consultant/Advisory Board; Modest; Medtronic, Boston Scientific, ReCor, Omron, Ablative Solutions. **D. Kandzari:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Medtronic. G. Consultant/Advisory Board; Modest; Medtronic. **M. Böhm:** H. Other; Modest; Medtronic, Abbott, Boehringer-Ingelheim, Servier, BMS, Astra-Zeneca. **F. Mahfoud:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Medtronic. H. Other; Modest; Medtronic, ReCor. **K. Kario:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Teijin Pharma Limited, Omron Helthcare Co., Ltd., FUKUDA DENSHI, Bayer Yakuhin Ltd., A & D Co., Ltd., Daiichi Sankyo Company, Limited, Mochida Pharmaceutical Co., Ltd, EA pharma, Boehringer Ingelheim Japan Inc., Tanabe Mitsubishi Pharma Corporation, Shionogi & Co., Ltd., MSD K.K., Sanwa Kagaku Kenkyusho Co.,LTD., Bristol-Myers Squibb K.K.. H. Other; Modest; Medtronic, Takeda Pharmaceutical Company Limited, Omron Healthcare Co., Ltd. **T. Walton:** G. Consultant/Advisory Board; Modest; Medtronic. **I. Hopper:** None. **M. Patel:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Medtronic, Bayer, Jansen, NHLBI. G. Consultant/Advisory Board; Modest; Bayer, Jansen, Astra Zeneca. **C. Tyson:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Medtronic. **C.M. Devireddy:** G. Consultant/Advisory Board; Modest; Medtronic. **J.P. Lea:** None. **J. Weil:** G. Consultant/Advisory Board; Modest; Medtronic. **T. Agdirlioglu:** None. **M. Meyer:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Medtronic. **M. Fahy:** A. Employment; Modest; Medtronic. **R. Townsend:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Medtronic.

Funding: No

Funding Component:

071

Increased Short-Term Blood Pressure Variability in Patients With Isolated Systolic Hypertension

Authors: Alejandro De La Sierra, Hosp Mutua Terrassa, Terrassa, Spain; Gianfranco Parati, Hosp San Luca, Milano, Italy; Jose R Banegas, Juan J de la Cruz, Univ Autónoma, Madrid, Spain; Manuel Gorostidi, Hosp Central Asturias, Oviedo, Spain; Ernest Vinyoles, CAP la Mina, Barcelona, Spain; Julian Segura, Luis M Ruilope, Hosp 12 de Octubre, Madrid, Spain

Isolated systolic hypertension (ISH) is a clinical condition derived from increased arterial stiffness and associated with a worse cardiovascular prognosis. Increased blood pressure variability (BPV) is also associated to cardiovascular risk. We aimed to estimate short-term BPV in patients with ISH compared to those with systolic and diastolic hypertension (SDH). From the Spanish ABPM Registry database containing 63910 identified patients, we selected 20497 with ISH and 23269 with SDH. BPV estimates were obtained from ABPM records, and included standard deviation (SD), coefficient of variation (CV), weighted SD and average real variability (ARV). Comparisons were carried out by Student's t test, and by general linear models adjusted for age, gender and office BP. Due to important differences in age between groups, a case-control matching was performed obtaining 14707 pairs matched by gender and with an age difference < 2 years. Comparisons were repeated in this dataset. Patients with ISH were older, more frequently women, with diabetes and with a previous history of CV disease. Office BP was lower (155±13/81±7 vs. 159±16/98±7 mmHg; p<0.001) in comparison to those with SDH. As shown in the table, systolic BPV was increased in ISH (p<0.001 for all comparisons), with respect to SDH. Results were confirmed after adjustments for age, gender and office BP and in the case-control matching comparison (also adjusted for office BP). No differences in diastolic BPV were observed between groups. We conclude that ISH is associated with an increased short-term BPV. This increased BPV could be one of the factors explaining the high CV risk of ISH patients.

Systolic BP	ISH (N=20497)	SDH (23269)	Unadjusted p value	Adjusted p value
SD daytime	13.1 ± 3.7	12.5 ± 3.6	<0.001	<0.001
CV daytime	9.8 ± 2.7	9.2 ± 2.7	<0.001	<0.001
SD nighttime	12.0 ± 4.1	11.6 ± 3.9	<0.001	<0.001
CV nighttime	9.8 ± 3.5	9.6 ± 3.4	<0.001	0.005
Weighted SD	12.7 ± 3.2	12.1 ± 3.1	<0.001	<0.001
ARV	10.4 ± 2.5	9.8 ± 2.3	<0.001	<0.001

Disclosures: A. De La Sierra: None. **G. Parati:** None. **J.R. Banegas:** None. **J.J. de la Cruz:** None. **M. Gorostidi:** None. **E. Vinyoles:** None. **J. Segura:** None. **L.M. Ruilope:** None.

Funding: No

Funding Component:

072

Return on Investment of Self-Measured Blood Pressure: An Economic Model from the Insurers' Perspective

Authors: Alejandro Arrieta, Florida Intl Univ, Miami, FL; John Woods, Indiana Univ Purdue Univ Indianapolis, Indianapolis, IN; Gregrory Wozniak, Stavros Tsipas, Michael Rakotz, American Medical Association, Chicago, IL; Stephen J Jay, Indiana Univ Purdue Univ Indianapolis, Indianapolis, IN

Introduction: About half of the 70 million adults with hypertension in the U.S. do not have their blood pressure (BP) controlled. An effective strategy to diagnose hypertension and improve BP control is to use self-measured blood pressure (SMBP) devices. Despite evidence supporting the *clinical* effectiveness of SMBP, most insurers remain

unconvinced that the cost of the devices would yield a positive *financial* return.
Objective: We adopted the perspective of private insurers to estimate return-on-investment (ROI) and net present value (NPV) of SMBP devices used to diagnose hypertension (including treatment selection and medication titration) and to manage BP.
Methods: We developed a decision-analytic model using Framingham risk predictions and reported SMBP and clinic blood pressure measurement (CBPM) sensitivity and specificity values to simulate health outcomes and their associated annual and lifetime projections of costs and savings for the U.S. population.
Results: Compared to CBPM, SMBP benefits exceed investment, producing large positive ROIs and NPVs. SMBP is cost-beneficial in the short-run and at lifetime horizon, but the return declines with patient age (see table).
Conclusions: A strong business case exists for reimbursing SMBP, but only when it is used both to diagnose and to manage hypertension. Its primary economic value stems from its diagnostic role in ruling out white coat hypertension. If SMBP is used solely to manage (but not to diagnose) hypertension, our model indicates that its incremental effects on blood pressure reduction and cardiovascular event rates are not large enough to produce positive financial gains.

Self-measured Blood Pressure, ROI and NPV

Age group	Panel size[1/]	ROI		NPV			
		3-year horizon	Lifetime	3-Year horizon	Lifetime	Lifetime: when used for diagnosis only	Lifetime: when used for management only
Age 25-34	200	847.1%	663.0%	$698	$801	$884	-$119
Age 35-44	190	773.9%	617.9%	$615	$691	$770	-$110
Age 45-54	200	624.3%	479.5%	$512	$574	$667	-$118
Age 55-64	190	386.8%	263.3%	$344	$361	$481	-$135
Age 65-74	130	270.0%	177.8%	$243	$241	$360	-$129
Age 75-84	60	241.2%	164.8%	$210	$201	$310	-$117
Age 85+	30	36.6%	9.9%	$30	$10	$115	-$108
Total investment	**1,000**			**$469,204**	**$518,924**	**$616,733**	**-$120,962**

[1/] Results assuming a panel of 1,000 individuals with specified age groups.

Disclosures: A. Arrieta: None. **J. Woods:** None. **G. Wozniak:** None. **S. Tsipas:** None. **M. Rakotz:** None. **S.J. Jay:** None.

Funding: No

Funding Component:

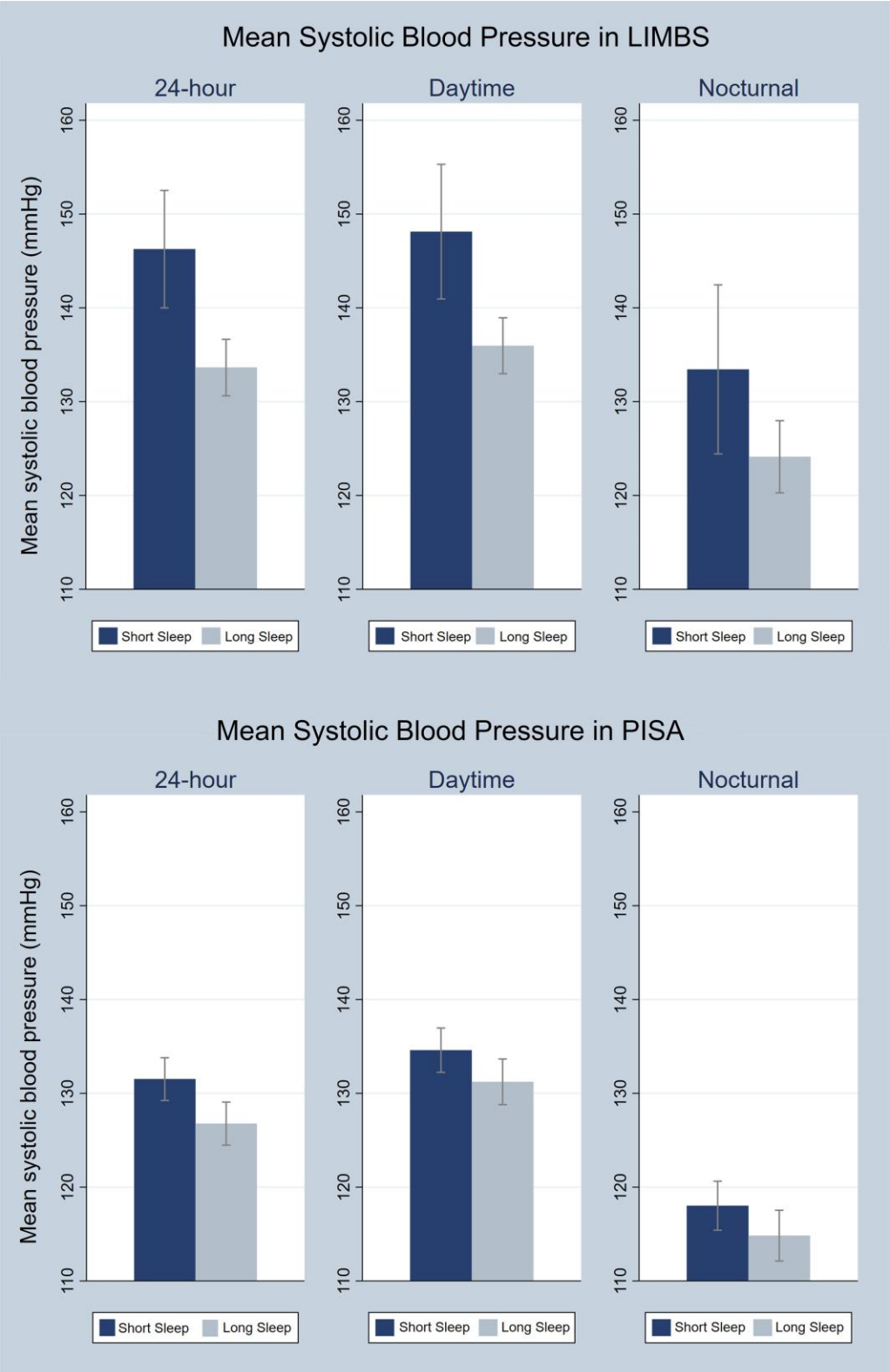
073

Sleep Duration and 24-hour Ambulatory Blood Pressure in Adults not on Antihypertensive Medications

Authors: Jordana B. Cohen, Rachel Shulman, Debbie L. Cohen, Univ of Pennsylvania, Philadelphia, PA; Michael A. Grandner, Univ of Arizon, Tuczon, AZ; Thorarinn Gislason, Univ of Iceland, Reykjavik, Iceland; Allan I. Pack, Samuel T. Kuna, Raymond R. Townsend, Univ of Pennsylvania, Philadelphia, PA

Background: Short sleep duration has been widely linked to increased cardiovascular morbidity and mortality, although the underlying mechanisms for this relationship remain unclear. Maladaptive circadian variations in blood pressure (BP) may be an important mediator of adverse outcomes in people with short sleep duration. Our goal was to evaluate the

association between sleep duration and 24-hour BP control. **Methods:** We performed a post-hoc analyses of 24-hour ambulatory BP monitoring in 219 non-hypertensive and pre-hypertensive adults in the Lifestyle Modification in BP Lowering Study (LIMBS) and Penn Icelandic Sleep Apnea (PISA) Study. **Results:** The 24-hour mean systolic BP was 12.7 mmHg higher in LIMBS (see Figure; $p<0.001$) and 4.7 mmHg higher in PISA ($p=0.005$) among participants with shorter sleep duration (<7 hours) compared to those with longer sleep duration (≥7 hours). There was a 1 mmHg higher 24-hour mean systolic BP for every 2.57 minute shorter sleep duration in LIMBS (95% CI -4.56 to -0.58; $p<0.001$), and a 1 mmHg higher 24-hour mean systolic BP for every 1.99 minute shorter sleep duration in PISA (95% CI -3.48 to -0.50; $p=0.009$). There was no significant association between sleep duration and BP variability, nocturnal dipping, or in-office systolic BP. **Conclusions:** Shorter sleep duration is strongly associated with higher systolic BP on 24-hour ambulatory BP monitoring; the association seems to be independent of nocturnal and in-office BP. Adults with shorter sleep duration may benefit from screening with 24-hour ambulatory BP monitoring to promote earlier detection of hypertension and potentially mitigate their increased risk for future cardiovascular disease.



Disclosures: **J.B. Cohen:** None. **R. Shulman:** None. **D.L. Cohen:** None. **M.A. Grandner:** None. **T. Gislason:** None. **A.I. Pack:** None. **S.T. Kuna:** None. **R.R. Townsend:** None.

Funding: No

Funding Component:

074

Non-Adherence to Prescribed Blood Pressure Lowering Drugs in Patients With Suspected "Resistant" Hypertension: A Call for Rigorous Adherence Testing

Authors: Marcel Ruzicka, Swapnil Hiremath, Ottawa Hosp, Ottawa, ON, Canada; Frans H Leenen, Univ of Ottawa Heart Inst, Ottawa, ON, Canada

Background. Pseudoresistance from non-adherence to blood pressure (BP) lowering drugs can cause unnecessary medical visits and invasive diagnostic tests and procedures. It also leaves patients at a high risk for vascular outcomes. There is currently no unified approach to the diagnosis of non-adherence. Direct questioning by medical personnel and review of pharmacy filling do not reveal the full extent of non-adherence. Direct observational therapy (DOT), a test where a patient's BP response is monitored after supervised administration of BP lowering drugs, has never been prospectively evaluated for diagnosis of suspected resistant hypertension (HTN). **Methods.** We conducted a prospective study to estimate the prevalence of pseudoresistant HTN using the DOT test. Eligible patients were adults with suspected resistant HTN, defined as daytime average systolic blood pressure (SBP) >135 mmHg on 24-hour ambulatory blood pressure monitoring (ABPM) while on 3 or more BP lowering drugs. Patients confirmed adherence at the clinic, and they had their pharmacy records reviewed. For the DOT, prescribed BP lowering drugs were administered by a nurse at the HTN clinic. BP response was monitored at the office until peak BP effect was reached followed by immediate 24-hour BP ambulatory monitoring, which was repeated at 1 month. **Results.** 60 patients were enrolled, and 50 patients completed this study. 30 patients had confirmed resistant HTN. 20 patients had a large SBP decrease during observation immediately post drug administration (≥ 20 mmHg) as well as daytime average SBP on 24-hour ABPM (>10 mmHg) as compared to their baseline SBPs. Of these 20 patients, 15 continued to have controlled BP at the 24-hour ABPM done at 1 month post DOT, indicating medium term change in in adherence. **Conclusions.** A large proportion of patients with suspected resistant HTN have pseudoresistant HTN from non-adherence. The diagnosis of pseudoresistance was associated with subsequent cure of resistant HTN in 75% of cases. Proper assessment of adherence is essential to avoid unnecessary drug escalation, and/or investigations for secondary HTN. The DOT represents a simple test for diagnosis and management of suspected resistant HTN, with important ramifications for health care policy makers.

Disclosures: M. Ruzicka: None. **S. Hiremath:** None. **F.H.H. Leenen:** None.

Funding: No

Funding Component:

075

Does the Benefit From Treating to Blood Pressure Targets Vary With Age?

Authors: George Roush, Kevin Singh, Ayla Zubair, NYU Sch of Med, New York, NY; William Kostis, Rutgers Robert Woods Johnson Medical Sch, New Brunswick, NJ; Domenic Sica, Dept of Med and Pharmacology, Virginia Commonwealth Univ, Richmond, VA, Richmond, VA; John Kostis, Rutgers Robert Woods Johnson Medical Sch, New Brunswick, NJ

Hypertension guidelines differ regarding how blood pressure targets vary with age. Crucial to this debate is how the benefit of treatment might vary with age. Therefore, a systematic search was conducted for trials randomizing treatment in the more intensive arm to systolic blood pressure (SBP) targets generally recommended, i.e., 140 mmHg or less. Meta-analysis examined the effects of age on risk reduction. Sixteen trials met criteria with SBP targets of 120-140 mmHg. Relative to high targets, low targets reduced risk for cardiovascular events (CVEs), 0.86 (95% CI 0.75,0.98), P=0.019, but treatment benefit differed among trials. This heterogeneity was explained by patient age. Relative to high targets, low targets reduced risk in older patients (mean ages 74+): 0.77 (0.61,0.97), P=0.025, but not in younger patients (mean ages 55-67): 0.90 (0.78,1.03), P=0.121, even though the latter had much greater statistical power. Risk

reduction from low targets was greater in older patients than in younger patients: Relative risk in older patients versus relative risk in patients 16 years younger (2 standard deviations of age) = 0.78 (95% CI 0.63,0.98), P=0.036 (see Figure). Although generally not statistically significant, similar patterns were seen for specific CVEs and total mortality. Risks for adverse effects also tended to decrease with age. The number needed to treat to low targets to prevent 1 CVE in 10 years for 2 typical populations declined with age from 45 to 14 and from 110 to 13. In conclusion, for both relative risk and absolute risk, treating to SBP targets of 120-140 mmHg was more beneficial in older patients than in younger patients with no age-related increase in adverse effects.

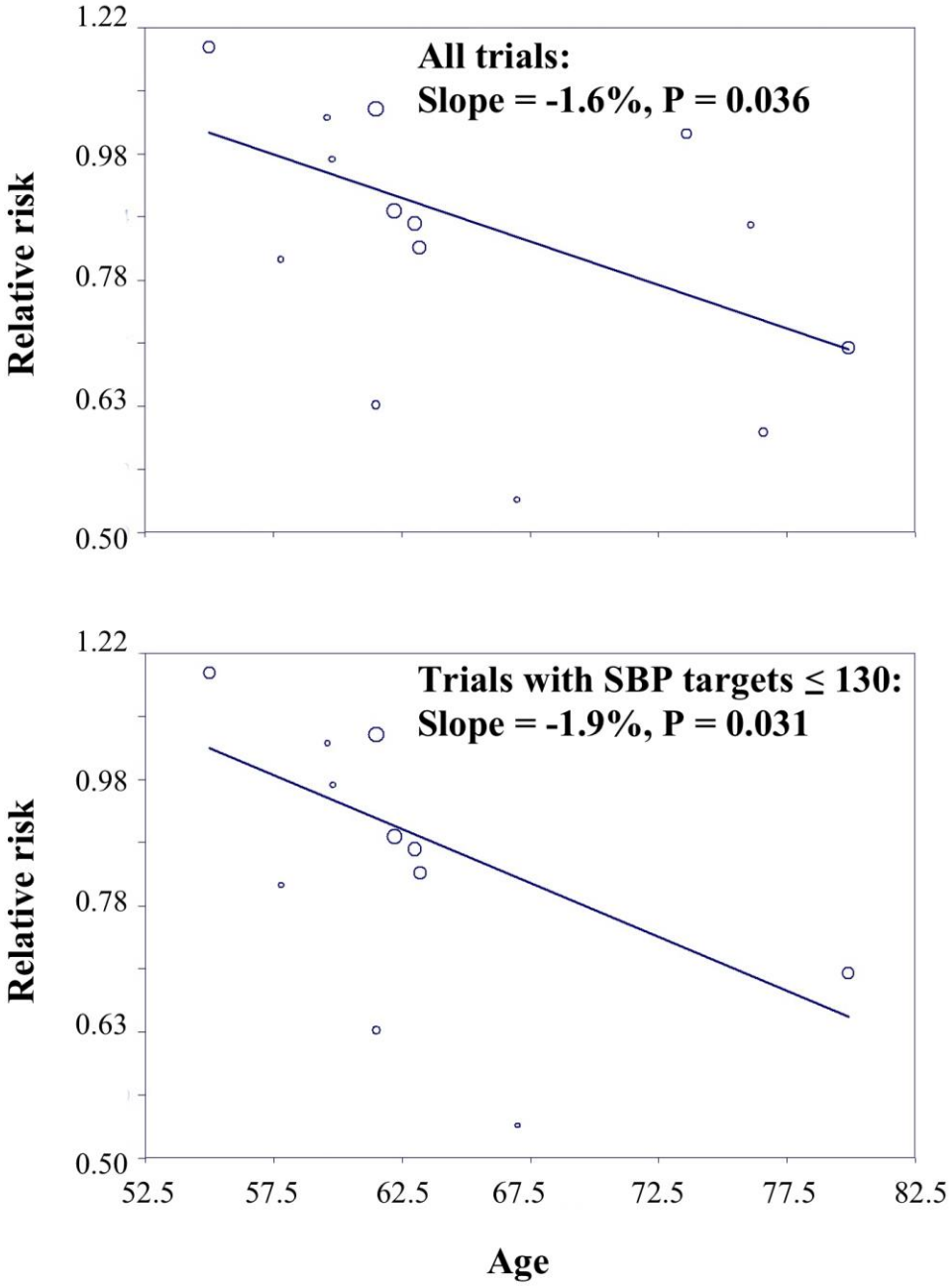


Figure. Change with age in relative risk for cardiovascular events from treating to SBP targets of 120-140 mmHg versus higher targets. Among all trials, the relative risk in older patients versus the relative risk in patients 16 years younger (2 standard deviations of age) was 0.78 (0.63,0.98), P=0.036.

Disclosures: **G. Roush:** None. **K. Singh:** None. **A. Zubair:** None. **W. Kostis:** None. **D. Sica:** None. **J. Kostis:** None.

Funding: No

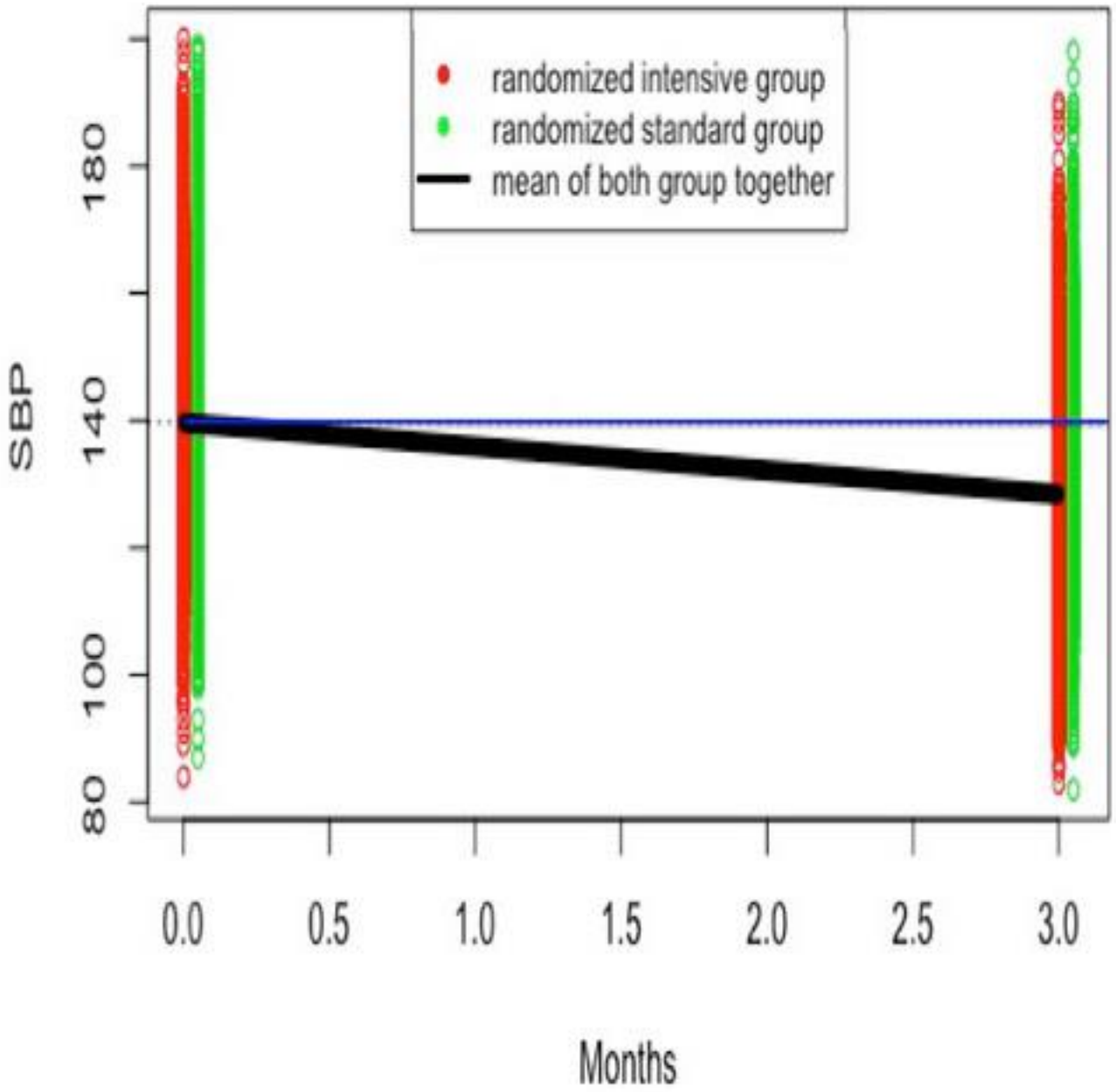
Funding Component:

076

All Cause Mortality is Higher for Patients Who Have Bigger Blood Pressure Drops on Antihypertensive Therapy

Authors: John B. Kostis, Rutgers Robert Wod Johnson Medical Sch, New Brunswick, NJ; Chun Pang Lin, Rutgers Robert Wood Johnson Medical Sch, New Brunswick, NJ; Javier Cabrera, Rutgers Univ, Piscataway, NJ; Daniel Blickstein, Nora M. Cosgrove, William J. Kostis, Rutgers Robert Wood Johnson Medical Sch, New Brunswick, NJ

Objectives: In the Systolic Blood Pressure Intervention Trial (SPRINT) patients were randomized to the intensive blood pressure group targeting systolic blood pressure (SBP) less than 120 mm Hg or to the standard group targeting SBP less than 140 mm Hg. Here we present data on the effect of SBP drop between randomization and after 3 months of treatment in 8919 patients. Methods: We evaluated the association of SBP drop (SBP at randomization minus SBP after 3 months of treatment) on all-cause mortality until the end of the study (approximately 1090 days after the 3-month visit). Logistic regression of all-cause death as affected by the blood drop, age, gender, SBP at 3 months, diastolic BP at 3 months and randomization group was performed. Results: Bigger SBP drop was associated with higher all-cause mortality (black line in the lower figure, p=0.0347) regardless of randomization to intensive (red) or standard (green) group and after adjustment for the other factors shown in the bottom figure. Mortality was associated with age (p>0.0001), male gender (p>0.0001), SBP at 3 months (p=0.0031), and randomization to intensive treatment (p=0.0269). In addition, patients receiving more than 1 drug were more likely to die (p=0.0179). Conclusion: In SPRINT, for a given achieved blood pressure patients with bigger SBP drop from baseline had higher all-cause mortality.



Disclosures: **J.B. Kostis:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Principal Investigator. **C. Lin:** None. **J. Cabrera:** None. **D. Blickstein:** None. **N.M. Cosgrove:** None. **W.J. Kostis:** None.

Funding: No

Funding Component:

077

Captopril-Induced Sustained Reduction in Blood Pressure is Associated With Alterations in Gut-Brain Axis in the Spontaneously Hypertensive Rats

Authors: Tao YANG, Victor P Aquino, Gilberto O Lobaton, Luis Colon-Perez, Jasenka Zubcevic, Marcelo Febo, Elaine M Richards, Carl J Pepine, Mohan K Raizada, Univ of Florida, Gainesville, FL

Objective The anti-hypertensive effect of captopril (CAP) is prolonged even when the treatment is discotinued. Our recent findings indicate the beneficial effect of CAP on hypertension-linked gut pathology. Given the roles of brain and gut/microbiota in hypertension, we hypothesized that prolonged anti-hypertensive effect of CAP is through impacts on the brain-gut axis. Accordingly, we tested if CAP induced alterations in the brain and the gut, and if the changes are preserved in the SHR following CAP withdrawal.
Methods Male SHR and normotensive Wistar Kyoto (WKY) rats were treated with CAP (250mg/kg/day) in water for 4 weeks followed by its withdrawal for 16 weeks (weeks 5-20). Systolic BP was measured by tailcuff at weeks 3, 5, and 9. Fecal microbiota was analyzed at week 4 and week 8. Brain neuronal activity was measured by manganese-enhanced MRI (MEMRI) at weeks 4 and 8. Gut pathology was assessed at week 20.
Results Unweighted principal coordinate analysis showed that CAP treatment significantly altered gut microbial composition, characterized by separate clusters between untreated and 4 weeks CAP-treated SHR. The composition change was preserved 4 weeks post-withdrawal of CAP. Sustained improvement in fibrotic area (untreated: 12.98±0.81% vs week 20: 10.23±0.69%, P=0.036), goblet cell number (untreated: 39±3.57 cells/villi vs week 20: 65±5.29 cells/villi, P=0.0006), and villi length (untreated: 271.4±9.85 µm vs week 20: 388.8±12.29 µm, P<0.0001) was observed at week 20 compared with untreated SHR. MEMRI demonstrated sustained decrease in neuronal activity in autonomic brain regions, with the most pronounced decrease in the posterior pituitary area in SHR treated with CAP (week 4) and withdrawal of CAP (week 8), compared with untreated control (untreated: 1.57±0.11 vs week 4: 1.14±0.074 vs week 8: 1.05±0.14, P=0.0162). In contrast, CAP-treated WKY showed little change in BP, gut pathology or neuronal activity, but distinct changes in gut microbiota.
Conclusion Oral CAP persistently lowered BP, altered gut microbiota, improved gut pathology and reduced posterior pituitary neuronal activity in the SHR even post-withdrawal of CAP, indicating persistent antihypertensive effect of CAP may result from altered brain-gut communication.

Disclosures: T. Yang: None. **V.P. Aquino:** None. **G.O. Lobaton:** None. **L. Colon-Perez:** None. **J. Zubcevic:** None. **M. Febo:** None. **E.M. Richards:** None. **C.J. Pepine:** None. **M.K. Raizada:** None.

Funding: No

Funding Component:

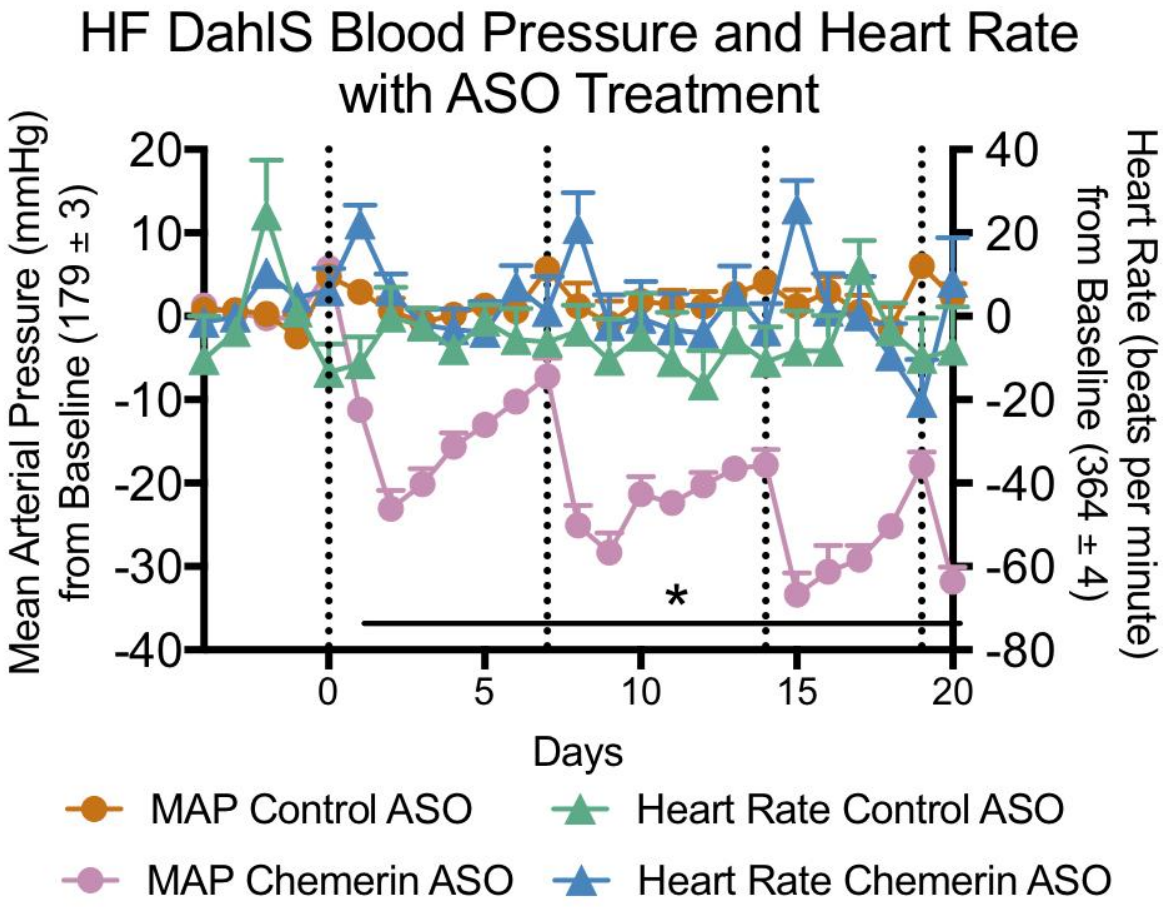
078

Chemerin Antisense Oligonucleotide Lowers Blood Pressure in a Rat Model of Adiposity-Associated Hypertension.

Authors: David Ferland, Emma Darios, Hannah Garver, Michigan State Univ, East Lansing, MI; Steve Yeh, Adam Mullick, Ionis Pharmaceuticals, Carlsbad, CA; Gregory D Fink, Stephanie W Watts, Michigan State Univ, East Lansing, MI

Chemerin is an adipokine, produced in liver and white adipose, whose human plasma levels have been positively associated with hypertension and obesity. In Sprague Dawley rats with normal adiposity and blood pressure (MAP), knockdown of chemerin throughout the body by antisense oligonucleotides (ASO) chronically reduced MAP by ~ 7 mmHg. Thus, we hypothesized that in high-fat-fed animals with hypertension and more visceral white adipose tissue, chemerin ASO would cause a greater reduction of blood pressure. Dahl salt-sensitive rats were fed a high fat diet (60% calories from fat) for 24 weeks after weaning. Radiotelemeters then were implanted for measuring MAP, temperature, heart rate and activity. After recovery and a one-week baseline recording period, animals received subcutaneous injections of either chemerin ASO (25 mg/kg) or scrambled control ASO (25 mg/kg) on days 0, 7, 14, 19. On day 21 the animals were euthanized for tissue and plasma analyses (chemerin PCR and Western). When compared to animals receiving scrambled control ASO, chemerin mRNA expression in animals receiving chemerin ASO was reduced by 99.5 ± 0.1% in liver, 90.6 ± 0.6% in mesenteric perivascular adipose tissue, and 99.3 ± 0.2% in retroperitoneal fat. Chemerin protein was undetectable in plasma from animals receiving chemerin ASO. MAP in rats receiving control ASO did not deviate significantly from baseline (179 ± 3 mmHg), while MAP in rats receiving chemerin ASO dropped by 32 ± 2 mmHg

after four injections without a significant change in heart rate (abstract figure). These data support the involvement of chemerin in adiposity-associated hypertension and suggest a possible new approach to treating hypertension.



Disclosures: **D. Ferland:** None. **E. Darios:** None. **H. Garver:** None. **S. Yeh:** None. **A. Mullick:** None. **G.D. Fink:** None. **S.W. Watts:** None.

Funding: No

Funding Component:

079

Long-Lasting RNAi Therapeutics Targeting Angiotensinogen Induces a Robust and Durable Antihypertensive Effect

Authors: Estrellita Uijl, Erasmus MC, Rotterdam, Netherlands; Katrina M Mirabito Colafella, Monash Univ, Melbourne, Australia; Richard van Veghel, René de Vries, Ingrid M. Garrelds, Ewout J Hoorn, Erasmus MC, Rotterdam, Netherlands; Marko Poglitsch, Attoquant Diagnostics, Vienna, Austria; Jae Kim, Don Foster, Alnylam Pharmaceuticals, Cambridge, MA; A.H. Jan Danser, Erasmus MC, Rotterdam, Netherlands

All angiotensin stems from angiotensinogen (AGT). A single dose of small interfering ribonucleic acids (siRNA) targeting AGT may provide long-lasting blood pressure reductions, as it would abolish angiotensin generation. Here we assessed efficacy of AGT siRNA in spontaneously hypertensive rats (SHRs). SHRs were treated for 4 weeks with vehicle, siRNA (10 mg/kg; s.c. every 2 weeks), valsartan (31 mg/kg/day; oral), captopril (100 mg/kg/day; oral), valsartan+siRNA, or captopril+valsartan (all groups n=8). Mean arterial pressure (MAP) was measured via radiotelemetry. Baseline MAP was 137±2 mmHg. ΔMAP was largest after valsartan+siRNA (-67±3 mmHg; P<0.01 vs. captopril+valsartan), followed by captopril+valsartan, captopril, siRNA and valsartan (-55±4, -24±2, -14±1, and -9±2 mmHg, respectively). Valsartan+siRNA

reduced cardiac hypertrophy the most (P<0.05 vs. captopril+valsartan). After 4 weeks, siRNA lowered AGT by 98.6%, which increased to 99.9% in combination with valsartan. All treatments increased renin, the highest rise occurring after valsartan+siRNA. Yet, only valsartan+siRNA lowered angiotensin II. No treatment altered aldosterone. Plasma K^+ tended to increase in all groups, significance being reached only in the valsartan+siRNA group. Both types of dual blockade attenuated normal growth from the second week of treatment onwards. In conclusion, due to renin upregulation, circulating angiotensin II remained intact even with only 1.4% of AGT left, relative to pretreatment. Consequently, AGT siRNA caused a similar antihypertensive effect as valsartan and captopril. Importantly, when combining siRNA+valsartan, angiotensin II collapsed, and blood pressure decreased synergistically. Given the potential for low dosing frequency suggested by this study, this novel treatment may address medication adherence problems in patients with resistant hypertension and further development is warranted.

Disclosures: E. Uijl: None. **K.M. Mirabito Colafella:** None. **R. van Veghel:** None. **R. de Vries:** None. **I.M. Garrelds:** None. **E.J. Hoorn:** None. **M. Poglitsch:** None. **J. Kim:** A. Employment; Modest; Alnylam Pharmaceuticals. **D. Foster:** A. Employment; Modest; Alnylam Pharmaceuticals. **A.H.J. Danser:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Alnylam Pharmaceuticals.

Funding: No

Funding Component:

080

Characterization of Blood Pressure Response to Spironolactone in Patients With Resistant Hypertension using Electronic Medical Records

Authors: Megan M Shuey, Hui Nian, Chang Yu, James M. Luther, Nancy J. Brown, Vanderbilt Univ Medical Ctr, Nashville, TN

Spironolactone is a recommended add-on therapy for BP control in patients with resistant hypertension (RH). We hypothesized that we could use electronic medical records (EMR) to assess the BP response to the addition of spironolactone in patients with apparent RH who were on a stable anti-hypertensive regimen of at least three antihypertensive medications including a thiazide or dihydropyridine CCB.

Patients with RH were identified using a previously published algorithm. We developed an algorithm to identify patients initiated on spironolactone during a period of otherwise stable medication use from up to six months before and after the start of spironolactone to evaluate BP response.

We identified 977 RH patients (751 white and 226 black) prescribed spironolactone during a stable medication window. The median dose of spironolactone prescribed was 25 mg and 724 patients were prescribed 25 mg (74.1%). The mean decrease in SBP following spironolactone was 8.5$\pm$18.1 mmHg and the mean decrease in DBP was 4.0$\pm$9.9 mmHg, consistent with data from clinical trials.

Using a mean decrease in SBP of 5 mmHg or in DBP of 2 mmHg to define “responders,” we found that 29% (283 of 977) of patients were nonresponders. Responders had significantly higher baseline BPs (p<0.001), were older (p=0.04), and had larger decreases in the eGFR (p<0.001) and serum Na^+ (p<0.004) and larger increases in creatinine (p<0.001) and K^+ (p<0.001) after starting spironolactone than nonresponders. In blacks, glucose increased following spironolactone in responders compared to non-responders (2.27 $\pm$40.77 vs -15.47$\pm$62.94 mg/dL, p=0.03); this relationship was not observed in whites.

When response was evaluated as a continuous variable, the decrease in SBP and DBP correlated with the decrease in serum Na^+ (p=0.04) and with the increases in serum K^+ (p=0.01) and creatinine (p<0.001). In blacks, there was a significant correlation between decrease in SBP and increase in glucose (p=0.04).

We have developed an algorithm to assess the BP response to spironolactone in patients with RH using the EMR.

Electrolyte changes associated with the BP response to spironolactone are consistent with its mechanism of action to block the mineralocorticoid receptor and decrease activity of the epithelial Na^+ channel.

Disclosures: M.M. Shuey: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; F31DK108444. **H. Nian:** None. **C. Yu:** None. **J.M. Luther:** None. **N.J. Brown:** None.

Funding: No

Funding Component:

081

Renal Sensory Nerves Increase Blood Pressure and Sympathetic Nerve Activity in 2-Kidney 1-Clip Hypertensive Mice

Authors: Jason Ong, Alan F Sved, Roderick J Tan, Brittney M Rush, Marcelo D Carattino, Sean D Stocker, Univ of Pittsburgh, Pittsburgh, PA

Renal denervation lowers arterial blood pressure (ABP) in multiple clinical trials and some experimental models of hypertension. These antihypertensive effects have been attributed to the removal of renal afferent nerves. The purpose of the present study was to determine whether renal sensory nerves contributed to the 2-Kidney-1-Clip (2K1C) model of hypertension. 2K1C hypertension was produced in male C57Bl6 mice (12-14 weeks, Jackson Laboratories) by placement of a 0.5mm length of PTFE tubing (ID: 0.008" x OD: 0.014") around the left renal artery. 2K1C mice (n=6) displayed an elevated ABP measured via telemetry (Day 0: 96±4mmHg vs Day 14: 115±3mmHg, P<0.05). Ganglionic blockade with hexamethonium (30mg/kg, ip) produced a greater fall in mean ABP at Day 14 vs Day 0 (Day 0: -38±4mmHg vs Day 14: -51±4mmHg, P<0.05). Ipsilateral vs contralateral kidneys of 2K1C mice had lower mass (0.072±0.01g vs 0.163±0.02g, respectively; P<0.05) and higher mRNA levels of several pro-inflammatory cytokines (IL1B, IL2, IL10, TNFa; P<0.05). Both total renal denervation (10% phenol) or selective denervation of renal afferent nerves (periaxonal application of 33mM capsaicin) at time of clipping resulted in a lower ABP than 2K1C mice at Day 14 (2K1C: 115±3mmHg, phenol: 104±2mmHg, capsaicin: 105±3mmHg; P<0.05). Direct recording of renal afferent nerve activity showed significantly greater discharge in 2K1C versus control mice (control: 2.2±1.3Hz vs 2K1C: 61±12Hz, n=3/group; P<0.05). Furthermore, electrical stimulation of renal afferent nerves in control mice produced a frequency-dependent increase in ABP (5Hz: 3±1mmHg, 10Hz: 7±1mmHg, 20Hz: 12±2mmHg, n=4/group; P<0.05). These responses were eliminated after ganglionic blockade with 5mg/kg chlorisondamine (5Hz: 1±1mmHg, 10Hz: 1±1mmHg, 20Hz: 1±1mmHg; P<0.05). Stimulus-triggered averaging of SNA during stimulation of renal afferent nerves (1 Hz, 200uA) revealed significant (P<0.05, n=4/group) increases in splanchnic (221±28%), renal (195+15%), and lumbar (234±32%) SNA. Interestingly, the latency to the peak SNA (142±10ms) suggests supraspinal pathways mediate the sympathoexcitatory response. These findings suggest 2K1C hypertension depends on renal sensory nerves and elevated SNA via supraspinal pathways.

Disclosures: J. Ong: None. **A.F. Sved:** None. **R.J. Tan:** None. **B.M. Rush:** None. **M.D. Carattino:** None. **S.D. Stocker:** None.

Funding: No

Funding Component:

082

Intrarenal Nacl Boli Cause Longlasting Inhibition of Renal Sympathetic Nerve Activity (RSNA)

Authors: Martin Hindermann, Medical Dept 4 (FAU), Erlangen, Germany; Kristina Rodionova, Medical Dept 4 (FAU &PMU), Erlangen and Nuremberg, Germany; Amelie Dietz, Medical Dept 4 (FAU), Erlangen, Germany; Tilmann Ditting,

Christian Ott, Medical Dept 4 (FAU &PMU), Erlangen and Nuremberg, Germany; Roland Schmieder, Kerstin Amann, Medical Dept 4 (FAU), Erlangen, Germany; **Roland Veelken**, Medical Dept 4 (FAU &PMU), Erlangen and Nuremberg, Germany

Afferent renal nerve fibers from the kidney likely counterregulate salt sensitive blood pressure increases by decreasing renal sympathetic nerve activity. We recently reported on a long-lasting tonic sympatho-inhibition due to intrarenal afferent renal nerve stimulation eliciting a TRPV1 dependent neuro-humoral pathway. We wanted to test the hypothesis that sodium influences this afferent sympatho-depressory mechanism. Groups of anesthetized SD rats (n=8) were equipped with femoral catheters (blood pressure (BP) & heart rate (HR) recording, drug application), a renal arterial catheter for intrarenal administration (IRA) of high salt (10 % NaCl, 10 µl) or Capsaicin (CAP 3.3, 6.6, 10, 33*10-7 M, 10 µl) and a bipolar electrode for RSNA recordings; eventually an intravenous (iv) bolus of the NK1-receptor blocker RP67580 (10*10-3M, 15 µl) was given. Cultured dorsal root ganglion neurons (Th11-L2) of rats with renal afferents were investigated in current clamp mode to assess action potential generation or in voltage clamp mode to investigate inward currents during 10 sec superperfusion to 4.5 % NaCl or equiosmotic mannitol. solution Results are given in mean±SEM. IRA high salt and IRA CAP decreased RSNA from baseline 4.1±0.6 µV*sec to 2.2±0.8 µV*sec (10% NaCl, $p<0.05$) and 3.9±0.5 µV*sec to 0.9±0.2 µV*sec (CAP, $p<0.01$). Suppressed RSNA in high salt groups and CAP could be unmasked by systemic (i.v.) administration of the NK1-blocker (2.7±1.8 µV*sec to 5.8±2.2 µV*sec; $p<0.05$ (10% NaCl); 1.0±0.2 µV*sec to 6.1±1.5 µV*sec; $p<0.01$ (CAP)). Cultured renal neurons exhibited production of action potentials (3.5±0.8*, from baseline, $p<0.05$) and increased sustained inward currents from baseline during exposure to NaCl 4.5 % (-10708.8±3546.5 pA*, from baseline, $p<0.05$). No responses to equiosmolar mannitol. Increased intrarenal sodium concentrations might induce long-lasting sympatho-depression via a neuro-humoral TRPV1 dependent and tachykinin mediated afferent nerve pathway from the kidney. Impairment of this sympatho-depressory mechanism could be involved in salt sensitive hypertension.

Disclosures: **M. Hindermann:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Universtiy of Erlangen. **K. Rodionova:** None. **A. Dietz:** None. **T. Ditting:** None. **C. Ott:** None. **R. Schmieder:** None. **K. Amann:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Deutsche Forshcungsgemeinschaft, University of Erlangen. **R. Veelken:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Deutsche Forschungsgemeinschaft, University of Erlangen.

Funding: No

Funding Component:

083

Role of Alpha2a-Adrenoceptors in the Development of Hypertensive Nephropathy

Authors: Johannes Stegbauer, Masudur Rahman, Mina Yakoub, Henning Hoch, Oliver Vonend, Lars C Rump, Lydia Hering, Heinrich-Heine-Univ, Düsseldorf, Germany

Objective and methods: Hypertensive nephropathy is associated by increased renal sympathetic nerve activity. Alpha2A-adrenoceptors (alpha2A-AR) regulate sympathetic tone by controlling norepinephrine (NE) release by a negative feedback mechanism. Recently, we have shown that deletion of alpha2A-adrenoceptors increases renal sympathetic neurotransmission. To investigate whether deletion of alpha2A-AR play a role in the development of hypertensive nephropathy, we induced hypertension by infusing angiotensin (Ang)II (1000ng/kg/min) for 4 weeks in uninephrectomized wild-type (WT) and alpha2A-AR knockout (alpha2A-AR-/-) mice on an FVB background. Blood pressure was assessed by radiotelemetry. **Results:** NE release stimulated by renal nerve stimulation 5Hz was significantly higher in untreated kidneys of alpha2A-AR-/- compared controls. During AngII infusion (1000ng/kg/min) urinary NE

excretion was significantly higher in alpha2A-AR-/- than control mice (4.3±0.6 vs. 2.1±0.4mg/dl creatinine, *P*<0.05). In line with an increased sympathetic NE release, the severity of hypertension caused by AngII infusion was significantly higher in alpha2A-AR-/- mice compared to control mice (176±4 vs. 163±3mmHg, *P*<0.01). Moreover, albuminuria (140±24 vs. 66±19mg/mg creatinine, *P*<0.05) as well as kidney injury assessed by glomerular injury score and renal fibrosis were accelerated and kidney function measured by cystatin C (1082±66 vs. 906±52ng/ml, P<0.05) was significantly decreased in alpha2A-AR-/- compared to WT mice. To test whether reduced renal sympathetic activity reversed this effect, renal nerve denervation (RDN) was performed in alph2A-AR-/- mice. RDN reduced renal NE content by 70%. In alpha2A-AR-/- mice, RDN reduced the severity of AngII induced hypertension 138±9 vs. 169± 11mmHg, P<0.05) and albuminuria compared to non-denervated alpha2A-AR-/- mice. **Conclusion:** Deletion of alpha2A-ARs exaggerates hypertensive nephropathy by increasing sympathetic NE activity. Reduction of NE release by RDN reduced blood pressure and albuminuria in alpha2A-AR-/- mice suggesting an important role of renal sympathetic tone in the pathogenesis of hypertensive nephropathy.

Disclosures: J. Stegbauer: None. **M. Rahman:** None. **M. Yakoub:** None. **H. Hoch:** None. **O. Vonend:** None. **L.C. Rump:** None. **L. Hering:** None.

Funding: No

Funding Component:

084

Selective Renal Afferent Denervation Enhances Diuretic and Natriuretic Response to Glucagon-like Peptide-1 in Rats With Heart Failure

Authors: Kenichi Katsurada, Neeru M Sharma, UNMC, Omaha, NE; Hong Zheng, Xuefei Liu, USD, Vermillion, SD; Kaushik Patel, UNMC, Omaha, NE

Glucagon-like peptide-1 (GLP-1), incretin hormone, has diuretic and natriuretic effects and its receptor agonist reduces cardiovascular events. The present study was designed to determine whether GLP-1-induced diuresis is regulated by renal nerve in the rats with a coronary ligation model of heart failure (HF). Our immunohistochemistry and immunoblotting studies demonstrate that GLP-1 receptors were expressed in the renal pelvic wall, densely innervated by afferent renal nerves (ARN). Basal ARN activity (ARNA) was increased in HF compared to normal rats (1.3 ± 0.2* vs. 0.6 ± 0.1 μV·s). GLP-1 (3μM) infused into left renal pelvis increased ipsilateral ARNA in both normal and HF rats. The change in basal ARNA and the maximum ARNA represented as percent activation by injection of capsaicin (transient receptor potential V1 receptor agonist, 100μM) were higher in HF than in normal rats (ΔARNA 227 ± 27%* vs. 131 ± 26%, and ARNAmax 64.2 ± 3.4%* vs. 45.1 ± 6.6%). Intravenous infusion of GLP-1 (1μg/kg/min) for 30 min increased renal urine flow and sodium excretion in normal rats and these effects were blunted in HF (Urine flow 1577 ± 122* vs. 1981 ± 101 μl/gkw, Sodium excretion 221 ± 19* vs. 284 ± 15 μEq/gkw). GLP-1-induced diuresis and natriuresis were enhanced by total renal denervation (T-RDN) with acute surgical cutting of the renal nerves in both normal and HF rats. The degree of diuretic and natriuretic responses to GLP-1 were greater in HF than in normal rats (increased % rate: Urine flow 167%* vs. 135%, Sodium excretion 169%* vs. 150%). Selective afferent renal denervation (A-RDN) was performed by bilateral perivascular application of capsaicin (33mM) on the renal nerves. Ten days after A-RDN, there was an enhanced diuretic and natriuretic response to GLP-1 in both normal and HF rats. These effects were greater in HF than in normal rats (increased % rate: Urine flow 187%* vs. 130%, Sodium excretion 147%* vs. 138%). Urine flow and sodium excretion responses to GLP-1 were not significantly different between T-RDN and A-RDN in both groups. Taken together our results indicate that the diuretic and natriuretic effects of GLP-1 are partly governed via ARN activation. GLP-1-induced diuresis and natriuresis enhanced by RDN have potential therapeutic implication for HF.

Disclosures: K. Katsurada: None. **N.M. Sharma:** None. **H. Zheng:** None. **X. Liu:** None. **K.P. Patel:** None.

Funding: No

Funding Component:

085

Renal and Neural Mechanisms of Age-Related Hypertension

Authors: Alissa A. Frame, Richard D. Wainford, Boston Univ, Boston, MA

Aim Hypertension (HTN) is positively correlated with age and sympathetic tone in humans. We hypothesize that sympathetically driven sodium chloride cotransporter (NCC)-mediated sodium retention contributes to age-related HTN.
Methods Three, 8 and 16 month old male SD rats on a normal salt (NS; 0.6% NaCl) diet underwent an acute IV volume expansion (VE; 5% body weight – mechanoreceptor stimulus) or 1M NaCl infusion (20µL/min, 2hr – chemoreceptor stimulus) and mean arterial pressure (MAP), natriuresis (UNaV) and paraventricular nucleus (PVN) neuronal activation (c-Fos expression) were assessed. In separate groups of rats fed a 21 day NS or high salt (HS; 4% NaCl) diet, 1) ex vivo afferent renal nerve (ARN) activity (norepinephrine-evoked substance P release) or 2) in vivo MAP, NCC activity (ΔUNaV to IV hydrochlorothiazide, 2mg/kg) and sympathetic tone were assessed. N=4-6/gp.
Results The natriuretic and PVN sympathoinhibitory parvocellular neuronal responses to VE, but not 1M NaCl infusion, are blunted in aged rats. Aged rats exhibit reduced ARN activity on a NS diet and fail to increase ARN activity on a HS diet. Aged rats develop salt sensitive HTN accompanied by elevated NCC activity and sympathetic tone on a NS diet and impaired suppression of NCC activity and sympathetic tone on a HS diet.
Conclusion Aging is associated with a selective impairment in the mechanosensitive ARN and salt sensitive HTN. We speculate that an age-related decrease in the mechanosensitive ARN sympathoinhibitory reno-renal reflex promotes sympathoexcitation, perhaps via reduced activity of PVN sympathoinhibitory parvocellular neurons, driving NCC-mediated sodium retention and salt sensitive HTN.

EXPERIMENT	ENDPOINT	PARAMETER	3 month old		8 month old		16 month old	
			NS	HS	NS	HS	NS	HS
Intravenous volume expansion	Natriuretic response	% of total sodium load excreted	78±6	N.D.	60±7*	N.D.	22±9*τ	N.D.
	Diuretic response	% of total water load excreted	96±7	N.D.	66±5*	N.D.	33±7*τ	N.D.
	PVN medial parvocellular neuronal activation	c-Fos+ cells (count)	59±4	N.D.	42±7*	N.D.	13±5*τ	N.D.
Intravenous 1M NaCl infusion	Natriuretic response	Peak UNaV (µeq/min)	17±2	N.D.	19±2	N.D.	16±2	N.D.
	Diuretic response	Peak rate (µL/min)	62±10	N.D.	68±6	N.D.	56±8	N.D.
Ex vivo renal pelvis assay	ARN activity	1250pM NE-evoked SP release (pg/min)	14±2	23±4#	8±2*	6±3#	N.D.	N.D.
21 day experimental diet: NS (0.6% NaCl) vs HS (4% NaCl)	Salt sensitivity of blood pressure	MAP (mmHg)	124±2	126±3	135±4*	143±5*	149±3*τ	169±1*τ#
	NCC activity	ΔUNaV to hydrochlorothiazide (µeq/min)	9±1	7±1#	18±2*	16±1*	15±5*	16±6*
	Global sympathetic tone	Plasma NE (nmol/L)	44±4	28±4#	55±3*	42±4*#	54±4*	41±4*#
	Renal sympathetic tone	Renal NE (pg/mg)	612±36	368±32#	835±48*	722±44*#	974±13*τ	1019±134*τ
	Vascular sympathetic tone	ΔMAP to hexamethonium (mmHg)	-33±4	-24±3#	-64±5*	-65±6*	-60±3*	-67±7*

Table 1 *P<0.05 vs respective 3 month old group, τP<0.05 vs respective 8 month old group, #P<0.05 vs respective NS group. NS = normal salt (0.6% NaCl), HS = high salt (4% NaCl), N.D. = not determined, PVN = paraventricular nucleus, ARN = afferent renal nerve, NE = norepinephrine, MAP = mean arterial pressure, UNaV = urinary sodium excretion.

Disclosures: A.A. Frame: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; F31DK116501. **R.D. Wainford:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA 17GRNT33670023, NIH K02HL112718, NIH R01HL107330, NIH R01HL141406.

Funding: Yes

Funding Component: Founders Affiliate (Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Rhode Island, Vermont)

086

Neuroanatomical Basis of PVN MC4R-Expressing Neurons for Sympathetic Cardiovascular Control

Authors: Uday Singh, Brandon A. Toth, Kevin C. Davis, Kenji Saito, Donald A. Morgan, Kamal Rahmouni, Huxing Cui, The Univ of Iowa, Iowa City, IA

It is well established that the central melanocortin system is critical for autonomic functions and energy homeostasis mainly via signaling at melanocortin-4 receptor (MC4R). Importantly, while obesity is commonly associated with elevated sympathetic tone and blood pressure, severely obese humans and rodents due to genetic MC4R deficiency exhibit normal to low sympathetic tone and blood pressure, suggesting a significant role of MC4R pathway in mediating obesity-associated sympathoexcitation and hypertension. MC4R is widely expressed in the brain including hypothalamic paraventricular nucleus (PVN) which regulates feeding and sympathetic traffic. However, the neuroanatomical basis of PVN MC4R neurons for sympathetic regulation is unclear. The goal of current study is to map the PVN MC4R neural circuits affecting sympathetic tone in mice. To this end, we injected Cre-dependent AAV driving eYFP-fused channel rhodopsin-2 expression into the PVN of MC4R-t2a-Cre knock-in mice, which allow targeted anterograde tract-tracing of PVN MC4R neurons throughout the brain. In addition to known brain region for feeding behavior (i.e. parabrachial nucleus), we found broad innervations of PVN MC4R neurons to various brain regions important for autonomic-cardiovascular control, including, but not limited to, nucleus of solitary tractus, dorsomotor nucleus of vagus, and ventrolateral medulla. Considerable innervation was also evident in spinal cord, which is further confirmed by Fluoro-gold (FG)-mediated retrograde tracing in the spinal cord (thoracic T6-10) of MC4R-GFP transgenic mouse. Double immunofluorescence labeling of GFP and FG revealed that ~50% (58 out of 116) MC4R neurons in the posterior parvocellular subdivision of PVN project to thoracic spinal cord. Furthermore, microinjection of synthetic MC4R agonist (MTII) into the PVN evokes ~42% increase (from baseline) in renal sympathetic nerve activity in anesthetized mice. These results provide important insights into understanding the divergent neural circuits by which PVN MC4R signaling differentially regulates metabolic and cardiovascular functions. Functional dissection of these diverging neural pathways using optogenetic/chemogenetic approaches is ongoing.

Disclosures: U. Singh: None. **B. Toth:** None. **K. Davis:** None. **K. Saito:** None. **D. Morgan:** None. **K. Rahmouni:** None. **H. Cui:** None.

Funding: No

Funding Component:

087

NPFF Receptors NPFF-R1 and NPFF-R2 in the Kidney: A Target for the Negative Control of Sodium Transport and Blood Pressure

Authors: Laureano D Asico, Van Anthony M Villar, Michael Bishop, Gerald Gomes, Momina Mazhar, Pedro A. Jose, George Washington Univ, Washington, DC

The kidney is critical in the overall regulation of fluid, electrolyte balance and blood pressure (BP). The renal dopaminergic system inhibits sodium transport in almost all nephron segments and is responsible for ≥50% of renal sodium excretion under moderate sodium excess conditions and is considered anti-hypertensive. Neuropeptide FF (NPFF), a morphine-modulating peptide, regulates food consumption and cardiovascular function through its interaction with two receptors, NPFFR1 and NPFFR2. NPFFR2 is anti-hypertensive under "normal" salt intake but becomes pro-hypertensive when salt intake is "high". The ultimate phenotype depends upon protein/protein interaction. Hence, we tested NPFFR2 and dopamine receptor interaction *in vitro* using renal proximal tubule cells (RPTCs) and blood pressure *in vivo* in C57Bl/6J mice. NPFF is synthesized in RPT where NPFFR2 is expressed and co-localize with D_1R. In RPTCs (n= 3-4/group), the D_1R/D_5R agonist fenoldopam (1 µM/30 min), increased cAMP production (4.23±0.56 pmol/mg/min *vs*. 2.54±0.19, vehicle; P<0.05) but was abrogated by co-treatment with NPFF (1 µM/30 (2.36±0.29 pmol/mg/min). Low NPFF (10^{-10} and 10^{-9} M) concentrations increased while higher concentrations had no effect (10^{-8} and 10^{-7} M), and slightly decreased (10^{-6} M) cAMP production in RPTCs. Fenoldopam (1 µM/30 min) added to the basolateral side of polarized RPTCs grown in Transwells, increased intracellular Na^+ (117±4% versus the vehicle-treated control), indicating inhibition of basolateral Na^+ transport. This effect was prevented by co-treatment with NPFF (98±5%). Basolateral NPFF treatment alone decreased the intracellular Na^+ (-86.10±4.6%), indicating increased basolateral Na^+ transport and that renal NPFF may be a negative regulator of dopamine. In C57Bl/6J mice on 0.9% NaCl diet, renal-selective silencing of *Npffr2* increased BP (115±3 vs 102±1 mmHg; P<0.05; n=3-5/group). By contrast, on 4% NaCl diet, renal-selective silencing of *Npffr2* decreased BP (98±4 vs 113±3 mmHg; P<0.05;
n=3-5/group), indicating that *Npffr2* is antihypertensive on normal NaCl diet but pro-hypertensive on high NaCl diet. These contrasting effects may be due to D_1R/D_5R negative regulation of *Npffr2* on normal salt diet which is lost when NaCl intake is increased.

Disclosures: L.D. Asico: None. **V.M. Villar:** None. **M. Bishop:** None. **G. Gomes:** None. **M. Mazhar:** None. **P. Jose:** None.

Funding: No

Funding Component:

088

The Immune Mechanisms of Salt-Sensitivity

Authors: Natalia Ruggeri Barbaro, Justin Van Beusecum, Roxana Loperena, Aseel Alsouqi, Mingfang Ao, Fernando Elijovich, Cheryl L. Laffer, Alp Ikizler, Vanderbilt Univ, Nashville, TN; Alicia A. McDonough, Keck Sch of Med of USC, Nashville, TN; Heitor Moreno Jr, Unicamp, Campinas, Brazil; David G. Harrison, Annet Kirabo, Vanderbilt Univ, Nashville, TN

Salt-sensitivity is present in 50% of all hypertensive individuals. Prior studies have focused on the roles of kidney, vasculature and sympathetic activity in salt-sensitivity but the contribution of immune cells is poorly understood. We recently found that in murine dendritic cells amiloride sensitive channels sense salt and trigger NADPH oxidase-dependent formation of isolevuglandin-(IsolG)-adducts. We tested the hypothesis that human monocytes exhibit salt-sensitivity leading to activation via IsoLG-adduct formation and this is associated with cardiovascular risk factors. In a cohort of 18 subjects, we found that the sodium intake, measured by 24 hours urine excretion (UNa) was positively correlated with plasma levels of IsolGs. We also measured accumulation of interstitial sodium in 70 subjects by Magnetic Sodium Resonance and evaluated their circulating monocytes by flow cytometry. Subjects with high skin sodium had higher levels of IsoLGs in their monocytes (24±6 vs 38±6 %, p<0.05) and higher expression of CD83, an activation and dendritic cell marker (0.04 ± 0.009 vs 0.12± 0.04%, *p*=0.04). To investigate the ability of monocytes to respond to salt, we

cultured monocytes from 17 subjects in high salt environment (HS:190 mM NaCl) or normal media (NS:150mM NaCl) for 48 hours. In culture, 47% of the subjects respond to salt, denoted by an increase of at least 20% in IsoLG formation (NS: 1327±240 *vs.* HS: 2217±653, p=0.009) as well as increased expression of the activation markers CD83 and CD86. The subjects' cardiovascular risk factors including pulse pressure, BMI, glucose and total cholesterol positively correlated with the amount of IsolGs produced (ΔHS-NS) in response to salt (r=0.51 p<0.05, r=0.66 p=0.005, r=0.55 p<0.05, p=0.72 p=0.003, respectively). Interestingly, 5 pM of Ouabain, a Na-K-ATPase blocker, increased intracellular sodium and expression of CD86 (NS: 98±11, HS: 203 ±16 vs, NS+ Ouabain: 476 ± 58 MFI, p=0.001) and CD83 (NS: 778±90, HS: 1529 ± 94 vs, NS+ Ouabain: 1649 ± 209 MFI, p=0.003). We suggest that in addition to the kidney and vasculature, human monocytes and monocyte derived cells exhibit salt sensitivity, and that this is conveyed by cardiovascular risk factors and activity of the Na-K-ATPase.

Disclosures: N. Ruggeri Barbaro: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 16POST29090001. **J. Van Beusecum:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 5T32HL069765-13. **R. Loperena:** None. **A. Alsouqi:** None. **M. Ao:** None. **F. Elijovich:** None. **C.L. Laffer:** None. **A. Ikizler:** None. **A.A. McDonough:** None. **H. Moreno Jr:** None. **D.G. Harrison:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; SFRN20420, R01HL125865, R01HL039006. **A. Kirabo:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; K01HL130497.

Funding: Yes

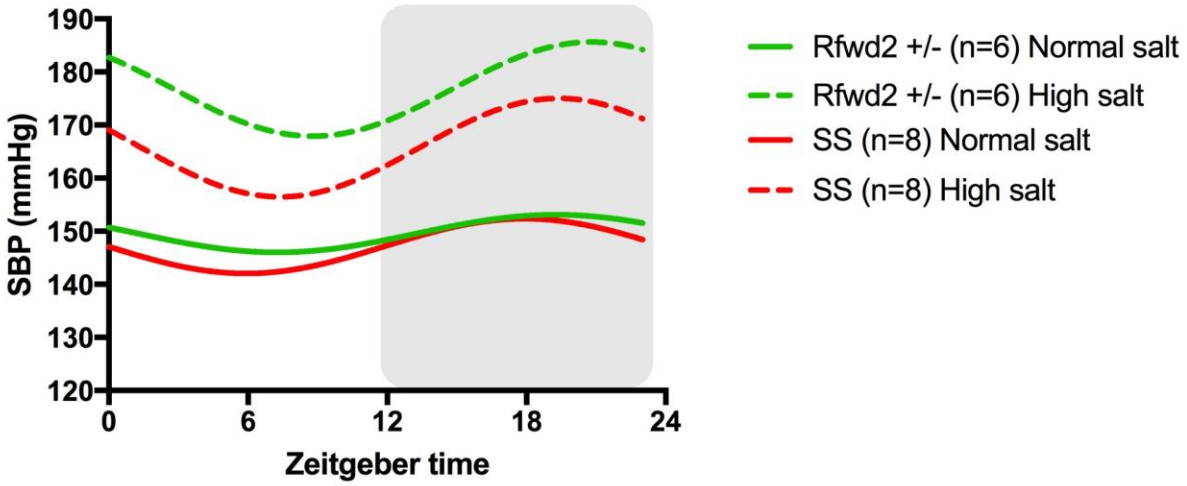
Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

089

Deletion of Circadian Clock Associated Gene, *Rfwd2*, Alters Rhythm in Blood Pressure Timing Without Changes in Salt-Sensitivity

Authors: Daian Chen, John N Booth III, Bryan K Becker, Jackson C Colson, Chunhua Jin, Univ Alabama Birmingham, Birmingham, AL; Allen W Cowley Jr, Aron Geurts, Medical Coll of Wisconsin, Milwaukee, WI; Paul Muntner, Martin E Young, David M Pollock, Jennifer S Pollock, Univ Alabama Birmingham, Birmingham, AL

Healthy individuals display a diurnal blood pressure (BP) rhythm and salt-sensitivity increases the susceptibility for dysfunctional BP rhythms. Previously, bayesian modeling found that *Rfwd2* gene was associated with circadian clock gene pathways in the Dahl Salt-sensitive rats (SS). SS rats lacking *Rfwd2* were generated to test the hypothesis that *Rfwd2* alters the circadian BP rhythm and salt-sensitivity in SS rats. *Rfwd2* total knockout rats on the SS background are embryonic lethal, thus experiments utilized male *Rfwd2* heterozygous SS (*Rfwd2*$^{+/-}$; n=6) and SS rats (n=8). All rats were maintained on Teklad (NIH-31) normal salt diet (NS; 0.4% NaCl) and then fed high salt diet (HS; 4% NaCl). BP was recorded via telemetry and systolic BP analyzed on days 4-7 of diet. On NS, the amplitude (difference between peak and mean) and mesor (rhythm-adjusted mean) were similar between genotypes. However, HS diet increased the amplitude (SS: 5.2 ± 0.4 to 9.3 ± 0.5, P<0.0001; Rfwd2$^{+/-}$: 3.7 ± 0.5 to 9.0 ± 0.5, P<0.0001) and mesor (SS: 147 ± 1.2 mmHg to 166 ± 1.7 mmHg, P<0.0001; Rfwd2$^{+/-}$: 150 ± 1.6 mmHg to 177 ± 3.1 mmHg, P<0.0001) in both genotypes. On HS, Rfwd2$^{+/-}$rats compared to SS had greater mesor (178 ± 3.1 mmHg vs. 166 ± 1.7 mmHg, P=0.003, respectively) and similar amplitude. Rfwd2$^{+/-}$ had significant 1.4hr delay in trough (lowest value) SBP timing compared to SS within both salt diets (NS: P=0.005; HS: P=0.008). In addition, HS caused a delay in trough SBP timing in both genotypes. These results (figure) show that *Rfwd2* has a role in trough BP timing, and the lack of this gene results in an exaggerated mesor on HS diet and a 1.4hr delay on NS and HS diets, which could potentially be coupled with renal injury.



Disclosures: **D. Chen:** None. **J.N. Booth:** None. **B.K. Becker:** None. **J.C. Colson:** None. **C. Jin:** None. **A.W. Cowley:** None. **A. Geurts:** None. **P. Muntner:** None. **M.E. Young:** None. **D.M. Pollock:** None. **J.S. Pollock:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

090

Does Dietary Sodium Contribute to the Discordance in Blood Pressures among Monozygotic Twins?

Authors: Vidya Kidambi, Medical Coll of Wisconsin, Milwaukee, WI; Mary F. Donohue, Michigan State Univ, East Lansing, MI; Yannick Munyara, Gerard Coly, Purushottam Laud, Medical Coll of Wisconsin, Milwaukee, WI; Tao Wang, Medical Coll of Wisconsin, Milwaukee, TN; Rakesh Reddy, Michigan State Univ, East Lansing, WI; S A. Burts, Kelly E. Klump, Michigan State Univ, East Lansing, MI; Theodore A. Kotchen, Allen W Cowley, Medical Coll of Wisconsin, Milwaukee, WI; Supratik Rayamajhi, Michigan State Univ, East Lansing, MI; David L Mattson, Medical Coll of Wisconsin, Milwaukee, WI; Ralph E. Watson, Michigan State Univ, East Lansing, MI; Mingyu Liang, Medical Coll of Wisconsin, Milwaukee, WI

Background: Dietary sodium (Na) and central adiposity are considered important risk factors for hypertension (HTN). Blood pressure (BP) levels are discordant among 40-50% of monozygotic (MZ) twin pairs. One prior study of MZ twins using questionnaires reported that hypertensive (HT) MZ twins differed in their intake of meat and milk. However, none of the previous studies have evaluated the impact of Na intake on BP in MZ twins. Methods: Eighty-eight pairs of MZ twins were enrolled from Milwaukee, WI and Michigan State University Twin Registry, East Lansing, MI (age- 44 ± 9 years, 63% women, and 96% Caucasian). BPs (measured in triplicate and averaged) and anthropometrics were measured. Na intake was calculated using Block Sodium Screener. Twin pairs were considered concordant or discordant based on systolic (SBP) or diastolic BP (DBP) difference of 10 mm Hg between co-twins, off BP medications. Results: Thirty-seven twin pairs were discordant in their BP levels. Mean (± SD, mm Hg) SBP/DBPs among concordant and discordant twins were 120 ± 12 / 77 ± 9 and 131 ± 18 / 81 ± 10 respectively. Mean differences in SBP/DBP among concordant and discordant twins were 5 ± 7 / 4 ± 4 and 14 ± 8 / 10 ± 6 respectively. Discordant twins as a group had higher waist circumference (WC) compared to concordant twins ($p<0.05$), but with no differences in BMI or Na intake. However, among the discordant twin pairs, the co-twin with higher BPs (138 ± 18 / 85 ± 9) had higher dietary salt intakes (3900 ± 1437 vs. 3261 ± 1058 mg/d) and higher WC (105 ± 15 vs. 98 ± 18 cm) ($p=0.02$) compared to their co-twin with lower BPs (121 ± 16 / 77 ± 9). The average Na intake among the concordant twins was 3443 ± 1189 mg/day. Differences in Na intake among discordant co-twins remained statistically significant after adjustment for age, gender, BMI, WC, and waist-to-hip ratio. Conclusions: Higher Na intake is associated with higher BPs among MZ twins with discordant BPs.

Differences in Na intakes were significant independent of central adiposity indicating significant Na sensitivity in discordant MZ twin pairs. The effect of ~500mg less Na intake/day in resulting in lower BPs in genetically identical individuals, suggests that modest decreases in Na-intake in Na-sensitive individuals may be sufficient to see the desired effect on BP levels.

Disclosures: V. Kidambi: None. **M.F. Donohue:** None. **Y. Munyara:** None. **G. Coly:** None. **P. Laud:** None. **T. Wang:** None. **R. Reddy:** None. **S.A. Burts:** None. **K.E. Klump:** None. **T.A. Kotchen:** None. **A.W. Cowley:** None. **S. Rayamajhi:** None. **D.L. Mattson:** None. **R.E. Watson:** None. **M. Liang:** None.

Funding: Yes

Funding Component: National Center

091

Knock Out of *Pappa2* Enhances Blood Pressure Salt-Sensitivity and Renal Injury

Authors: Allen Cowley, Aron Geurts, Louise Evans, Theresa Kurth, Chun Yang, Nadezda Zheleznova, Clayton Wollner, Vikash Kumar, Medical Coll Wisconsin, Milwaukee, WI

Pappa2 (pregnancy associated plasma protein A2) is a protease of insulin-like growth factor binding protein 5 (IGFBP5) which has been primarily studied in pregnancy and post-natal growth. Our recent studies using contiguous congenic strains indicate that *Pappa2* is linked to salt-sensitive hypertension in Dahl salt-sensitive (SS) rats. The role of *Pappa2* in cardiovascular and renal function has not been studied previously. We therefore mutated *Pappa2* in a salt-resistant subcongenic strain 26-P using the CRISPR-Cas9 method. Mutation of the *Pappa2* gene resulted in a 60-bp deletion and the absence of the wild type protein in the urine was verified by Western blot analysis. We hypothesized that deletion of *Pappa2* in the salt-insensitive strain would return or enhance BP salt-sensitivity as found in SS rats. Both parental rats and weaned offspring were maintained on a 0.4% NaCl diet until 9 weeks of age, then surgically instrumented with radiotelemeters for 24 hr BP recordings obtained while fed 0.4% NaCl and during 21 days of a 4% NaCl diet. Results: Mean 24 hr arterial pressure at 21 days of a 4.0% salt diet was significantly higher ($P<0.05$) in male *Pappa2-/-* (164 $\pm$ 5 mmHg; n=11) compared to *Pappa2+/+* WT (137 $\pm$ 4 mmHg; n=9) rats. Body weight (9 wks) of *Pappa2-/-* was less ($P<0.05$) than weight of *Pappa2+/+* WT rats (217 $\pm$ 7 g vs 281 $\pm$ 11 g). Although less than expected compared to previously studied SS rats, indices of renal injury were only moderately increased in *Pappa2-/-* compared to *Pappa+/+* WT rats including tubular protein casts (6.509 ± 0.789, n=11 vs 3.8 ± 0.718, n=9; $P<0.05$), cortical T-lymphocyte ($CD3^+/mm^2$; 96.1 ± 9.9, n=11 vs 70.9 ± 6.8, n=9; $P=0.06$), macrophage ($CD68^+/mm^2$; 74.6 ± 9.4, n=11 vs 47.6 ± 5.2, n=8; $P<0.05$), and interstitial smooth muscle actin (4.5 ± 0.4, n=11 vs 3.4 ± 0.2, n=8; $P<0.05$). In summary, the “rescue” of the BP salt-sensitivity trait with mutation of *Pappa2* provides the first direct evidence that this gene plays an important role in renal fluid and electrolyte homeostasis and the regulation of arterial pressure.

Disclosures: A. Cowley: None. **A. Geurts:** None. **L. Evans:** None. **T. Kurth:** None. **C. Yang:** None. **N. Zheleznova:** None. **C. Wollner:** None. **V. Kumar:** None.

Funding: No

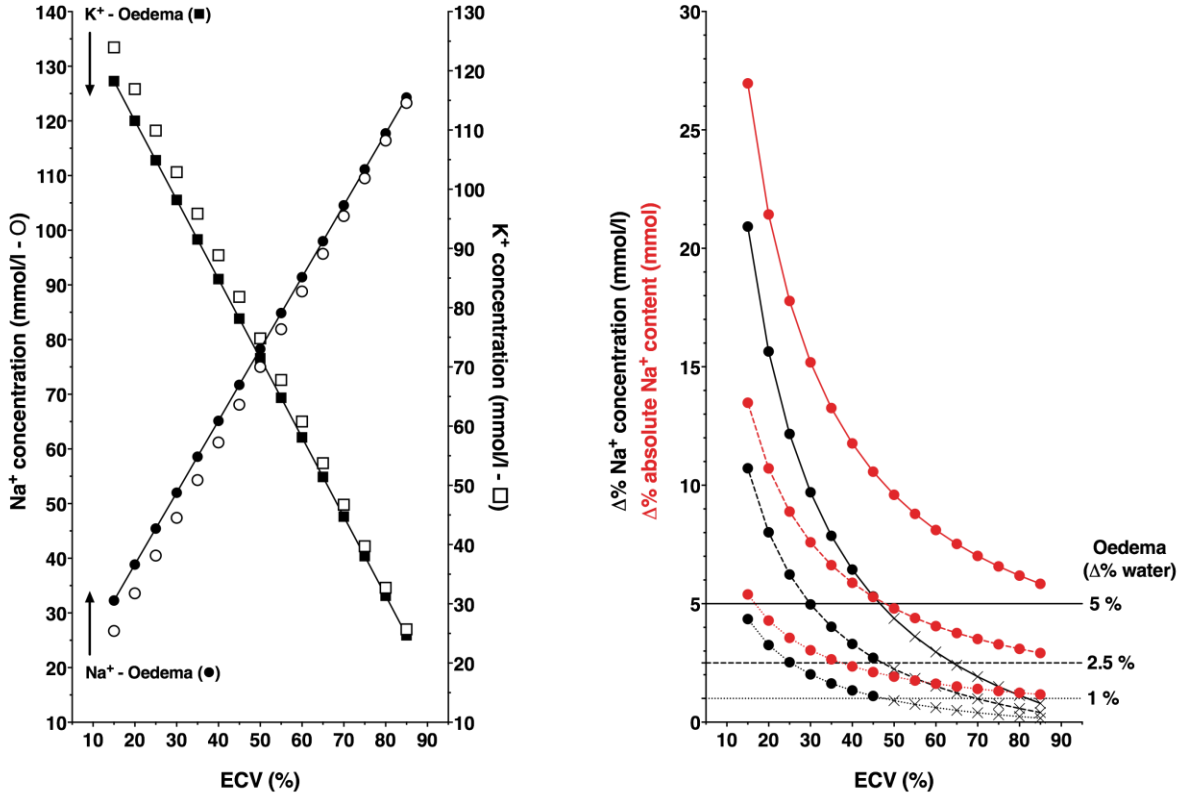
Funding Component:

092

Tissue Sodium is a Highly Sensitive Marker of Subclinical and Localised Edema

Authors: Giacomo Rossitto, Mark Petrie, Rhian Touyz, Christian Delles, Univ of Glasgow, Glasgow, United Kingdom

At variance with the classic physiology concept of constant balance between salt intake and excretion, hypertonic Na^+ accumulation has been shown to occur in peripheral tissues, particularly in the skin. In humans, higher ^{23}Na MR signal from skin and skeletal muscle has been associated with aging, hypertension, CKD and diabetes.In a rodent model of aging and hypertension, we recently identified an increase in absolute Na^+ content and concentration in the myocardium, but paralleled by water accrual and an associated decrease in K^+, thus pointing to an overall isotonic accumulation. To further investigate the phenomenon, we developed a mathematical model for expected chemical composition of tissues, as a function of extracellular volume fraction (ECV%) and the amount of fluid isotonic to the extracellular space added to the system (i.e. edema; *fig, closed circles*).Total Na^+ concentration is higher in tissues with larger ECV%; the opposite occurs relative to K^+ (*fig A, open symbols*). The proportional increase in Na^+ concentration due to different degrees of edema is higher than the increase in water content in tissues where ECV% is < 45% (3-4 times higher for skeletal muscle and myocardium, where ECV% is 18-30%; *fig B, black*). When absolute Na^+ content is assessed, similarly to ^{23}Na MR signal analysis, the proportionally higher increase occurs across the whole spectrum of ECV% (*fig B, red*).We conclude that Na^+ is more sensitive than water to detect edema. This could justify some of the recent evidence of high tissue Na^+ content in multiple conditions, including hypertension, CKD and diabetes, and holds the broad potential for early diagnosis of subclinical congestion and localised edema.



Disclosures: **G. Rossitto:** None. **M. Petrie:** None. **R. Touyz:** None. **C. Delles:** None.

Funding: No

Funding Component:

093

Salt Resistance and Renal Gpr83 Function

Authors: Xiaoxu Zheng, Prasad Konkalmatt, Laureano Asico, Pedro A Jose, Ines Armando, George Washington Univ, Washington, DC

The G protein coupled receptor 83 (Gpr83) is an orphan receptor that has been associated with essential hypertension. Gpr83 is expressed in the kidney but its function is unknown. We found that Gpr83 is expressed in mouse renal proximal and distal convoluted tubules, as well as in human renal proximal tubule cells (hRPTCs). In C57Bl/6J mice on normal salt diet, the lack of one (Gpr83+/-) or both Gpr83 (Gpr83-/-) alleles resulted in an increase in systolic blood pressure (SBP, ~20 mm Hg ($P<0.05$; n=4/group, measured under anesthesia) compared with Gpr83+/+ littermates, suggesting that Gpr83 is needed to keep a normal BP. Renal specific Gpr83 silencing by the renal subcapsular infusion of Gpr83 siRNA (3 µg/day; 7 days) increased SBP in C57Bl/6J mice on a normal salt diet, relative to mice treated with nonsilencing siRNA (120±5 vs 98±6 mmHg; $P<0.05$; n=4/group). A high salt (4% NaCl) diet increased renal Gpr83 transcription by 2 fold ($P<0.05$; n=4/group) in SJL/J and BALB/c salt resistant mice, relative to C57Bl/6J salt sensitive mice. *Gpr83* gene down-regulation in mouse and human renal proximal tubule cells decreased about 50% the expression of nuclear factor of activated T cells 5 (NFAT5). The renal expressions of NFAT5 and the NFAT5 targets UT-A3, Tau T and BGT1 were increased by a high salt diet in salt resistant mice. The neuropeptide PEN (PEN), a ligand of Gpr83, is also expressed in hRPTCs (0.43±0.03 mRNA PEN/mRNA GAPDH). We found that Gpr83 stimulation with PEN (1 µM, 3 hr) decreased the transcription of NFAT5 (0.59±0.05 vs 1.0±0.2fold, $P<0.05$; n=3-4/group), which was abolished by Gpr83 silencing. Our results suggest that Gpr83 may protect against the development of salt sensitivity. Gpr83 mediated regulation of NFAT5 may play a role in salt-sensitive hypertension.

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

Funding: No

Funding Component:

094

Smooth Muscle PPARγ Mutation Causes Impaired Renal Blood Flow and Salt-Sensitive Hypertension

Authors: Jing WU, Univ Iowa, Coralville, IA; Larry N. Agbor, Masashi Mukohda, Anand R. Nair, Pablo Nakagawa, Donald A. Morgan, Kamal Rahmouni, Univ Iowa, Iowa City, IA; Katherine H. Gotlinger, Michal L. Schwartzman, New York Medical Coll, Valhalla, NY; Curt D. Sigmund, Univ Iowa, Iowa City, IA

Mutations in PPARγ cause hypertension (HT) while PPARγ activation lowers blood pressure (BP) in humans. To determine if vascular smooth muscle (VSM) PPARγ regulates salt sensitivity, we studied transgenic mice selectively expressing a HT-causing PPARγ mutant in VSM (S-P467L) and non-transgenic littermates (NT) fed a 4% high salt (HS) diet for 4 weeks. Salt equally suppressed plasma renin in both strains, but S-P467L mice exhibited increased systolic BP (S-P467L 136±3 mmHg vs NT 124±2 mmHg, $p<0.01$) and pulse wave velocity (3.1±0.1 vs 2.7±0.1 m/s, $p<0.01$) in response to HS. The salt-induced HT was not associated with changes in diastolic BP, sympathetic nerve activity, heart rate, or cardiac output. Thus, the pressor effect of HS was likely due to higher peripheral vascular resistance. HS-fed S-P467L mice developed impaired acetylcholine (ACh)- and sodium nitroprusside (SNP)-induced vasorelaxation in carotid (Max ACh relaxation: 31±4.9% vs 90±1.8%, $p<0.01$; Max SNP relaxation: 38±2.8% vs 89±2.6%, $p<0.01$) and basilar artery (Max ACh relaxation: -3.2±9.3% vs 57±5.9%, $p<0.01$). The impaired vasodilation rapidly developed after 3-day HS diet, preceding salt-induced BP elevation. Pre-incubation with a cyclooxygenase inhibitor indomethacin normalized ACh/SNP relaxation responses, and preliminary mass spectrometry indicated HS increased prostaglandin E2 in S-P467L aortas. HS-fed S-P467L mice had smaller renal artery luminal diameter (322±21 vs 389±22 µm, $p<0.05$) and blunted renal blood flow (36±3.6 vs. 50±6.4 µL/min/g, $p<0.05$). During the 4^{th} week of HS diet, S-P467L mice produced 31% less nitrate/nitrite in 24 hour urine compared to NT controls (2.2±0.3 vs 3.2±0.4 µmol, $p<0.05$), suggesting blunted renal bioavailability of nitric oxide, a potent inhibitor of Na-K-2Cl cotransporter (NKCC2). This was associated with a declined capacity of HS-fed S-P467L mice to excrete an acute volume/Na^+ load, which was rescued by an NKCC2 inhibitor furosemide, but not by the Na-Cl-cotransporter inhibitor hydrochlorothiazide. Our data support the novel concept that

smooth muscle PPARγ regulates systemic vascular resistance, renal perfusion and tubular sodium transport, and loss of these protective actions of PPARγ predisposes to salt sensitivity and hypertension.

Disclosures: J. Wu: None. **L.N. Agbor:** None. **M. Mukohda:** None. **A.R. Nair:** None. **P. Nakagawa:** None. **D.A. Morgan:** None. **K. Rahmouni:** None. **K.H. Gotlinger:** None. **M.L. Schwartzman:** None. **C.D. Sigmund:** None.

Funding: Yes

Funding Component: National Center

095

Renal Transcriptomic Analysis of Transgenic Mice Containing Human Cyp11b2 Gene

Authors: Brahmaraju Mopidevi, Indu Sivankutty, Sravan Perla, Sudhir Jain, Ashok Kumar, New York Medical Coll, Valhalla, NY

The hCYP11B2 gene encodes aldosterone synthase, the rate-limiting enzyme in the biosynthesis of aldosterone. Inappropriate excess levels of aldosterone induce positive sodium balance and predisposes to hypertension and other cardiovascular problems. Epidemiological studies have suggested that the variant -344T of the Cyp11B2 gene is associated with hypertension and myocardial hypertrophy. We have found that hCyp11B2 gene has three SNPs in 1 Kb of its promoter that are in complete linkage disequilibirium. These SNPs are T/C at -344, C/T at -470 and C/A at -663. Thus variant -344T always occurs with variants -470C, -663A (Hap-I), and variant -344C almost always occurs with variants, -470T, -663T (Hap-II). We have generated novel transgenic (TG) mice with the hCYP11B2 gene, targeted to the mHPRT locus, with either haplotype I or II variants. We observed increased baseline blood pressure in Hap I TG mice (Hap-I 117±2.5 vs. Hap-II 109±1.9 mm Hg, $p<0.05$), an effect accentuated by high-salt diet (Hap-I 135±2.6 vs. Hap-II 122±2.2 mm Hg, $p<0.05$). In order to identify the downstream genes that are involved in Cyp11B2 induced hypertension and kidney damage, we have performed transcriptome analysis in kidneys of TG animals using RNAseq. Our results indicate that the highly significant differentially expressed genes are involved in the neuro-inflammation signaling pathway (p=2.71E-10), complement system (p=2.83E-07) and IL-7 signaling pathway (p=1.43E-04) with Z scores 2.333, 0.816 and 2.714 respectively. Using Ingenuity pathway analysis, we have identified genes that are associated with renal fibrosis. We have also found that expression of CTSW, CCL5, CCR2, CX3CR1, IRF1, and IRF7 are increased by 12.27 fold (p=3.49E-05), 4.23 fold (p= 3.78E-08), 2.9 fold (p= 3.45E-11), 3.37 fold (p=2.60E-12), 2.1 fold (p= 3.20E-06) and 2.0 fold (p= 1.21E-04) respectively in kidneys of hypertensive animals. Our RNA seq results delineate the regulatory molecules involved in the aldosterone mediated nephrotoxicity. These findings not only provide the valuable information on the pathophysiology of kidney damage but also can be used to identify novel therapeutic targets to reduce high blood pressure and end organ damage.

Disclosures: B. Mopidevi: None. **I. Sivankutty:** None. **S. Perla:** None. **S. Jain:** None. **A. Kumar:** None.

Funding: No

Funding Component:

096

Progesterone Upregulates Endothelial Mineralocorticoid Receptor Expression Which Predisposes Female Mice to Obesity-Induced Endothelial Dysfunction

Authors: Jessica L Faulkner, Simone Kennard, Galina Antonova, Zsolt Bagi, Augusta Univ, Augusta, GA; Iris Jaffe, Tufts Univ, Boston, MA; Vijay S Patel, Eric J Belin de Chantemèle, Augusta Univ, Augusta, GA

Compelling evidence indicates a higher efficacy of mineralocorticoid receptor (MR) blockade for the treatment of cardiovascular disease in females with obesity and diabetes than in males, however, the origin of this sex-specific effect is unknown. We have shown that leptin induces endothelial dysfunction in obese female mice via aldosterone-dependent activation of MR and that only female mice develop endothelial dysfunction (vasorelaxation to acetylcholine) in response to aldosterone *ex vivo*. Therefore, we hypothesized that females express higher endothelial MR (ECMR) expression than males which predisposes females to obesity-associated endothelial dysfunction. RT-PCR analysis in isolated aortic endothelial cells of Balb/C mice revealed a higher NR3C2 (MR gene) expression in females compared to males (2.9±0.5-fold from male, $P<0.05$), however, no such difference was observed in non-endothelial cells. Similarly, human adipose tissue endothelial cells exhibited higher ECMR mRNA expression than males (2.1±0.1 fold from male). Female sex-hormone suppression (ovariectomy) decreased ECMR expression in female mice (-0.8±0.2-fold from sham), which was restored by progesterone supplementation (-0.1±0.1-fold from sham). NR3C2 mRNA gradually increased with progesterone in diestrous (1.6±0.1-fold from estrous) phase and increased further in pregnancy day 16 (9.2±0.2-fold from estrous). Furthermore, progesterone dose-dependently increased ECMR protein expression in human endothelial cells *in vitro* ($P<0.05$). Increases in ECMR associated with higher progesterone levels in diestrus and pregnant females were associated with an increased sensitivity to leptin-induced endothelial dysfunction in mice. In parallel, while leptin induced endothelial dysfunction in intact ECMR female mice ($P<0.05$), specific deletion of MR in endothelial cells protected female mice from leptin-induced endothelial dysfunction. These data indicate that progesterone drives the sex-difference in the levels of ECMR expression and predisposes female mice to leptin-induced endothelial dysfunction. In addition, these data provide a rational for the higher efficacy of MR blockade in obese and diabetic women suffering from cardiovascular disease.

Disclosures: J.L. Faulkner: None. **S. Kennard:** None. **G. Antonova:** None. **Z. Bagi:** None. **I. Jaffe:** None. **V.S. Patel:** None. **E.J.B. Belin de Chantemèle:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

097

Interaction of miRNA, Endothelial Mineralocorticoid Receptor and Epithelial Sodium Channel in Endothelium Stiffness and Aortic Dysfunction

Authors: Guanghong Jia, Habibi Javad, Annayya R. Aroor, Yan Yang, Michael A Hill, Adam Whaley-Connell, Univ of Missouri Sch of Medcine, Columbia, MO; Frederic Jaisser, Sorbonne Univ, Paris, France; James R Sowers, Univ of Missouri Sch of Medcine, Columbia, MO

Excessive endothelial cell (EC) mineralocorticoid receptor (ECMR) and epithelial sodium channel activation in endothelium (EnNaC) increase oxidative stress and inflammation with associated cardiovascular abnormalities. Previous studies implicate elevations in circulating aldosterone (Aldo) in obesity enhance ECMR/EnNaC signaling that contribute to the development of vascular stiffness, in part, by reducing endothelial nitric oxide (NO) synthase (eNOS) activity and NO production. However, the specific role of ECMR/EnNaC signaling and its molecular mechanisms have not been explored. We hypothesized interactions between ECMR, dysregulation of miRNA expression, and EnNaC contribute to obese-/Aldo-induced endothelium dysfunction and aortic stiffness. Six week-old $ECMR^{-/-}$ and wild type littermate mice were fed a mouse chow or Western diet (WD) containing excess fat (46%) and fructose (17.5%) for 16 weeks. The EnNaC alpha subunit KO ($EnNaC^{-/-}$) and $EnNaC^{+/+}$ female mice were administrated Aldo (250 µg/kg/day) via osmotic minipumps for 3 weeks beginning at 25-28 weeks of age. RNA sequencing showed that in $ECMR^{+/+}$ mice WD induced increased miR-7a, miR-34, and miR-6973a and reduced miR-99, miR-486a, miR-6904, miR-6916, miR-6240 that potentially target EnNaC expression. For EnNaC in vascular function, $EnNaC^{-/-}$ significantly reduced inward sodium currents by patch clamp in

isolated mouse ECs. Meanwhile, EnNaC$^{-/-}$ significantly inhibited Aldo-induced endothelium stiffness and endothelium dependent relaxation observed in EnNaC$^{+/+}$. Further, chronic Aldo infusion induced aortic endoplasmic reticulum stress, reduced eNOS activation, endothelium permeability, expression of pro-inflammatory cytokines IL1 and IL6, oxidative stress, and aortic collagen 1 deposition and these abnormalities were attenuated in EnNaC$^{-/-}$ mice. These data indicate that interaction of endothelial specific ECMR/miRNA/EnNaC mediates vascular endothelium dysfunction and prompts aortic stiffness.

Disclosures: G. Jia: None. **H. Javad:** None. **A. Aroor:** None. **Y. Yang:** None. **M. Hill:** None. **A. Whaley-Connell:** None. **F. Jaisser:** None. **J. Sowers:** None.

Funding: No

Funding Component:

098

Aldosterone-Producing Cell Clusters in Essential Hypertension

Authors: Kei Omata, Ryo Kusaka, Yuto Yamazaki, Sadayoshi Ito, Fumitoshi Satoh, Tohoku Univ, Sendai, Japan; William Rainey, Scott Tomlins, Univ of Michigan, Ann Arbor, MI; Hironobu Sasano, Tohoku Univ, Sendai, Japan

Introduction: Primary aldosteronism (PA) is one of the major causes of secondary hypertension and has two major subtypes. One is unilateral aldosterone-producing adenoma (APA), which is positive for aldosterone synthase (CYP11B2) by immunohistochemistry (IHC), and the other is bilateral idiopathic hyperaldosteronism (IHA), which has image-undetectable CYP11B2-positive cell clusters (termed aldosterone-producing cell clusters, APCC) beneath the adrenal capsule. We and others have previously shown that APCC present in normotensive adrenals and the number is significantly less than IHA adrenals, supporting the disease-causing role of APCC in hyperaldosteronism of IHA. Furthermore, we have also shown that most of APCCs in the IHA and normotensive adrenals harbor somatic mutations in a L-type calcium channel, *CACNA1D*, whereas most of APA has been previously shown to harbor those in a potassium channel, *KCNJ5*. These mutations are reported to increase intracellular calcium levels, resulting in aldosterone overproduction. Here, we seek for the first time to identify the potential role of APCC in non-PA hypertensive patients and to clarify whether these adrenals could be precursor of APA or IHA by performing next-generation sequencing (NGS) on observed APCC.**Method and Results:**We selected fifteen adrenal glands with the evidence of hypertension and/or antihypertensive agents from a cohort of serial Japanese autopsy cases. None of the cases harbored adrenal tumors, but instead nine cases harbored 23 APCCs. We then isolated DNA from each APCC and performed NGS targeting genes mutated in APA. Of observed APCCs, 9 (39%) APCCs harbored somatic mutations in *CACNA1D*.**Interpretations:**These results show that autonomous aldosterone production in a part of patients with essential hypertension is caused by APCCs with aldosterone-driving somatic mutations. In addition, the mutation spectrum observed in this cohort suggests that essential hypertension could potentially develop into IHA through aldosterone overproduction by APCC.

Disclosures: K. Omata: None. **R. Kusaka:** None. **Y. Yamazaki:** None. **S. Ito:** None. **F. Satoh:** None. **W. Rainey:** None. **S. Tomlins:** None. **H. Sasano:** None.

Funding: No

Funding Component:

099

Carvedilol Selectively Stimulates Beta-arrestin2-dependent Serca2a Activity in Cardiomyocytes to Augment Contractility

Authors: Anastasios Lymperopoulos, Barbara M Parker, Ava Brill, Katie A McCrink, Jennifer Maning, Victoria L Desimine, Shelby L Wertz, Nova Southeastern Univ, Fort Lauderdale, FL

Background: Heart failure (HF) is the number one killer in the western world and β-blockers are part of its cornerstone pharmacotherapy. We recently showed that β_1-adrenergic receptor (AR)-activated βarrestin2, a G protein-coupled receptor (GPCR) adapter protein, promotes Sarco(endo)plasmic reticulum Ca-ATPase (SERCA)2a SUMO (small ubiquitin-like modifier)-ylation & activity, thereby increasing cardiac contractility. Given that carvedilol, unlike other β-blockers, has been shown to activate βarrestins and SERCA2a in the heart, we investigated herein its effects on βarrestin2-dependent SERCA2a modulation and contractility.
Methods: We studied SERCA2a-βarrestin interactions, SUMOylation & activity in response to therapeutic doses of carvedilol or metoprolol in H9c2 cardiac cells. We also measured contraction amplitude (cell shortening) of neonatal rat ventricular myocytes (NRVMs).
Results: Carvedilol, unlike metoprolol, acutely induces βarrestin2 (not βarrestin1) interaction with SERCA2a in H9c2 cardiomyocytes, resulting in enhanced SERCA2a SUMOylation. This translates into enhanced SERCA2a activity in the presence of the β_2AR-selective inverse agonist ICI 118,551 (ICI), indicating an opposing effect of β_2AR on carvedilol-stimulated, βarrestin2-dependent SERCA2a activation in H9C2 cells. In addition, the amplitude of spontaneous cell shortening of βarrestin2-overexpressing NRVMs is enhanced by carvedilol, especially in the presence of ICI (122+9% of control, ICI only-treated NRVMs; $p<0.05$, n=10). In contrast, metoprolol alone or with ICI had no effect again (98+6% of control, ICI only-treated NRVMs; $p<0.05$, n=10).
Conclusions: Carvedilol uniquely stimulates βarrestin2-mediated SERCA2a SUMOylation/activity through the β_1AR in cardiac myocytes. This translates into direct positive inotropic effects of this β-blocker, which may be opposed by the cardiac β_2AR subtype. These findings challenge the conventional wisdom that all β-blockers exert negative inotropy in the heart, complicating their use in human chronic HF treatment, and highlight the particular usefulness of carvedilol for treating this disease, as this drug may have positive inotropic properties on top of its classic reverse remodeling effects.

Disclosures: A. Lymperopoulos: None. **B.M. Parker:** None. **A. Brill:** None. **K.A. McCrink:** None. **J. Maning:** None. **V.L. Desimine:** None. **S.L. Wertz:** None.

Funding: Yes

Funding Component: National Center

100

Mechanistic Insight into Tannic Acid Mediated Angiotensin Type 1 Receptor and PCSK 9 Downregulation

Authors: Laxmi Iyer, Yong Zhang, Thomas Thekkumkara, Texas Tech Univ Health Sciences Ctr, Amarillo, TX

Deregulated angiotensin type 1 receptor (AT1R) and low-density lipoprotein receptor (LDLR) are implicated in cardiovascular disorders. AT1R is over expressed in hypertension and LDLR is involved in lowering plasma LDL cholesterol. In this study we investigate the role of tannic acid (TA), a hydrolyzable polyphenol, on AT1R and LDLR in rat aortic smooth muscle cells (RASMC) and rat liver epithelial cells (RLEC) respectively. TA (10 µg/ml) treatment exhibited down-regulation of AT1R specific [3H] AngII binding compared to untreated control (3628.32 ± 289.42 vs. 7244.66 ± 40.26 DPM/mg protein, $P < 0.001$). TA mediated AT1R downregulation was dose (0-40 µg/ml) and time dependent (0-24 hours) without significant change in receptor affinity (K_d 1.302 ± 0.152 nM for TA treated vs. K_d 1.391 ± 0.048 nM for untreated). Consistently, TA inhibited mRNA expression (TA 0.023± 0.0012 vs. control 0.051± 0.0045 mRNA fold).

Furthermore, TA activated of ERK p42/44, which was inhibited by MEK inhibitor PD98059 (20μM) and an EGFR inhibitor AG1478 (5μM) suggesting a role for EGFR induced ERK p42/44 in AT1R downregulation. A similar effect was observed with EGF (0.5 μg/ml) alone (EGF; 3788.04 ± 206.51 vs. Control; 6549.22 ± 115.48 DPM/mg protein, P < 0.001). LY294002 (10μM), an inhibitor of phosphoinositide 3-kinase (PI3K), inhibited both TA and EGF induced pERK and reversed AT1R downregulation suggesting that TA mediated AT1R downregulation is through EGFR-PI3K-ERK pathway. Consistent with the receptor downregulation significant inhibition of AngII induced intracellular calcium (TA 203.4 ± 7.79nM vs control 408.5 ± 5.33 nM) was observed. Under similar conditions proprotein convertase subtilisin/kexin type 9 (PCSK9), a crucial enzyme responsible for LDLR turn over, was decreased 2.8 fold at the protein level and mRNA levels (reduced 46.78± 2.3%) in RLEC. Alternatively, we observed 3.6 fold increased cell surface expression of LDLR and induced LDL-c uptake in human hepatocyte cells (2677 ± 26.39 untreated vs. 3894 ± 40.1 for TA treated). Our study provides a mean to control hypertension and hypercholesteremia associated cardiovascular disorders.

Disclosures: L. Iyer: None. **Y. Zhang:** None. **T. Thekkumkara:** None.

Funding: No

Funding Component:

101

Transforming Growth Factor-Beta-Receptor Antagonist Blocks the Cardiac Fibrosis and Remodeling in Guanylyl Cyclase/Natriuretic Peptide Receptor-A Gene-Disrupted Mice

Authors: Umadevi Subramanian, Ramachandran Samivel, Hanqing Zhao, Venkateswara Gogulamudi, **Kailash Nath Pandey**, Tulane Som Physiology, New Orleans, LA

Targeted-disruption of guanylyl cyclase/natriuretic peptide receptor-A (GC-A/NPRA) gene (*Npr1*) exhibits hypertension and provokes congestive heart failure in mice; however, the underlying mechanisms are not well clear. The objective of this study was to determine whether transforming growth factor-beta receptor (TGF-βR) antagonist, GW788388 inhibits the development of cardiac fibrosis and remodeling in *Npr1* gene-disrupted mice. The adult male (16-20 weeks) *Npr1* null mutant (*Npr1*$^{-/-}$, 0-copy), heterozygous (*Npr1*$^{+/-}$, 1-copy), and wild-type (*Npr1*$^{+/+}$, 2-copy) mice were orally administered with TGF-βR antagonist, GW788388 (1 mg/kg/day) for 28 days. The expression of cardiac fibrotic markers was analyzed using real-time PCR and Western blot. Heart weight-to-body weight (HW/BW) ratios were determined and heart functions were measured by echocardiographic analysis. The *Npr1*$^{-/-}$ mice showed markedly increased cardiac fibrosis and HW/BW ratio with increased expression of collagen-1α (3.5-fold), monocyte chemoattractant protein (4-fold), connective tissue growth factor (CTGF, 5-fold), α-smooth muscle actin (α-SMA, 4-fold), TGF-βRI (4-fold), TGF-βRII (3.5-fold) and SMAD proteins (SMAD-2, 5-fold; SMAD-3, 3-fold) compared with *Npr1*$^{+/+}$ mice. The expression of phosphorylated extracellular-regulated kinase (pERK1/2) was also up-regulated by 68% (P<0.001) in *Npr1*$^{-/-}$ mice. The treatment of *Npr1*$^{-/-}$ mice with GW788388 prevented the development of cardiac fibrosis and down-regulated the expression of fibrotic markers and SMAD proteins by 70-75% (P<0.001) compared to vehicle-treated mice. In contrast, the expression of pERK1/2 proteins was unaffected in GW7885388-treated mice excluding the involvement of non-genomic pathway. The left ventricular dimensions (systole and diastole) and fractional shortening were significantly (P<0.001) improved in the drug-treated *Npr1*$^{-/-}$ mice. The results suggest that the cardiac fibrosis, remodeling, and dysfunction in *Npr1*$^{-/-}$ mice are regulated through TGF-βR-mediated SMAD-dependent canonical pathway. The findings will be important for the development of new molecular therapeutic targets for the treatment of cardiac fibrosis, remodeling, and dysfunction in humans.

Disclosures: U. Subramanian: None. **R. Samivel:** None. **H. Zhao:** None. **V. Gogulamudi:** None. **K.N. Pandey:** None.

Funding: No

Funding Component:

102

G-Protein Coupled Protein Kinase 4 Variants Differentially Modulate Response of Breast Cancer Cells to Dopamine 1 Receptor Agonist

Authors: Wei Yue, Hanh Tran, Jiping Wang, Katherine A Schiermeyer, Peng Xu, John J Gildea, Robin A Felder, Univ of Virginia, Charlottesville, VA

Breast cancer incidence is higher in hypertensive women yet no biochemical basis has been reported for this link. Dopaminergic D1-like receptor (D_1R) regulates renal sodium excretion and blood pressure. Uncoupling of D_1R from its G protein/effector enzyme complex resulted from constitutive activation of GRK4 impairs the regulation and leads to hypertension. Our prior studies have shown the essential role of GRK4 gene variants in hypertension. Elevated expression of D_1R is associated with aggressive breast cancer. On the other hand, GRK4 expression is also reported higher in breast cancer than in normal breast tissues. The current study tested our hypothesis that GRK4 variants differentially affect breast cancer cell viability. We first used MCF-7 breast cancer cells as a model to determine the effect of GRK4 expression on baseline growth. To avoid interference of endogenous GRK4, we used shRNA to knockdown cMyc, the transcription factor that regulates GRK4 and expressed GRK4α, GRK4γ, and GRK4γ with 3 SNPs, R65L, A142V, A486V (GRK4γ TM). cMyc KD significantly reduced the growth rate of MCF-7 cells (doubling time is 3-fold longer than the wild type cells). Expression of 3 isoforms of GRK4 offset the growth retardation of cMyc KD. The effect of GRK4γ was weaker than that of GRK4α and GRK4γ TM. Interestingly, while all offered a growth advantage the cells with different GRK4 subtypes showed different responses to the D_1R agonist SKF38393. Wild type and GRK4γ expressing MCF-7 were resistant to SKF with less than 30% inhibition (286545/364447, 67909/91592) at 40 μM. In contrast, GRK4α and GRK4γ TM expressing cells were inhibited by more than 80% (9878/72721, 19228/118371) at the same concentrations. To confirm the role of GRK4, we chose 3 breast cancer cell lines that naturally express GRK4 with 0, 1, and 2 SNPs and compared their responses to SKF38393. Similar to the MCF-7 expressing GRK4α and GRK4γ TM, MDA-MB-468 cells containing 2 SNPs were more sensitive to SKF38393 with 98% inhibition (24937/1143137). BT-20 cells which have no SNP showed the least growth inhibition (29%, 96245/353854). While unexpected and with unknown mechanism, our data implicate a new therapeutic potential for use of a D_1R agonist for breast cancer treatment with aberrant expression of GRK4.

Disclosures: W. Yue: None. **H. Tran:** None. **J. Wang:** None. **K.A. Schiermeyer:** None. **P. Xu:** None. **J.J. Gildea:** None. **R.A. Felder:** None.

Funding: No

Funding Component:

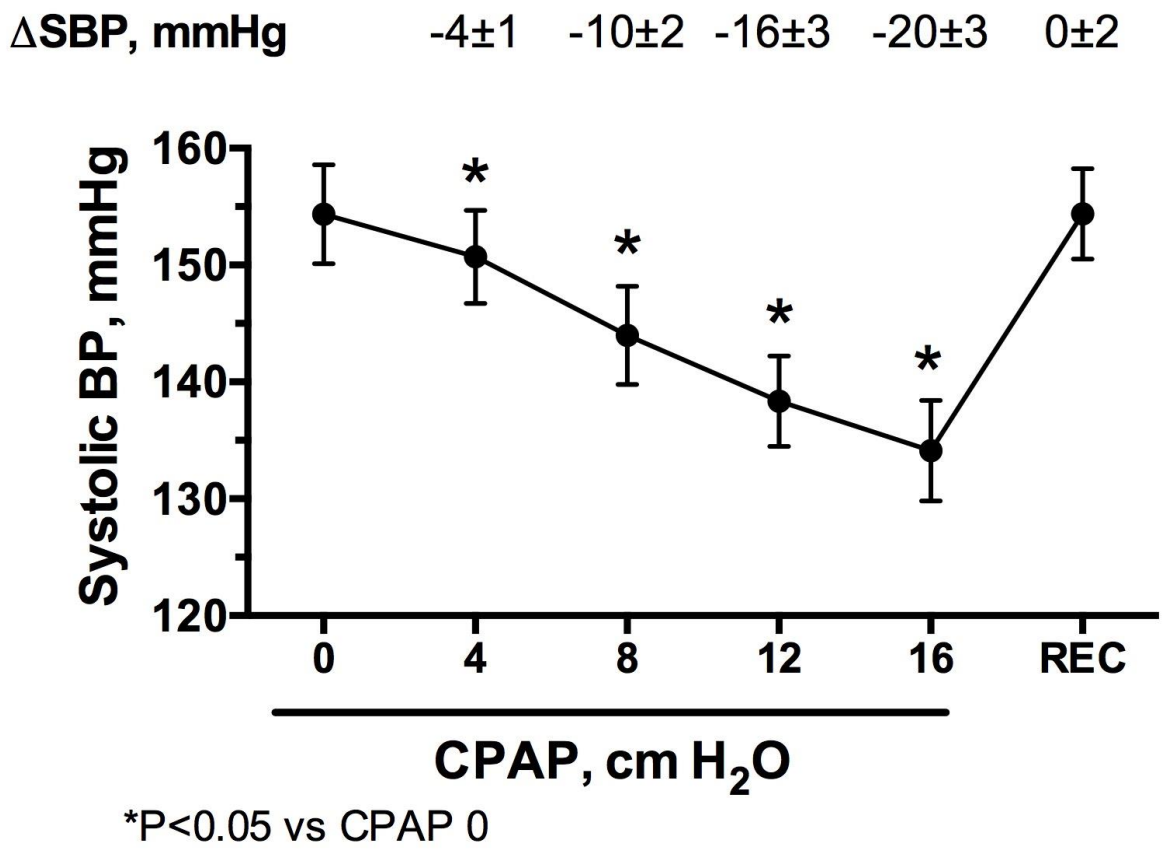
103

Acute, Dose-Dependent, Blood Pressure-Lowering Effect of Continuous Positive Airway Pressure in Autonomic Failure Patients With Supine Hypertension

Authors: Luis E Okamoto, Jorge E Celedonio, Andre Diedrich, Cyndya A Shibao, Alfredo Gamboa, Bonnie K Black, Sachin Y Paranjape, David Robertson, Italo Biaggioni, Vanderbilt Univ Medical Ctr, Nashville, TN

Primary autonomic failure (AF) is characterized by disabling orthostatic hypotension but half of these patients also have supine hypertension. We have previously shown that continuous positive airway pressure (CPAP 4, 8, 12 and 16 cm H_2O, each for 2 min) applied during autonomic blockade with trimethaphan acutely decreased systolic blood pressure (SBP) in hypertensive and normotensive subjects (-17±2 and -8±2 mmHg at 16 cm H_2O) due to decreases in cardiac output (CO)

and stroke volume (SV). In this study, we hypothesized that CPAP would have an acute, dose-dependent, blood pressure-lowering effect in patients with autonomic failure with supine hypertension. Five CPAP levels (0, 4, 8, 12 and 16 cm H_2O, each for 2-3 min) were applied sequentially to 15 AF patients with supine hypertension (12 males, 70±1 yr). Hemodynamic parameters were measured at the end of each CPAP level. We found that CPAP levels of 4, 8, 12 and 16 cm H_2O significantly decreased SBP in a dose-dependent manner (Figure). The maximal SBP drop was -20±3 mmHg with CPAP 16 (from 154±4 to 134±4 mmHg, P<0.01), and was associated with a decrease in CO (-12±4%) and SV (-13+4%). Neither systemic vascular resistance nor heart rate changed significantly with CPAP. SBP returned to baseline levels within 5 minutes after removal of the CPAP (REC). We conclude that, in AF patients with supine hypertension, CPAP acutely decreases SBP in a dose-dependent manner, due to decreases in CO and SV likely reflecting a decrease in venous return. These results suggest that CPAP could be used as a non-pharmacologic approach to treat the supine hypertension of AF. Future studies are needed to test the safety and efficacy of this approach.



Disclosures: **L.E. Okamoto:** None. **J.E. Celedonio:** None. **A. Diedrich:** None. **C.A. Shibao:** None. **A. Gamboa:** None. **B.K. Black:** None. **S.Y. Paranjape:** None. **D. Robertson:** None. **I. Biaggioni:** None.

Funding: No

Funding Component:

104

DOCA Induced Natriorexigenic and Hypertensive Effects are Blunted by Central Infusion of AT1 Receptor Biased Agonist TRV120027

Authors: Fernando Seara, Pablo Nakagawa, Javier A. Gomez, Guorui Deng, Julien A. Sebag, Univ of Iowa, Iowa city, IA; Sadashiva S. Karnik, Cleveland Clinic Lerner Res Inst, Cleveland, OH; Justin L. Grobe, Curt D. Sigmund, Univ of Iowa, Iowa city, IA

TRV120027 (TRV) is biased agonist at the angiotensin AT1 receptor that activates the β-arrestin pathway, causing receptor internalization. AT1 signaling within the hypothalamus is required for the blood pressure, polydipsia, and sodium appetite phenotypes in the deoxycorticosterone acetate (DOCA)-salt model of salt-sensitive hypertension (HTN). We hypothesized that TRV action within the hypothalamus would antagonize the effects of DOCA-salt. Using a novel luciferase-based HiBit tagging assay, we confirmed that TRV decreased AT1 surface density in immortalized mouse hypothalamic N43/5 cells by 35±4% compared to vehicle (n=4, p<0.05), and this was prevented by pretreatment with losartan. TRV (0.05 µg/h, icv n=5-10) or aCSF (n=4-8) were chronically infused into C57BL/6J mice implanted with DOCA (50 mg, sc) pellets, compared with mice undergoing sham surgeries (CTL, n=5-8), and animals were offered chow, water and NaCl *ad libitum*. DOCA increased fluid intake at baseline (CTL 4±0 vs 15±1 mL/day DOCA+aCSF, p<0.05), 0.15 mol/L NaCl (3.8±0 vs 20±2, p<0.05), 0.3 mol/L (4±0 vs 26±2, p<0.05), and 0.45 mol/L (4±0 vs 28±3, p<0.05). DOCA-induced polydipsia was not affected by TRV at baseline (DOCA+aCSF 19±1 vs 15±0 mL/day, p>0.05), 0.15 mol/L NaCl (27±4 vs 20±2 mL/day, p>0.05), 0.3 mol/L (25±4 vs 26±2 mL/day, p>0.05), or 0.45 mol/L (25±3 vs 28±3 mL/day, p>0.05). However, TRV prevented DOCA-induced increase of NaCl preference at NaCl 0.15 mol/L (DOCA+aCSF 29±1 vs 14±2% DOCA+TRV, p<0.05), 0.30 mol/L (29±3 vs 5±1%, p<0.05), and 0.45 mol/L (29±3 vs 5±1%, p<0.05). DOCA+aCSF exhibited increased heart weight / tibia length (CTL 8±0 vs 10±0 g/m DOCA+aCSF, p<0.05), which was prevented by TRV (8±0, p<0.05). In a separate cohort, DOCA-implanted mice were provided only 0.9% NaCl drink for one week, and mean arterial pressure (MAP) was recorded after acute injection of TRV (2 µg icv n=5) or aCSF (n=5). MAP was elevated with DOCA-salt (141±5 mmHg), and at 40 minutes post injection, TRV decreased MAP by 17±4 mmHg (p<0.05). These data underscore the potential for AT1 receptor-biased agonists as tools to treat subtypes of HTN characterized by salt sensitivity and low circulating renin activity.

Disclosures: F. Seara: None. **P. Nakagawa:** None. **J.A. Gomez:** None. **G. Deng:** None. **J.A. Sebag:** None. **S.S. Karnik:** None. **J.L. Grobe:** None. **C.D. Sigmund:** None.

Funding: Yes

Funding Component: National Center

105

PVN Inflammation Contributes to Brain GαI2 Protein Dependent Sympathetically Mediated Salt Sensitive Hypertension

Authors: Richard D Wainford, **Jonique George**, Boston Univ Sch Med, Boston, MA

Hypothesis: PVN inflammation contributes to salt sensitive hypertension (HTN) that develops following central Gαi2 protein down regulation.
Methods: Male Sprague-Dawley (SD) rats receiving a continuous I.C.V. scrambled (S) or a targeted Gαi2 oligodeoxynucleotide (ODN) infusion (25µg/5µl/day) were fed a normal (NS; 0.6% NaCl) or high salt (HS; 4% NaCl) diet for 7-days (N=4-6/group). Additional groups received a S or Gαi2 ODN and minocycline (120 µg/day) co-infusion and a 7-day HS diet. On day 7 24h sodium balance, MAP, plasma norepinephrine content (via ELISA) and PVN specific mRNA levels of IL1β, IL6, IL10 and TNFα (via RT-PCR) were assessed. Separate groups underwent IHC analysis of PVN astrocytes and PVN microglia activation.
Results: Central Gαi2 protein down regulation, but not control S ODN treatment, results in HS evoked HTN, sympathoexcitation, sodium retention, increased PVN mRNA levels of pro-inflammatory cytokines IL1β, IL6 and TNFα, decreased PVN anti-inflammatory 1L10 mRNA levels and no change in astrocyte PVN levels. Significantly, Gαi2 protein down regulation increased PVN microglial activation in rats on a NS diet. I.C.V. minocycline (anti-inflammatory antibiotic) co-infusion abolished the HS-evoked pro-inflammatory mRNA cytokine response and attenuated HTN, sympathoexcitation and sodium retention following central Gαi2 protein down regulation.
Conclusion: PVN inflammation contribute to central Gαi2 protein dependent salt sensitive HTN by modulating

sympathetic outflow and sodium homeostasis. We speculate central Gαi2 protein downregulation evokes microglial activation to prime a pro-inflammatory PVN response to HS intake.

	ICV SCR ODN INFUSION (25μg/5μl/day)			ICV Gai_2 ODN INFUSION (25μg/5μl/day)		
	Normal Salt (0.6% NaCl)	High Salt [HS] 4% NaCl	HS + ICV MINO (120μg/5μl/day)	Normal Salt (0.6% NaCl)	High Salt [HS] 4% NaCl	HS + ICV MINO (120μg/5μl/day)
MAP (mmHg)	123±4	121±3	119±3	118±4	144±3*	130±4*#
Plasma NE (nmol/l)	62±7	38±4*	42±5*	59±5	92±7*	62±6#
24h Na+ balance (mEq)	0.3±0.1	0.4±0.1	0.3±0.1	0.3±0.1	1.8±0.3*	0.9±0.2*#
IL-1β mRNA (fold increase)	0.9±0.2	0.8±0.1	1.1±0.2	1.1±0.2	2.4±0.4*	1.3±0.3#
IL-6 mRNA (fold increase)	1.0±.2	0.9±0.1	1.1±0.1	1.0±0.1	2.2±0.3*	1.2±0.2#
TNFα mRNA f(old increase)	1.1±0.1	1.0±0.2	1.0±0.1	1.3±0.2	2.3±0.5*	1.1±0.2#
IL-10 mRNA (fold increase)	1.0±0.1	1.3±0.1	1.3±0.2	1.1±0.2	0.4±.0.1*	1.1±0.2#
% Astrocytes present in PVN	42.7±2	37.5±3	N.D.	34.9±3	35.9±4	N.D.
% activated microglia in PVN	9.345±2	N.D.	N.D.	18.75±2	N.D.	N.D.

*p<0.05 vs. respective normal salt (0.6% NaCl) group; #p<0.05 vs. respective high salt (4% NaCl) group (N=4/group ± SEM). MINO = minocycline. N.D. = not determined

Disclosures: **R.D. Wainford:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; R01 HL141406, R01 HL107330, K02 HL112718, AHA 17GRNT33670023. **J. George:** None.

Funding: Yes

Funding Component: Founders Affiliate (Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Rhode Island, Vermont)

106

Central Angiotensin II Mediates Neurogenic Hypertension Through Post-Translational Regulation of Neuronal Nitric Oxide Synthase in the Paraventricular Nucleus of the Hypothalamus

Authors: Neeru M Sharma, UNMC, Omaha, NE; Andrea S Haibara, Univ of Minas Gerais, Belo Horizonte, Brazil; Kenichi Katsurada, Xuefei Liu, Kaushik P Patel, UNMC, Omaha, NE

Activation of renin-angiotensin- system, nitric oxide (NO) bioavailability and subsequent sympathoexcitation plays a pivotal role in the pathogenesis of many cardiovascular diseases including hypertension. Previously we have shown Ang II enhances PIN (a protein inhibitor of nNOS: neuronal nitric oxide synthase, known to dissociate nNOS dimers into monomers) mediated ubiquitination of nNOS in the PVN of rats with heart failure. To further elucidate the mechanism by which Ang II increases sympathetic outflow by regulating PIN mediated nNOS ubiquitination, we used Sprague-Dawley rats (250-300 g) subjected to intracerebroventricular infusion of Ang II (20 ng/min, 14days, 0.5μl/h) through osmotic mini-pumps and NG108-15 hybrid neuronal cell line treated with Ang II as an *in vitro* model. Ang II infusion

increased baseline mean arterial pressure (126 ± 9* vs. 84 ± 4 mmHg) and renal sympathetic nerve activity (20.5 ± 2.3* vs. 6.4 ± 1.9 % of Max. Activity). Ang II infusion increased the expression of PIN (1.36 ± 0.04* Ang II vs. 0.81 ± 0.02 Veh) with a concomitant 52% decrease in dimeric nNOS and PIN-Ub conjugates (0.73 ± 0.04* Ang II vs. 1 ± 0.03 Veh) in the PVN. Further, Ang II-mediated increase in PIN expression(0.67±0.06* CHX AngII vs. 0.40±0.08 CHX 0h) was independent of CHX (protein synthesis inhibitor) mediated decrease in PIN expression (0.41±0.06* CHX AngII vs. 0.19±0.04 CHX 4h) suggesting Ang II-mediated post-translational stabilization of PIN. Substrate-dependent ligase assay in cells transfected with pCMV-(HA-Ub)8 vector revealed a reduction of HA-Ub-PIN conjugates after Ang II and proteasome inhibitor lactacystin (LC) treatment (4.5 ± 0.6* LC Ang II vs. 9.2 ± 2.2 LC). TUBE (Tandem Ubiquitin-Binding Entities) assay showed decrease PIN-Ub conjugates in Ang II-treated cells (0.82 ± 0.12* LC Ang II vs. 1.23 ± 0.05 LC) while AT1R blocker Losartan(Los) treatment diminishes the Ang II-mediated stabilization of PIN (1.21 ± 0.07 LC Los vs. 1.14 ± 0.04* LC AngII Los). Taken together, our studies suggest that increased central levels of Ang II contribute to the enhanced expression of PIN leading to reduced expression of the dimeric form of nNOS, thus diminishing the inhibitory action of NO on pre-autonomic neurons in the PVN resulting in an increase in sympathetic outflow.

Disclosures: N.M. Sharma: None. **A.S. Haibara:** None. **K. Katsurada:** None. **X. Liu:** None. **K.P. Patel:** None.

Funding: Yes

Funding Component: National Center

107

Neurons in the Organum Vasculosum of the Lamina Terminalis Sense NaCl and Angiotensin II to Regulate Sympathetic Nerve Activity and Arterial Blood Pressure

Authors: Sean D Stocker, Brian J Kinsman, Kirsteen N Browning, Univ of Pittsburgh, Pittsburgh, PA; Megan M Wenner, William B Farquhar, Univ of Delaware, Newark, DE

The organum vasculosum of the lamina terminalis (OVLT) contains specialized neurons that sense changes in extracellular NaCl concentrations to regulate sympathetic nerve activity (SNA) and arterial blood pressure (ABP). However, OVLT neurons also abundantly express angiotensin II (AngII)-type I receptors. Therefore, we performed a series of in vitro and in vivo studies in adult male Sprague-Dawley rats to determine whether OVLT neurons sense both NaCl and AngII to impact SNA and ABP. First, in vitro whole-cell recordings revealed the majority of OVLT neurons (66%, 23/35) showed an increased discharge frequency to both +7.5mM NaCl (1.1±0.2 to 2.2±0.3Hz, P<0.001) and 100pM AngII (1.3±0.3 to 2.2±0.3Hz, P<0.001). Second, in vivo single-unit recordings demonstrate the majority of OVLT neurons (61%, 11/18) displayed an increase in cell discharge to intracarotid injection of 0.5M NaCl (3.6±0.6 to 9.0±1.6Hz, P<0.01) and 200ng AngII (3.2±0.7 to 9.2±1.9Hz, P<0.01). Third, optogenetic inhibition of OVLT neurons (rAAV2-CaMKII-eNpHR3.0-mCherry, $5x10^{12}$ particles/mL, 561nm, 10mW) attenuated thirst responses to IV infusion of 2M NaCl (1.25mL/30min; Laser OFF: 6.1±0.5mL vs ON: 2.0±0.7mL, P<0.05) and AngII (Laser OFF 7.2±0.7mL vs ON: 2.2±1.2mL; n=5 per group, P<0.05). Fourth, OVLT injection (20nL) of 0.5M NaCl or 10pmol AngII increased SNA (121±6% or 117±5%, n=4/group; P<0.05 respectively) and mean ABP (7±1 or 9±2mmHg, n=4/group; P<0.05 respectively). To determine whether chronic activation of OVLT neurons produces hypertension, rats received an OVLT injection of rAAV2-hSyn-HM3D(Gq)-mCherry ($3x10^{12}$ particles/mL, 50nL) and given access to 0.1% NaCl chow and 0.9% NaCl drinking solution. After 4 weeks, daily administration of clozapine-N-oxide to the drinking solution (3mg/kg/day) significantly increased 24-h fluid intake (baseline: 60±10mL vs Day 7: 178±29mL; n=6, P<0.01) and 24-h mean ABP (baseline: 97±2mmHg vs Day 7: 108±1 mmHg; n=6, P<0.05). Ganglionic blockade with hexamethonium (30mg/kg, ip) produced a greater fall in mean ABP at Day 7 (baseline: -38±6 mmHg vs Day 7: -53±5 mmHg, P<0.05). These findings suggest OVLT neurons sense both NaCl and AngII to regulate body fluid homeostasis and SNA to produce a sympathetically-mediated hypertension.

Disclosures: S.D. Stocker: None. **B.J. Kinsman:** None. **K.N. Browning:** None. **M.M. Wenner:** None. **W.B. Farquhar:** None.

Funding: No

Funding Component:

108

Sympathetic Inhibition Prevents the Homing of Specific Memory T Cells to the Bone Marrow and the Development of Repeated Hypertension

Authors: Liang Xiao, Luciana S Carmo, Hana A Itani, Jason D Foss, Wei Chen, David G Harrison, Vanderbilt Univ Medical Ctr, Nashville, TN

Effector memory T cells (T_{EM} cells) play a crucial role in hypertension. Formed during an initial blood pressure surge, T_{EM} cells can reside in bone marrow (BM) in a quiescent state for prolonged periods. Upon re-exposure to a hypertensive stimulus, these cells can be reactivated and aggravate hypertension and renal damage. Hypertension is also associated with elevated sympathetic outflow. We hypothesized that sympathetic nerves promote accumulation and reactivation of hypertension-specific T_{EM} cells in the BM. We performed unilateral superior cervical ganglionectomy (SCGx) in C57BL/6 mice, causing sympathectomy of the forelimb on the surgical side. After 2-week Ang II infusion (490 ng/kg/min s.c.), 5×10^6 BM cells from both the SCGx and control limbs were isolated and co-cultured with 0.5×10^6 dendritic cells from other hypertensive mice. We found 30% fewer $CD8^+$ T cell proliferation in the SCGx BM than intact side (1.9±0.2 vs. 2.8±0.1×10^4, $p<0.01$), but no difference in $CD4^+$ T cells. To study mechanisms involved in T cell homing, 10^7 pan T cells were isolated from CD45.2 mice after Ang II infusion and adoptively transferred to CD45.1 mice with unilateral SCGx. Flow cytometry indicated that 35% fewer donor $CD8^+$ T_{EM} cells homed to the SCGx than the intact BM 7 days after transfer (5.3±0.8 vs. 8.1±1.5 per 10^4 total T cells, $p<0.05$). We further determined if systemic sympatho-inhibition during T cell homing is protective against future hypertensive stimuli. We blocked systemic sympathetic outflow by injecting an inhibitory designer receptor exclusively activated by a designer drug (Gi-DREADD) into the rostroventral lateral medulla (RVLM) and performed pan-T cell adoptive transfer 10 days later. The DREADD ligand CNO was given in drinking water 3 days before till 7 days after adoptive transfer. After washout, mice received low dose Ang II infusion (140 ng/kg/min) for 2 weeks. As measured by radiotelemetry, mice with control injection developed hypertension (160±7 mmHg). However, mice with Gi-DREADD expressed in RVLM remained normotensive (136±3 mmHg, $p<0.05$ vs. control). These data define a novel role of sympathetic nerves in regulation of memory T cell trafficking, and sympathetic inhibition may have long term protective effect beyond lowering blood pressure.

Disclosures: L. Xiao: None. **L.S. Carmo:** None. **H.A. Itani:** None. **J.D. Foss:** None. **W. Chen:** None. **D.G. Harrison:** None.

Funding: Yes

Funding Component: National Center

109

Association Between Aortic Stiffness and Lower Retinal Microvascular Blood Flow Revealed by Laser Speckle Flowgraphy: Surrogate Measures of Cerebral Microcirculation in Humans

Authors: Seth W. Holwerda, Randy H. Kardon, Joseph M. Wetherell, Jan M. Full, Lyndsey E. Dubose, Lauren J. Points, Allene L. Gremaud, Rachel E. Luehrs, Brian Tong, Julie K. Nellis, Amy K. Stroud, Jess G. Fiedorowicz, Gary L. Pierce, Univ of Iowa, Iowa City, IA

The age-related increase in aortic stiffness increases systolic blood pressure (BP) and flow pulsatility and is associated with target organ damage such as ischemic cerebrovascular disease. However, the mechanistic link between aortic stiffness and cerebrovascular disease remains unclear because techniques available to non-invasively assess dynamic

changes in the human brain microcirculation with high spatial and temporal resolution are limited. Given the evidence that disease- and age- related alterations in the retinal microvasculature may mirror changes in the cerebral microvasculature, we assessed resting and dynamic changes in the retinal microcirculation using high resolution laser speckle flowgraphy. We tested the hypothesis that retinal microvascular blood flow at rest and during acute increases in BP would be associated with age-related increases in aortic stiffness (carotid-femoral pulse wave velocity, CFPWV). In 40 healthy subjects (35 ± 2 years; 25-60 years; 18 M/22 F; systolic BP: 123 ± 2 mmHg; diastolic BP: 77 ± 2 mmHg), retinal arterial blood flow was assessed at baseline (following dilation of the left pupil with 0.5% topicamide), and during a sympatho-excitatory stimulus (2-min cold pressor test). At baseline, lower retinal blood flow was significantly correlated with higher CFPWV (R=-0.35, P=0.03) and older age (R=-0.38, P=0.02) independent of mean BP (partial correlation). In response to the CPT, significant increases were observed in mean BP (BL: 92 ± 2 vs. CPT: 106 ± 2 mmHg, P<0.001) and retinal blood flow (BL: 215 ± 6 vs. CPT: 230 ± 8 AU, P<0.001). The relation between higher CFPWV and lower retinal blood flow observed at baseline was maintained during the CPT independent of mean BP (R=-0.37, P=0.03). However, no association was noted between CFPWV and % change in retinal blood flow. These preliminary data suggest that higher aortic stiffness is associated with lower retinal microvascular blood flow at rest and during sympatho-excitation independent of BP. These findings have important implications for age-related ocular degeneration, and lend support for future studies aiming to identify retinal microvascular abnormalities as markers of age-related cerebrovascular pathology.

Disclosures: S.W. Holwerda: None. **R.H. Kardon:** None. **J.M. Wetherell:** None. **J.M. Full:** None. **L.E. Dubose:** None. **L.J. Points:** None. **A.L. Gremaud:** None. **R.E. Luehrs:** None. **B. Tong:** None. **J.K. Nellis:** None. **A.K. Stroud:** None. **J.G. Fiedorowicz:** None. **G.L. Pierce:** None.

Funding: Yes

Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

110

Vascular Smooth Muscle RhoBTB1 Protects From Hypertension and Arterial Stiffness by Cullin-3 Dependent Ubiquitination of Phosphodiesterase 5

Authors: Masashi Mukohda, **Shi Fang**, Jing Wu, Larry N Agbor, Anand R Nair, Stella-Rita C Ibeawuchi, Chunyan Hu, Xuebo Liu, Ko-Ting Lu, Deng-Fu Guo, Debbie R Davis, Henry L Keen, Frederick W Quelle, Curt D Sigmund, Univ of Iowa, Iowa City, IA

We previously reported that vascular smooth muscle cell (VSMC) selective expression of hypertension (HT)-causing mutations in either PPARγ or the E3 Ring Ubiquitin Ligase Cullin-3 causes nitric oxide resistance and HT. Here we sought to assess the physiological role of RhoBTB1, a VSMC PPARγ target gene and Cullin-3 substrate adaptor. S-P467L mice which selectively express dominant negative PPARγ-P467L in VSMC exhibit RhoBTB1-deficiency. We bred S-P467L mice with mice inducibly expressing RhoBTB1 in response to Cre-recombinase. Inducible VSMC-specific restoration of RhoBTB1 in S-P467L mice fully corrected the HT (SBP, 141±6 vs 124±3 mmHg, p<0.01, n=8-10), arterial stiffness (Aortic Pulse Wave Velocity, 3.8±0.2 vs 2.5±0.1 mm/ms, p<0.01, n=11-13), and vasodilator function (Aorta, 46±5 vs 80±2% ACh-induced relaxation, p<0.01, n=6-9). Notably, the cardiovascular protection occurred despite preservation of increased agonist-mediated contraction and RhoA/Rho kinase activity, suggesting RhoBTB1 selectively controls vasodilation. Sodium nitroprusside-induced production of cGMP in aorta was severely impaired in S-P467L but restored by RhoBTB1. Consistent with this, phosphodiesterase 5 (PDE5) activity in aorta was augmented 2.5±0.3 fold in S-P467L but was returned to normal by RhoBTB1. PDE5 and RhoBTB1 reciprocally co-immunoprecipitated in HEK293 cells. Ubiquitination of PDE5 by Cullin-3 in HEK293 cells was RhoBTB1-dependent. Consistent with a role for Cullin-3 in mediating turnover of PDE5, PDE5 activity was augmented in MLN4924-treated aorta, a Cullin inhibitor, and abrogated by PDE5 inhibitor. The

beneficial cardiovascular effect of RhoBTB1 in S-P467L mice was phenocopied by PDE5 inhibition. Angiotensin-II infusion also causes RhoBTB1-deficiency and HT which was reversed by smooth muscle specific RhoBTB1 restoration. We conclude that RhoBTB1 augments the cGMP response to nitric oxide by restraining the activity of PDE5 by acting as a substrate adaptor delivering PDE5 to the Cullin-3 E3 Ring ubiquitin ligase complex for ubiquitination and proteasomal degradation. RhoBTB1 provides protection from HT, vascular smooth muscle dysfunction, and arterial stiffness in at least two models of HT.

Disclosures: M. Mukohda: None. **S. Fang:** None. **J. Wu:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; AHA Fellowship. **L.N. Agbor:** None. **A.R. Nair:** None. **S.C. Ibeawuchi:** None. **C. Hu:** None. **X. Liu:** None. **K. Lu:** None. **D. Guo:** None. **D.R. Davis:** None. **H.L. Keen:** None. **F.W. Quelle:** None. **C.D. Sigmund:** A. Employment; Modest; University of Iowa. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH, AHA. C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; Carver Trust.

Funding: Yes

Funding Component: National Center

112

Carotid Artery Stiffness Increases the Risk of Incident Depressive Symptoms: The Paris Prospective Study 3

Authors: Thomas T van Sloten, Pierre Boutouyrie, Muriel Tafflet, Inserm U970, Paris, France; Frédérique Thomas, Preventive and Clinical Investigation Ctr, Paris, France; Catherine Guibout, Rachel E Climie, Inserm U970, Paris, France; Cédric Lemogne, AP-HP, Georges Pompidou European Hosp, Dept of Psychiatry, Paris, France; Bruno Pannier, Preventive and Clinical Investigation Ctr, Paris, France; Stéphane Laurent, Xavier Jouven, Jean-Philippe Empana, Inserm U970, Paris, France

Introduction
Late-life depression is related to poor quality of life, functional impairment, and increased risk of mortality and cardiovascular disease. Effective interventions for prevention and treatment of late-life depression need to be developed, which requires a better understanding of late-life depression risk factors. Arterial stiffness, an important cardiovascular disease risk factor, may contribute to late-life depression via cerebrovascular damage, but evidence is scarce.
Aim
We investigated the association between carotid artery stiffness and incident depressive symptoms in a large community-based cohort study.
Methods
This longitudinal study included 7,013 participants (60 (SD 6) years; 36% women) free of depressive symptoms at baseline. Carotid stiffness (high-resolution echotracking) was determined at baseline. Presence of depressive symptoms was determined at baseline and at 4 and 6 years of follow-up, and was defined as a score ≥7 on a validated 13-item questionnaire (Q2DA) and/or new use of antidepressants. Cox regression and generalized estimating equations (GEE) were used.
Results
In total, 6.9% (n=484) of the participants had incident depressive symptoms at 4 or 6 years of follow-up. Greater carotid stiffness was associated with a higher incidence of depressive symptoms (Figure). Results were qualitatively similar when GEE was used instead of Cox regression.
Conclusions
Greater carotid stiffness is associated with a higher incidence of depressive symptoms. This study supports the hypothesis that carotid stiffness contributes to the development of late-life depression.

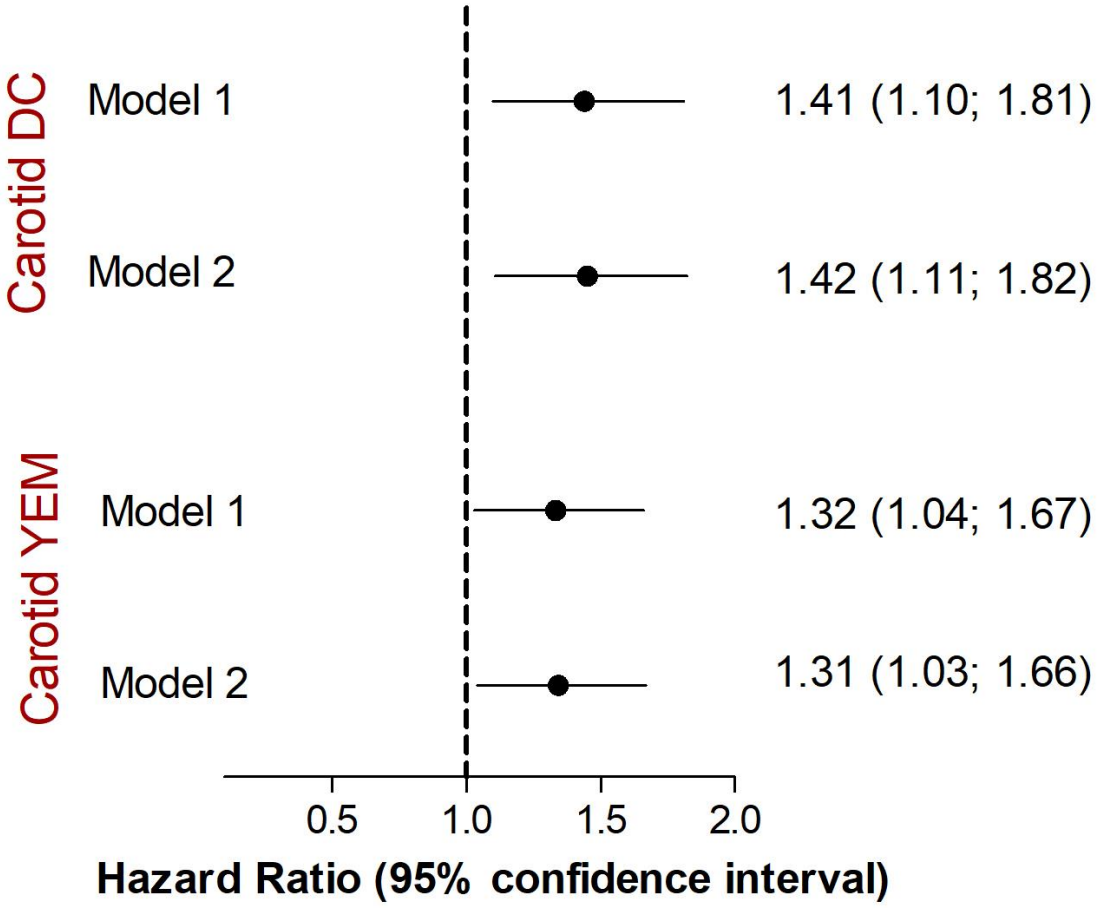


Figure. Association between carotid distensibility coefficient (DC) (tertile 1 vs. tertile 3) and Young's elastic modulus (YEM) (tertile 3 vs. tertile 1) and incident depressive symptoms. Model 1: adjusted for age, sex, living alone, education, smoking, systolic BP, HR, DM2, prior CVD, BMI, physical activity and antihypertensive and lipid-modifying medication. Model 2: model 1 plus baseline Q2DA score.

Disclosures: T.T. van Sloten: None. **P. Boutouyrie:** None. **M. Tafflet:** None. **F. Thomas:** None. **C. Guibout:** None. **R.E. Climie:** None. **C. Lemogne:** None. **B. Pannier:** None. **S. Laurent:** None. **X. Jouven:** None. **J. Empana:** None.

Funding: No

Funding Component:

113

Female Protection From Arterial Stiffness Diminishes With G Protein-Coupled Estrogen Receptor Deletion or Angiotensin II Hypertension

Authors: Benard O Ogola, Caleb M Abshire, Gabrielle L Clark, Dylan J Lawrence, Margaret A Zimmerman, Carolyn L Bayer, Kristin S Miller, Sarah H Lindsey, Tulane Sch of Med, New Orleans, LA

Arterial stiffness independently predicts cardiovascular mortality, coronary events, and stroke in hypertensive subjects and is exacerbated in women following menopause. Previously, our laboratory indicated that G protein-coupled estrogen receptor (GPER) plays a protective role in the vasculature. Therefore, the current study assessed the impact of sex and GPER on arterial stiffening in control and hypertensive conditions. We hypothesized that genetic deletion of GPER attenuates sex differences in arterial stiffness. Male and female wildtype (wt) and global GPER knockout (ko) mice (n=46) were used between 16-21 weeks of age. Angiotensin II (Ang II) infusion (700 ng/kg/day for two weeks) was used to induce hypertension, and systolic blood pressure (SBP) was measured using tail cuff plethysmography. Local pulse wave velocity (PWV) within the carotid artery was obtained via high frequency ultrasound in both color Doppler and M-mode. Statistical analysis was performed using two-way ANOVA with Sidak's multiple comparisons test. Baseline SBP was significantly lower in females (P=0.035) but was not impacted by genotype (P=0.78). Baseline PWV was significantly higher in male versus female wt mice (1.29 vs. 0.804 m/s, P=0.003) but was not different in ko mice (1.25 vs. 1.04 m/s, P=0.22). Ang II infusion significantly increased SBP (P<0.001) and PWV (P<0.001) in all groups, removing any impact of sex or genotype. In addition, significant correlations were found between Doppler and M-mode methods for obtaining carotid PWV (r=0.67, P<0.001) and between PWV and *ex vivo* carotid wall thickness (r=0.70, P=0.004). In contrast, SBP did not correlate with PWV (P=0.77) or carotid wall thickness (P=0.68). In conclusion, we found that GPER deletion did not impact blood pressure either in normotensive or hypertensive conditions. While arteries from female wt mice were less stiff at baseline, genetic deletion of GPER or Ang II infusion removed this protection independent of blood pressure. Moreover, we found that local carotid PWV provides information on vascular status that could not be obtained via blood pressure. This data indicates that GPER plays an important role in female vascular physiology that is absent in pathological conditions.

Disclosures: B.O. Ogola: None. **C.M. Abshire:** None. **G.L. Clark:** None. **D.J. Lawrence:** None. **M.A. Zimmerman:** None. **C.L. Bayer:** None. **K.S. Miller:** None. **S.H. Lindsey:** None.

Funding: No

Funding Component:

114

Increased Circulating Levels of Mitochondrial N-Formyl Peptides Leads to Vascular Dysfunction and High Blood Pressure in Spontaneously Hypertensive Rats

Authors: Camilla F Wenceslau, Cameron G. McCarthy, R. Clinton Webb, Augusta Univ, Augusta, GA

Aims: The most powerful signaling pathway that induces actin polymerization and movement in neutrophils is due to formyl peptide receptor (FPR) activation. FPR was originally identified by its ability to bind bacterial N-formyl peptides. Mitochondria carry hallmarks of their bacterial ancestry and one of these hallmarks is that this organelle uses an N-formyl-methionyl-tRNA as an initiator of protein synthesis. Consequently, mitochondrial N-formyl peptides (F-MITs) are recognized by FPR. Recently, we have observed that FPR is expressed in endothelial and vascular smooth muscle cells (VSMC). We hypothesized that increased plasma levels of F-MITs activates FPR and contributes to microvascular damage and hypertension. **Methods:** Male SHR (12-week old) were treated for 10 days with FPR antagonist cocktail: Cyclosporin H (CsH, 0.1 mg/kg/day *i.p.*) and WRW4 (0.1 mg/kg/day *i.p.*) or vehicle. Blood pressure (BP) was measured by telemetry. We also treated Wistar rats (12 week old) with F-MITs (formylated peptide corresponding to the NH2-terminus of mitochondria ND6; 0.02 mg/rat/day *i.p.*) or non-formylated peptides (control) for 10 days. Blood was collected to measure F-MITs. Intrarenal arteries and mesenteric resistance arteries (MRA) were isolated and mounted in a wire myograph. Wistar VSMCs were treated with F-MITs (20 min, 10 µM) or control in the presence or absence of FPR antagonists to evaluate actin polymerization. T-test *p<0.05; n=5-6. **Results and Conclusion:** F-MITs plasma levels were increased in SHR (1.7-fold* vs. Wistar). FPR blockade decreased BP (SHR + vehicle: 151 ± 7.2 vs. SHR+CsH+WRW4: 138 ± 0.4* mmHg) and ameliorated acetylcholine-induced relaxation (Intrarenal; Emax: SHR+vehicle: 10 ± 5 vs.

SHR+CsH+WRW4: 28 ± 6* %); (MRA; Emax: SHR+vehicle: 74±10 vs. SHR+CsH+WRW4: 99±0.6* %). F-MITs infusion increased BP in Wistar rats (Control: 133 ± 1.9 vs. F-MITs: 145 ± 4.7* mmHg) and decreased acetylcholine-induced relaxation (EC50; MRA: Control: -8.765 ± 0.05 vs. F-MIT: -8.348 ± 0.08*) (Emax; Intrarenal: Control: 63±4 vs. F-MIT: 46 ± 3* %). F-MITs induced VSMCs actin polymerization (1.8 fold* vs. control) and FPR blockers abolished this result. In conclusion, FMITs play a role in vascular dysfunction and hypertension via FPR activation.

Disclosures: C.F. Wenceslau: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; K99GM118885. **C.G. McCarthy:** None. **R. Webb:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; P01HL134604.

Funding: No

Funding Component:

115

Renal-Specific Expression of Human Grk4 R65L in Mice Lacking Grk4 Increases Blood Pressure

Authors: Selim Rozyyev, Prasad Konkalmatt, Laureano Asico, Megha Kumar, Jessica Hunt, Pedro A Jose, Ines Armando, George Washington Univ, Washington, DC

The genetic causes of salt (NaCl) sensitivity in humans are not well understood. The kidney is critical to the overall fluid and electrolyte balance and long-term regulation of blood pressure (BP). Therefore, the pathogenesis of salt sensitivity must involve a deficient renal NaCl handling. The renal paracrine inhibition of Na^+ transport by dopamine is impaired in salt-sensitive rats, mice, and humans due to decreased dopamine D1 receptor (D1R) function and not related to a primary defect of the D1R gene but rather due to its uncoupling from second messengers caused by activating variants of the human G protein-coupled receptor kinase type 4 (h*GRK4*) (R65L, A142V, and A486V). We have shown that global transgenic mice expressing hGRK4 R65L variant have salt-sensitive hypertension. To determine the role of the kidney in the salt-sensitivity of these mice, we developed a mouse model of kidney-specific expression of the R65L variant (KS hGRK4 R65L) by bilateral ureteral infusion of AAV vectors carrying the variant in GRK4 knockout mice. Mice infused with hGRK4 wild-type served as controls (KS hGRK4 WT). Systolic BP (SBP) was measured under anesthesia in the mice before and after infusion of the AAV vectors. SBP before AAV was similar in both groups. SBP post AAV increased in KS hGRK4 R65L (93±1 vs 117±4 mmHg, $P<0.05$, n=4) but not in KS hGRK4 WT (96±2 vs 105±6, n=5). Renal mRNA expressions of dopamine 1 (D1R, 0.81±0.01 vs 1.28±0.04, $P<0.01$), D3R (0.44±0.02 vs 1.27±0.07, $P<0.01$) and angiotensin AT1A (AT1A, 0.78±0.02 vs 1.02±0.02, $P<0.01$) receptors were decreased in renal cortex of KS hGRK4 R65L relative to KS hGRK4 WT. In addition, the expressions of Na^+/K^+ATPase (1.12±0.03 vs 1.0 ±0.007, $P<0.05$) and ENaC (1.4±0.14 vs 1.0±0.11, $P<0.05$) were increased while those of the proximal tubule Na^+ transporters NaPi2 (0.81±0.02 vs 1.04±0.02), SGLT2 (0.89±0.03 vs 1.07±0.05), and NBCe2 (0.50±0.07 vs 1.15±0.03) were decreased. Our results show that kidney specific expression of hGRK4 R65L increased SBP and produced changes in the expression of receptors and Na^+ transporters, pump, and channel but their roles in the increased SBP remain to be determined.

Disclosures: S. Rozyyev: None. **P. Konkalmatt:** None. **L. Asico:** None. **M. Kumar:** None. **J. Hunt:** None. **P.A. Jose:** None. **I. Armando:** None.

Funding: No

Funding Component:

116

Epigenetic Regulation of Human Angiotensinogen (hAGT) Gene by SNPs.

Authors: Sravan Perla, Brahmaraju Mopidevi, Indu Sivankutty, Sudhir Jain, Kavita Jain, Ashok Kumar, New York Medical Coll, Valhalla, NY

Hypertension is a complex disease caused by a combination of genetic and environmental factors. Renin angiotensin system (RAS) dysfunction leads to essential hypertension. Polymorphisms in the AGT gene increase RAS activity and cause blood pressure elevation. We and others have shown that SNPs in the promoter of hAGT gene can be arranged mainly in two haplotypes. Variants -1670A, -1562C, and -1561T always occur with -6A and form the haplotype-I (Hap-I), while variants -1670G, -1562G, and -1561G always occur with -6G and constitute haplotype-II (Hap-II). We have made transgenic animals containing these two haplotype blocks and shown that Haplotype-I is the risk haplotype and increases hAGT expression in TG animals. We hypothesized that these SNPs, when present together as Hap-I or Hap-II in transgenic mice (TG) may lead to haplotype dependent DNA methylation of CpG sites in the promoter of the hAGT gene. Methylation patterns may modulate gene transcription at an epigenetic level and this, in turn, could be dependent on individual polymorphisms. Hypo-methylation is associated with higher gene expression and hyper methylation is associated with lower gene expression. We have found that the number of methylated CpG sites in the hAGT promoter is decreased after high salt (HS) treatment in the liver of Hap-I TG animals as compared to the liver of Hap-I animals. In the liver of Hap-I animals, the hAGT promoter CpG sites are methylated at -460,-434,-386,-261,+42, whereas after high salt treatment, the promoter DNA methylation is observed at -460,-386,+65. On the other hand methylation of hAGT promoter CpG sites in the liver of Hap-II are increased as compared to the liver of Hap-II after high salt treatment. In the liver of Hap-II animals, the CpG sites are methylated at -386,-346,+65, whereas after high salt treatment, methylate sites are observed at -386,-261. Results of this experiment show that methylation of CpG sites is reduced in the DNA obtained from high salt treated liver of both haplotypes. However, DNA from high salt treated liver of Hap-II animals had least number of methylated CpG sites and therefore open chromatin that leads to higher expression of the hAGT gene in this haplotype.

Disclosures: S. Perla: None. **B. Mopidevi:** None. **I. Sivankutty:** None. **S. Jain:** None. **K. Jain:** None. **A. Kumar:** None.

Funding: No

Funding Component:

117

Inhibition of DNA Methyltransferase Attenuates Salt-Sensitive Hypertension & Renal Disease in Dahl Salt Sensitive Rats

Authors: John Henry H Dasinger, Xiaoqing Pan, Justine M Abais-Battad, Ammar J Alsheikh, Hayley Lund, Daniel J Fehrenbach, Pengyuan Liu, Mingyu Liang, David L Mattson, Medical Coll of Wisconsin, Milwaukee, WI

DNA methylation plays a key role in the regulation of gene expression. In the present study, we investigated the impact of changes in DNA methylation in Dahl Salt Sensitive (SS) rats fed a casein-based AIN-76A chow containing low salt (LS) or high salt (HS). In addition to the hypertension and renal damage which occurs following 14 days of HS feeding, we assessed 5-Methylcytosine levels at single-base resolution by reduced representation bisulfite genome sequencing (RRBS) of DNA from the renal outer medulla of SS rats. Several hundred differentially methylated regions (DMRs) were identified between the SS rats on the LS or HS diet. Approximately a quarter of the DMRs were located in transcriptional start site regions, a third in intragenic regions, and the remainder in intergenic regions. To investigate the functional role of methylation in SS hypertension, we administered decitabine (DNA methyltransferase inhibitor, 5mg/kg, ip) or vehicle at Days 0, 3, 5, and 7 during a 14 day HS period. While there was no difference in MAP during the LS period (127±3 vs.

126±6 mmHg, vehicle vs. decitabine, n=9), at the end of 2 weeks of HS there was a significant increase in MAP in the vehicle SS rats compared to the decitabine-treated rats (163±6 vs 141±4 mmHg, *p<0.0001*). This protective effect in MAP in the decitabine-treated group also exhibit a reduction in albumin excretion rate compared to controls (119±19 vs. 182±24 mg/day, *p<0.01*). Upon analysis of the immune cell infiltration profile, there was a reduction in number of CD45+ total leukocytes, CD11b/c+ monocytes/ macrophages, and CD45R+ B cells in the decitabine-treated animals compared to controls. Decitabine administration also significantly reduced renal histological damage as indicated by the presence of outer medullary protein casts compared to vehicle-treated rats (6.8±1.0% vs. 10.6±1.3%, $p<0.05$). Lastly, methylation rate was significantly reduced in decitabine-treated rats by 40% compared to the control levels. In summary, the inhibition of methylation by decitabine attenuated MAP and markers of renal damage in response to a HS diet suggesting that DNA methylation could be a potential therapeutic target for the treatment of hypertension and renal damage.

Disclosures: J.H. Dasinger: None. **X. Pan:** None. **J.M. Abais-Battad:** None. **A.J. Alsheikh:** None. **H. Lund:** None. **D.J. Fehrenbach:** None. **P. Liu:** None. **M. Liang:** None. **D.L.L. Mattson:** None.

Funding: Yes

Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

118

DNA de novo (de)Methylation in the Kidney Contributes to Salt-Induced Hypertension

Authors: Pengyuan Liu, Sir Run Run Shaw Hosp and Inst of Translational Med,Zhejiang Univ, Hangzhou, Zhejiang, China; **Yong Liu**, Xiaoqing Pan, Medical Coll of Wisconsin, Milwaukee, WI; Yingchuan Li, Intensive Care Unit, Shanghai Jiaotong Univ Affiliated Sixth Hosp, Shanghai, China; Kristie Usa, Mingyu Liang, Medical Coll of Wisconsin, Milwaukee, WI

Numerous adult diseases involving tissues that consist primarily of non-dividing cells are associated with changes in DNA methylation. It suggests a role for de novo methylation or demethylation of DNA, which is catalyzed by DNA methyltransferase 3 (Dnmt3) and ten-eleven translocases (Tet). However, the contribution of DNA de novo (de)methylation to these diseases remains nearly completely unproven. Broad changes in DNA methylation occurred within days in the renal outer medulla of Dahl SS rats fed a high-salt diet, a classic model of hypertension. Intra-renal administration of anti-Dnmt3a/Tet3 GapmeR's attenuated high salt-induced hypertension in SS rats (mean arterial pressure 153+/-4 vs. 141+/-2 mmHg, n=9-11, p<0.05). The high salt diet induced differential expression of 1,712 genes in the renal outer medulla. Remarkably, the differential expression of 76% of these genes were prevented by anti-Dnmt3a/Tet3 GapmeR's. The genes differentially expressed in response to the GapmeR's were involved in the regulation of metabolism and inflammation and were significantly enriched for genes showing differential methylation in response to the GapmeR's. These data indicate DNA de novo (de)methylation in the kidney contributes to the development of hypertension in SS rats. The findings should help to shift the paradigm of DNA methylation research in diseases involving non-dividing cells from correlative analysis to functional and mechanistic studies.

Disclosures: P. Liu: None. **Y. Liu:** None. **X. Pan:** None. **Y. Li:** None. **K. Usa:** None. **M. Liang:** None.

Funding: No

Funding Component:

119

Using Blood Pressure Values from Electronic Medical Records to Identify Hypertension During Pregnancy

Authors: Lu Chen, Kaiser Permanente Washington, Seattle, WA; Thomas Easterling, Univ of Washington, Seattle, WA; T. Craig Cheetham, Kristi Reynolds, Kaiser Permanente Southern California, Dept of Res & Evaluation, Pasadena, CA; Lyndsay Avalos, Kaiser Permanente Northern California, Div of Res, Oakland, CA; Victoria Holt, Univ of Washington, Seattle, WA; Romain Neugebauer, Kaiser Permanente Northern California, Div of Res, Oakland, CA; Susan M Shortreed, Mary Akosile, Aruna Kamineni, Rod Walker, Kaiser Permanente Washington, Seattle, WA; Sylvia E Badon, Kaiser Permanente Northern California, Div of Res, Oakland, CA; Sascha Dublin, Kaiser Permanente Washington, Seattle, WA

Objective: Hypertension is a major risk factor for poor pregnancy outcomes. Many observational studies have relied on diagnosis codes, particularly from the delivery hospitalization, to identify hypertension in pregnancy. We augmented diagnosis codes with electronic blood pressure (BP) data to improve the identification of pregnant women with hypertension.
Methods: We studied pregnant women aged 15-49 years enrolled in three Kaiser Permanente health plans who delivered during 2005-2014. Using diagnosis codes, BP values, and antihypertensive medication dispensings, we defined hypertension as: (1) ≥ 2 high BPs (≥ 140/90 mmHg) within 30 days of each other (2highBPs); or (2) ≥ 1 antihypertensive medication fill with ≥1 hypertension diagnosis code from 120 days prior to pregnancy through 20 weeks gestation (chronicHTN); or (3) ≥ 1 high BP, a hypertension diagnosis code, and an antihypertensive fill within 7 days (RapidTx). Among women meeting our study definition, we examined receipt of hypertension diagnosis codes and prevalence of severe hypertension (1+ BP ≥160/110 mmHg).
Results: Among 553,477 eligible women, 29,933 (5%) met our definition of hypertension, including 26,855 identified via 2highBPs, 5,774 via chronicHTN and 6,198 via RapidTx (not mutually exclusive). Among women meeting our hypertension definition overall, only 64% had 1+ hypertension diagnosis code assigned during pregnancy, and 49% had one at delivery. Among hypertensive women identified via 2highBPs, only 60% (16,057/26,855) had a hypertension diagnosis code in pregnancy and 45% (12,131/ 26,855) at delivery. However, 53% of our hypertensive women (14,972/ 29,933) overall and 56% (14,972/ 26,855) of the 2highBPs women had severe hypertension at some time during pregnancy.
Conclusion: Incorporating BP values identifies additional pregnant women with hypertension who would have been missed by approaches using diagnosis codes alone. Women identified by our method frequently had severely elevated BP, showing the importance of including these women in future studies of hypertension during pregnancy.

Disclosures: L. Chen: None. **T. Easterling:** None. **T. Cheetham:** None. **K. Reynolds:** None. **L. Avalos:** None. **V. Holt:** None. **R. Neugebauer:** None. **S.M. Shortreed:** None. **M. Akosile:** None. **A. Kamineni:** None. **R. Walker:** None. **S.E. Badon:** None. **S. Dublin:** None.

Funding: No

Funding Component:

120

Long-Term Stability of the Masked Hypertension Phenotype and Responsiveness of Masked Hypertension to Antihypertensive Treatment: Findings From the Masked Hypertension Study

Authors: Ian M Kronish, Justin Young, Daichi Shimbo, Joseph E Schwartz, Columbia Univ, New York, NY

Background: Prior studies have assessed the short-term reproducibility of the masked hypertension (MHT) phenotype. Less is known about the stability of MHT over time or its responsiveness to BP medication. **Methods:** We assessed BP phenotypes in 441 untreated subjects with a screening BP<160/105mmHg using office BP and 24-hr ambulatory BP

monitoring on 2 occasions, 6 years apart. MHT was defined as office BP<140/90mmHg and awake ambulatory BP≥135/85mmHg. Sustained hypertension at follow-up was defined as office BP≥140/90mmHg and ambulatory BP≥135/85mmHg or treatment with BP medication. Left ventricular mass (LVM) was assessed by 2D-echo. **Results:** Of subjects with MHT at baseline, 41%(24/59) continued to have MHT at 6 years, 44%(26/59) developed sustained hypertension, and 15%(9/59) became normotensive (**Table**). Of subjects with baseline office SBP 120-139mmHg, there was a trend toward a greater increase in LVM over 6 years in those with versus without MHT (change in LVM +25.2±37.5g vs +15.7±27.8g, p=0.25). Of subjects with MHT at baseline, those on BP treatment at 6 years had a decrease in ambulatory awake SBP compared to baseline whereas untreated subjects had an increase in ambulatory awake SBP (-5.4±7.2mmHg vs +7.2±8.0mmHg; p<0.001). MHT subjects who were treated also had a smaller increase in LVM (+21.5±41.6g vs +30.4±32.3g; p=0.58). **Conclusion:** Approximately half of MHT subjects progressed to sustained hypertension over 6 years, supporting the hypothesis that MHT is an intermediary phenotype on the way to sustained hypertension. Treatment with BP medication was associated with lower ambulatory BP, though randomized trials are needed to confirm efficacy and safety.

Table: Cross-Tabulation of BP Phenotypes Across Two Visits a Mean of 6 Years Apart

BP Phenotype		Follow-Up				
		Normotension (N=236)	White-coat hypertension (N=1)	Masked hypertension (N=110)	Sustained hypertension	
					Untreated (N=24)	Treated (N=70)
Baseline	Normotension (N=357)	226 (63.3%)	1 (0.3%)	84 (23.5%)	10 (2.8%)	36 (10.1%)
	White coat hypertension; (N=1)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)
	Masked hypertension (N=59)	9 (15.3%)	0 (0%)	24 (40.7%)	8 (13.6%)	18 (30.5%)
	Sustained hypertension (N=24)	1 (4.2%)	0 (0%)	2 (8.3%)	6 (25.0%)	15 (62.5%)

Disclosures: I.M. Kronish: None. **J. Young:** None. **D. Shimbo:** None. **J.E. Schwartz:** None.

Funding: No

Funding Component:

121

Endothelial Cullin3 Mutation Causes Vascular Dysfunction, Arterial Stiffening, and Hypertension

Authors: Jing WU, Univ Iowa, Coralville, IA; Larry N. Agbor, Shi Fang, Chunyan Hu, Xuebo Liu, Masashi Mukohda, Anand Nair, Curt D. Sigmund, Univ Iowa, Iowa City, IA

Mutations in CULLIN3 gene (causing in-frame deletion of exon 9) cause human hypertension (HT), which is likely to be driven by a combination of renal tubular and vascular mechanisms. We have recently shown that smooth muscle expression of Cul3Δ9 mutant causes vascular dysfunction and HT 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 Cul3Δ9 mice with tamoxifen-inducible Tie2-CRE^{ERT2} mice. The resultant mice (E-Cul3Δ9) developed nocturnal hypertension (Night time peak systolic BP, E-Cul3Δ9: 138±3 vs Control: 121±4 mmHg, p<0.01) and arterial stiffening (pulse wave velocity, 3.7±0.3 vs 2.7±0.1 m/s, p<0.01). No difference was seen in daytime BP. Nitric

oxide synthase (NOS) inhibitor L-NAME induced smaller increases in nocturnal peak SBP and DBP in E-Cul3Δ9 mice (15±1 vs 27±3 mmHg, p<0.01), suggesting their NOS activity is low. Of note, E-Cul3Δ9 mice exhibited impaired endothelial-dependent relaxation in carotid artery (Max ACh relaxation: 69% vs 84%, p<0.05) and cerebral resistance basilar artery (41% vs 77%, p<0.01). No difference in smooth muscle function was observed. To determine the molecular mechanisms, we isolated primary aortic endothelial cells from mice carrying the inducible Cul3Δ9 construct and induced Cul3Δ9 expression *in vitro* using adenovirus carrying Cre recombinase gene. Expression of Cul3Δ9 resulted in marked decreases in wild type Cul3 protein, phosphorylated eNOS, and nitric oxide production. Because protein phosphatase 2A (PP2A) is a known Cul3 substrate which dephosphorylates eNOS, we determined whether impaired eNOS activity was attributable to PP2A. Cul3Δ9-induced impairment of eNOS activity was rescued by a selective PP2A inhibitor Okadaic Acid (4 nM), but not by a Protein Phosphatase 1 inhibitor Tautomycetin (4 nM). These data define a novel regulatory pathway involving Cullin3/PP2A/phospho-eNOS in the endothelium. Selective endothelial expression of Cul3Δ9 partially phenocopies the hypertension observed in Cul3Δ9 patients, suggesting that mutations in Cullin-3 cause human hypertension in part through a vascular mechanism characterized by endothelial dysfunction.

Disclosures: J. Wu: None. **L.N. Agbor:** None. **S. Fang:** None. **C. Hu:** None. **X. Liu:** None. **M. Mukohda:** None. **A. Nair:** None. **C.D. Sigmund:** None.

Funding: Yes

Funding Component: National Center

122

Deletion of *Gstm1* Results in Poor Survival and Exaggerated Kidney Injury Associated With Increased Oxidative Stress and Apoptosis in a Mouse Model of Chronic Kidney Disease

Authors: Sylvia Cechova, Gabor Bodonyi-Kovacs, Fang Chan, Univ of Virginia, Charlottesville, VA; Phillip Ruiz, Univ of Miami Sch of Med, Miami, FL; Thu H. Le, Univ of Virginia, Charlottesville, VA

In humans, a common deletion variant of the *GSTM1* gene, the *GSTM1(0)* null allele, results in decreased GSTM1 enzymatic activity and is associated with higher levels of oxidative stress. We reported that *GSTM1(0)* is associated with increased risks of chronic kidney disease (CKD) progression and death in the African Americans Study of Kidney Disease. To establish a causal relationship between GSTM1 deficiency and CKD progression, we deleted *Gstm1* in the mouse, and determined its effect in the sub-total nephrectomy (Nx) model of CKD. After the 8th week after Nx, 4 out of 8 *Gstm1* knockout (KO) mice began to die, whereas wild-type (WT) mice had 100% survival by the study endpoint at 13th week. Remaining KO mice had significantly higher plasma creatinine ($p < 0.05$), suggestive of lower GFR. Due to early mortality in KO mice after the 8th week after Nx, we phenotyped mice at the 8th week (n=6 each). KO mice had significantly higher 1) mean systolic blood pressure (by radiotelemetry): KO 157.2 ± 5, WT 141.1 ± 2.4 mmHg, 2) urine albumin/creatinine ratio (µg/mg): KO 748.4 ± 187.9, WT 235.1 ± 24.6, and 3) renal superoxide (O2·-) levels (counts/min/mg dry tissue): KO 249.7 ± 63; WT 74.5 ± 14.7; $P \leq 0.02$. Hydrogen peroxide levels were not different. Renal mRNA levels of the superoxide dismutase isoforms SOD1, SOD2, and SOD3 were significantly lower in KO mice (KO 7.78 ± 0.047, 0.446 ± 0.062, 0.18 ± 0.03; WT 10.64 ± 0.43, 2.432 ± 0.386, 1.188 ± 0.124, respectively, $P \leq 0.003$. Total kidney pathology score (KO 18.7 ± 1.5; WT 8 ± 0.6; $P < 0.0001$) showed that KO mice had worse kidney injury, with severe glomerulosclerosis, proteinaceous casts, and chronic inflammation. Both glomerular surface area (SA)/total non-infarcted kidney area (KO 3.347 ± 0.301; WT 2.286 ± 0.268; $P = 0.02$) and mesangial SA/glomerular SA (KO 35.62 ± 2.219; WT 23.6 ± 2.569; $P = 0.005$) were significantly increased in KO mice. GSTM1 has also been shown to suppress ASK1 - the apoptosis signal-regulating kinase 1 that induces apoptosis via activation of caspase-3. Consistent with activated ASK1 pathway, KO mice had significantly higher renal TUNEL staining and caspase-3 protein levels (KO 3.594 ± 0.327; WT 2.511 ± 0.208; $P = 0.01$). In conclusion, in CKD, loss of GSTM1 leads to deleterious outcome associated with increased O2·- levels and apoptosis.

Disclosures: S. Cechova: None. **G. Bodonyi-Kovacs:** None. **F. Chan:** None. **P. Ruiz:** None. **T. Le:** None.

Funding: No

Funding Component:

123

Effect of Immune Cell-Derived Reactive Oxygen Species on Dahl SS Hypertension

Authors: Justine M Abais-Battad, Hayley Lund, John Henry Dasinger, Daniel J Fehrenbach, David L Mattson, Medical Coll of Wisconsin, Milwaukee, WI

Previous studies utilizing the SS$^{p67phox-/-}$ rat have demonstrated the importance of systemic NADPH oxidase NOX2-derived reactive oxygen species (ROS) production in the pathogenesis of Dahl Salt-Sensitive (SS) hypertension and renal damage. Our laboratory has established a key role for renal immune cell infiltration in the development of SS hypertension and observed an enrichment of NOX2 subunits in infiltrating T cells. The contribution of ROS specifically derived from immune cells in SS hypertension remains unknown. To assess the role of ROS in immune cells, SS$^{p67phox-/-}$ rats underwent total body irradiation and received bone marrow transfer from either SS$^{p67phox-/-}$ (p67/p67) or SS (p67/SS) donor rats. p67/SS rats would be capable of producing NOX2-derived ROS only in the transferred SS cells of hematopoietic origin while the p67/p67 control group would be unable to produce NOX2-derived ROS globally. Mean arterial pressure (MAP) was not different between the p67/SS and p67/p67 rats when fed a 0.4% NaCl diet (112.6±1.4 vs 110.7±2.4 mmHg, n=4-5). After 3 weeks of high salt, however, there was a significantly greater increase in MAP in the p67/SS rats (176.1±4.7 mmHg) compared to the p67/p67 control rats (147.9±8.4 mmHg), which was accompanied by a significant increase in albuminuria (230.3±13.9 vs 153.1±14.9 mg/day, p67/SS vs p67/p67, n=5-6). Interestingly, upon analysis of the immune cells in the kidney, no differences were detected in the number of CD45+ total leukocytes, CD11b/c+ monocytes/macrophages, CD3+ T cells, and CD45R+ B cells between the p67/SS and p67/p67 (n=4-5), indicating that the recruitment of immune cells into the kidney during hypertension and renal damage is independent of NOX2-derived ROS production from the renal parenchyma. The respiratory burst response to phorbol 12-myristate 13-acetate stimulus (135 μM) in peritoneal macrophages isolated from p67/SS was 4.0-fold greater compared to nonresponsive p67/p67 cells, and was completely inhibited by superoxide dismutase, demonstrating that the reconstituted bone marrow cells in the p67/SS are capable of producing superoxide. In summary, the production of NOX2-derived ROS from immune cells alone is sufficient for the development of Dahl SS hypertension and renal damage.

Disclosures: J.M. Abais-Battad: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 1F32HL136161. **H. Lund:** None. **J. Dasinger:** None. **D.J. Fehrenbach:** None. **D.L. Mattson:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; HL137748, HL116264, 15SFRN2391002.

Funding: Yes

Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

124

Nadph Oxidase 4 and Mitochondrial Abnormalities Contribute to Oxidative Stress and Endothelial Dysfunction in Young Normotensive Patients With Autosomal Dominant Polycystic Kidney Disease

Authors: Ali Kahveci, Fouad T Chebib, Alfonso Eirin, Amir Lerman, Lilach O Lerman, Peter C Harris, Vicente E Torres, **Maria V Irazabal**, Mayo Clinic, Rochester, MN

Background: Fifty percent of patients with autosomal dominant polycystic kidney disease (ADPKD) present hypertension (HTN) early on, which increases to nearly 100% at end-stage, and is associated with faster renal functional decline. Endothelial dysfunction (ED) accompanied by oxidative stress (OS) precedes the development of HTN, but the mechanisms responsible remain unknown. The aim of this study was to determine whether NADPH oxidase (NOX4) and mitochondrial dysfunction contribute to OS and ED preceding HTN in early ADPKD. Methods: We prospectively measured plasma levels of homocysteine (Hcy), 8-isoprostane, NOX4 and the mtDNA genes COX3 and ND1 in young normotensive (without BP medication) ADPKD patients, and age-matched healthy volunteers (HV) (n=10, each). Total kidney volume (TKV) and Renal Blood Flow (RBF) were evaluated by MRI. Results: BP levels were higher in ADPKD, and HtTKV was twofold higher in ADPKD vs. controls. eGFR and RBF were similar between the groups (Table). Plasma Hcy, 8-isoprostanes and NOX4 were higher in ADPKD, and correlated directly with HtTKV ($p<0.05$, Figure), but the correlation with RBF was not significant. Plasma mtDNA copy number were lower in ADPKD compared to controls, and correlated inversely with HtTKV(R^2 0.397 and 0.392 respectively). Conclusion: Early ADPKD is associated with elevation in Hcy, 8-isoprostane, and NOX4 levels, and decreased mtDNA copy numbers, which precede the reduction in RBF and the development of HTN, and are associated with disease severity. NOX4 and mitochondrial abnormalities may contribute to oxidative stress, endothelial dysfunction, and possibly the development of HTN and disease progression in ADPKD.

Table			
	HV	**ADPKD**	*p value*
Number of patients	10	10	N/A
Gender (Female/Male)	6/4	6/4	N/A
Age (years)	23.2 ± 3.2	22.5 ± 3.2	N/A
Systolic blood pressure (mmHg)	114.6 ± 9.0	123.6 ± 11.0	**0.008**
Diastolic blood pressure (mmHg)	70.2 ± 8.4	77.3 ± 8.3	0.063
Serum creatinine (mg/dL)	0.9 ± 0.1	0.8 ± 0.2	0.494
eGFR-CDK-EPI (ml/min/1.73m^2)	104.9 ± 19.3	110.6 ± 12.4	0.411
HtTKV (mL/m)	179 (160 - 189)	347 (270 - 460)	**<0.001**
RBF (cc/min/1.73m^2)	624 ± 100	619 ± 79	0.903

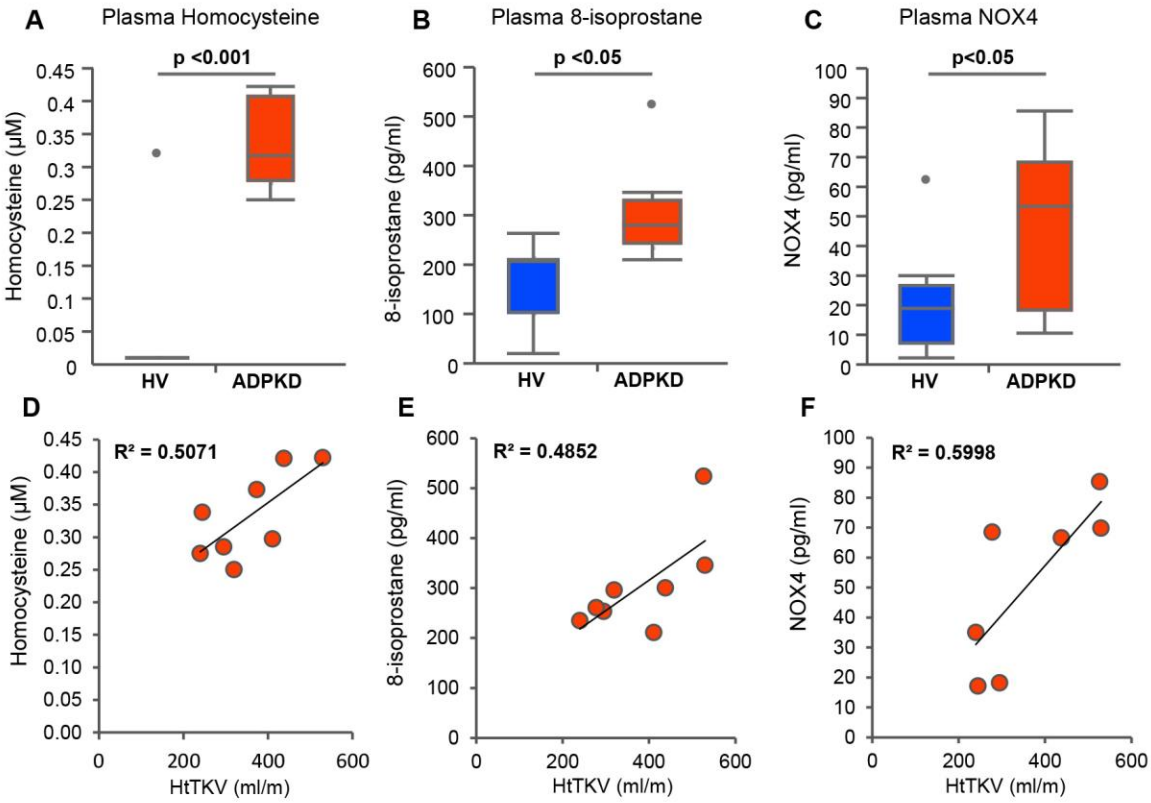


Figure.Top: Plasma levels of Hcy (A), 8-isoprostane (B) and NOX4 (C) in ADPKD patients and HV. Top and bottom boxes are estimated 75th and 25th percentiles, respectively. Vertical lines extend from the 75th percentile to the highest and from the 25th percentile to the lowest data points. **Bottom:** HtTKV correlated directly with plasma Hcy (D), 8-isoprostane (E), and NOX4 (F).

Disclosures: A. Kahveci: None. **F.T. Chebib:** None. **A. Eirin:** None. **A. Lerman:** None. **L.O. Lerman:** None. **P.C. Harris:** None. **V.E. Torres:** None. **M.V. Irazabal:** None.

Funding: No

Funding Component:

125

Hyperacetylation of CypD Contributes to Mitochondrial Dysfunction, Vascular Oxidative Stress and Hypertension, and Mitochondria-Targeted Isoketal Scavenger mito2HOBA Prevents CypD Hyperacetylation and Reduces Hypertension

Authors: Sergey I Dikalov, Vanderbilt Univ Medical Ctr, Nashville, TN; Vladimir Mayorov, Mercer Univ Sch of Med, Macon, GA; Arvind Pandey, Vanderbilt Univ Medical Ctr, Nashville, TN; Alexander Panov, Mercer Univ Sch of Med, Macon, GA; Hana Itani, Venkataraman Amarnath, Raymond Mernaugh, Olivier Boutaud, Sean Davies, John Oates, David G Harrison, Anna Dikalova, Vanderbilt Univ Medical Ctr, Nashville, TN

One third of adult population has hypertension and third of hypertensive patients remain hypertensive despite treatment with multiple drugs. There have been no mechanistically novel treatments for hypertension in the past 30 years. We propose that new agents targeting CypD activation, a regulatory subunit of mitochondrial permeability transition pore, could add to the currently available therapeutic armamentarium to improve treatment of hypertension. To test this hypothesis we have studied CypD acetylation which was associated with CypD activation. We found that angiotensin II-induced hypertension is linked to CypD-K166 hyperacetylation, mPTP opening, impaired mitochondrial respiration and reduced kidney ATP. CypD hyperacetylation may result from imbalance between GCN5L1 acetyltransferase and reduced Sirt3 deacetylase activity. Indeed, aortic mitochondrial level of GCN5L1 increased by 50% while Sirt3 was reduced by 40% in angiotensin II infused mice. Sirt3 can be inactivated by cytotoxic reactive lipid dicarbonyls derived from arachidonic acid, isoketals, leading to mitochondrial hyperacetylation. To test the role of mitochondrial isoketals we synthesized a novel mitochondria-targeted isoketal scavenger mito2HOBA. mito2HOBA supplementation in drinking water (0.1g/Liter) attenuates angiotensin II-induced hypertension, prevents accumulation of mitochondrial isoketal protein adducts in the aorta and kidney measured by LC/MS and D11 antibody, improves Sirt3/GCNL1 ratio, normalizes the CypD acetylation, attenuates mPTP opening, protects mitochondrial respiration and preserves normal kidney ATP level. Decreased NO bioavailability is a hallmark of endothelial oxidative stress in hypertension due to superoxide overproduction and NO oxidation. Interestingly, angiotensin II increases vascular superoxide by 2-fold and reduces endothelial NO level to 50% while mito2HOBA diminishes vascular $O_2^{\bullet-}$ and preserves NO bioavailability in angiotensin II-infused mice. These data support the role of isoketals-mediated CypD hyperacetylation in endothelial dysfunction and hypertension. We conclude that scavenging of mitochondrial isoketals may have therapeutic potential in treatment of hypertension and target-organ-damage.

Disclosures: S.I. Dikalov: None. **V. Mayorov:** None. **A. Pandey:** None. **A. Panov:** None. **H. Itani:** None. **V. Amarnath:** None. **R. Mernaugh:** None. **O. Boutaud:** None. **S. Davies:** None. **J. Oates:** None. **D.G. Harrison:** None. **A. Dikalova:** None.

Funding: Yes

Funding Component: National Center

126

Nox5 Regulation of Vascular Contraction Involves Oxidation of Endoplasmic Reticulum Calcium Channels and Calreticulin

Authors: Augusto C Montezano, Livia Lucca Camargo, Emma Carrick, Francisco Jose Rios, Laura Haddow, Wendy Beatie, Chet E Holterman, Christopher Kennedy, Rhian M Touyz, ICAMS - Univ of Glasgow, Glasgow, United Kingdom

The biological function of calcium-sensitive superoxide-generating Nox5 is unclear, but it may play a role in regulating contraction, as we previously demonstrated. Here we explored molecular mechanisms whereby Nox5 controls contraction. Human arteries and mice expressing human *NOX5* in smooth muscle cells (Nox5+SM22+) were studied. In arteries from hypertensive subjects, Nox5 expression, assessed by immunoblotting, was increased (50%, $p<0.05$ vs control). In human VSMCs, AngII-induced ROS generation (1 fold) and activation of myosin light chain (MLC) (2.5 fold)

were exaggerated in VSMCs from hypertensive subjects ($p<0.05$ vs control); an effect that was attenuated by Nox5 siRNA. In arteries from Nox5+/SM22+ mice, contraction to U46619 was increased in (5.8±0.3 mN vs WT: 4.2±0.2 mN, $p<0.05$). These hypercontractile responses were inhibited by NAC (ROS scavenger), calmidazolium (calmodulin inhibitor), dantrolene (ryanodine receptor Ca^{2+} channel inhibitors) and CDN1163 (SERCA channel activator), but not by a Nox1/Nox4 inhibitor (GKT137831). $ONOO^-$ levels were increased in vessels from Nox5+/SM22+ mice (5.8±0.9 vs WT 3.4±0.1 AU/mg, $p<0.05$). Inactivation of MYPT1 (181±1.8AU vs 164±1.9AU WT) and activation of MLC (207±10.3AU vs 155±2.7AU WT) were increased in VSMCs from Nox5+SM22+ ($p<0.05$). To assess the oxidative proteome in VSMCs, we immunoprecipitated reversibly oxidized proteins and observed oxidation of Nox5, decreased oxidation of MYPT1 and increased oxidation of SERCA2b in Nox5+/SM22+ mice . Proteome analysis of human VSMCs identified the ER Ca^{2+} sensor, calreticulin, as a potential Nox5 binding protein. Calreticulin reversible oxidation was increased in VSMCs from Nox5+SM22+ mice and hypertensive subjects. Our study unravels crosstalk between oxidative stress and Ca^{2+} in the vasculature, where Nox5 regulation of contraction involves ROS, Ca^{2+} and endoplasmic reticulum localized Ca^{2+} channels/proteins. We identify novel mechanisms whereby Nox5 influences pro-contractile signalling through processes involving oxidation of the ER Ca^{2+} sensor, calreticulin, and ER Ca^{2+} channels.

Disclosures: A.C. Montezano: None. **L.L. Camargo:** None. **E. Carrick:** None. **F.J. Rios:** None. **L. Haddow:** None. **W. Beatie:** None. **C.E. Holterman:** None. **C. Kennedy:** None. **R.M. Touyz:** None.

Funding: No

Funding Component:

127

Axl^+ $Siglec6^+$ Dendritic Cells: the Role of Salt, Stretch, and Hypertension

Authors: Justin P Van Beusecum, Natalia R Barbaro, Vanderbilt Univ Medical Ctr, Nashville, TN; Roxana Loperena, Vanderbilt Univ, Nashville, TN; David G Harrison, Vanderbilt Univ Medical Ctr, Nashville, TN

We have shown that monocyte-derived dendritic cells (DCs) are activated in hypertension to produce large amounts of cytokines and to activate T cells. DCs from hypertensive mice can convey hypertension when adoptively transferred to recipients. Single cell sequencing has recently identified a novel subset of DCs in humans that express Axl and $Sigelc6^+$ (AS DCs). These cells have been reported to potently drive T cell proliferation and to produce large amounts of IL-8 and IL12. The role of AS DCs in hypertension remains unknown. We isolated total peripheral blood mononuclear cells (PBMCs) from normotensive (n=23) and hypertensive (n=12) patients and assessed DC populations, including AS DCs, using flow cytometry. We found a significant increase in the AS DCs in hypertensive compared to normotensive patients (297 ± 73 vs. 108 ± 26/ml; P=0.0304). In contrast, there were no differences in $CD1c^+$ DCs (3398 ± 776 vs. 5245 ± 122/ml) or $CD141^+$ DCs (164 ± 20 vs. 218 ± 49/ml) between normotensive and hypertensive subjects. To investigate the mechanism by which AS DCs are formed in hypertension, we used two *in vitro* hypertensive stimuli: exposure to salt and hypertensive stretch of adjacent human endothelial cells. Human PBMCs were cultured in either normal NaCl (NS, 150 mM) or high NaCl (HS, 190 mM) for 48 hours. Flow cytometry indicated a striking increase in AS DCs by exposure to HS compared to NS (516 ± 181 vs 201 ± 57/ml) and this was prevented by co-treatment of cells with the salt-sensing Serum Glucocorticoid Kinase 1 inhibitor GSK650394. As a second approach, we co-cultured human aortic endothelial cells (HAECs) with PBMCs from normotensive donors and exposed the HAEC monolayer to either normal (5%) or hypertensive cyclical stretch (10%) for 24 hours. Co-culture of PBMCs with HAECs exposed to 10% stretch doubled AS DCs as compared to PBMCs cultured with HAECs undergoing 5% stretch (1.4 ± 0.5 vs 0.7 ± 0.3%; P=0.0217). These data show that AS DC population are increased in hypertensive patients and that known hypertensive stimuli *in vitro* promote formation of AS DCs. Thus, AS DCs seem to be an important immune cell subset in human hypertension and might be a novel therapeutic target for this disease.

Disclosures: J.P. Van Beusecum: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 5T32HL069765-13-JVB, F32HL143927-Pending-JPVB. **N.R. Barbaro:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHAPOST290900- NRB. **R. Loperena:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; F31HL132526. **D.G. Harrison:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA SFRN20420, R01HL125865, and R01HL039006, UL1TR000445.

Funding: Yes

Funding Component: National Center

128

Interleukin-17 Mediates Hypertension, Intrauterine Growth Restriction, Cytolytic Natural Killer Cells and Vascular Dysfunction in Pregnant Rats

Authors: Olivia Travis, Dakota White, William A Pierce, Ying Ge, Cassandra M Young, Jan Michael Williams, Frank Spradley, Denise C Cornelius, Univ Of Miss Med Ctr, Jackson, MS

Preeclampsia is a hypertensive disorder of pregnancy characterized by intrauterine growth restriction (IUGR), vascular dysfunction, and chronic immune activation including increased T_H17s and cytolytic NK cells (NK_C). We recently developed a novel model of preeclampsia in which placental ischemia (PI)-induced T_H17s cause a preeclampsia-like phenotype in pregnant rats characterized by hypertension, IUGR, oxidative stress (ROS), and increased cytolytic NK cells (NK_C). In the current study we investigated a novel role for IL-17, the main cytokine secreted from T_H17s, to directly induce IUGR, NK_C activation, and vascular dysfunction in pregnancy. IL-17 (150 pg/day) was chronically infused into a subset of normal pregnant (NP) rats from gestation day (GD) 12-19 (NP+IL-17) via i.p. minipump. On GD 18 carotid catheters were implanted and on GD 19 MAP, fetal weight, placental weight, placental NK_C, and NK_C-associated proteins were measured and vascular reactivity of uterine arteries was assessed. Data are expressed as mean±SEM. MAP significantly increased from 100±3 mmHg in NP (n=9) to 115±1 mmHg in NP+IL-17 (n=12). Fetal weight significantly decreased from 2.5±0.04 g in NP to 2.3±0.03 g in NP+IL-17 ($p<0.05$). Placental weight significantly decreased from 0.62±0.02 g in NP to 0.55±0.01 g in NP+IL-17 ($p<0.05$). Placental ROS significantly increased 1353±337 RLU/min/mg in NP to 2210±180 RLU/min/mg in NP+IL-17 ($p<0.05$). Placental NK_C increased from 2.6±1.6% of the total NK population in NP to 11.3±2.2% in NP+IL-17 ($p<0.05$). Placental granzyme B increased from 22.7±1.6 pg/mg in NP to 30.2±2 pg/mg in NP+IL-17 ($p<0.05$). Placental granzyme A increased from 3067±225 pg/mg in NP to 3926±210 pg/mg ($p<0.05$). Additionally, placental levels of VEGF, an important pro-angiogenic factor secreted by non-cytolytic uterine NK cells significantly decreased from 77.6±6.5 pg/mg in NP to 54.2±3.2 pg/mg in NP+IL-17 ($p<0.05$). We also observed impaired relaxation of uterine arteries in response to acetylcholine. These data suggest a shift from non-cytolytic NK to NK_C cells in the placentas of NP+IL17. In addition to hypertension and ROS, this study demonstrates novel roles for IL-17 to directly mediate IUGR, NK_C activation, and endothelial vascular dysfunction during pregnancy.

Disclosures: O. Travis: None. **D. White:** None. **W.A. Pierce:** None. **Y. Ge:** None. **C.M. Young:** None. **J.M. Williams:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; National Institutes of Health-NIDDK, NIGMS. **F. Spradley:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; National Institutes of Health-NHLBI. **D.C. Cornelius:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; National Institutes of Health-NHLBI, NIDDK.

Funding: No

Funding Component:

129

TRPM7 α-kinase Deficiency Causes Cardiovascular Inflammation and Fibrosis.

Authors: Francisco J Rios, Zou Zhi-guo, Livia L Camargo, Adam P Harvey, Univ of Glasgow, Glasgow, United Kingdom; Silvia Lacchini, Univ of Sao Paulo, Sao Paulo, Brazil; Panagiota Anyfanti, Aristotle Univ of Thessaloniki, Thessaloniki, Greece; Sarah McGrath, Carl S Goodyear, Augusto C Montezano, Rhian M Touyz, Univ of Glasgow, Glasgow, United Kingdom

We previously demonstrated that TRPM7, a Mg^{2+}/cation channel fused to an α-kinase domain, is regulated by vasoactive mediators and plays a protective role in hypertension. Here we questioned whether TRPM7-kinase influences vascular inflammation and fibrosis. We used Wild-type (WT) and heterozygote mutant mice for TRPM7-kinase (M7+/-). Vascular inflammatory responses were assessed *ex vivo* by intravital microscopy. Immune cells were investigated by flow cytometry. Fibrosis was investigated by sirius-red staining. Bone-marrow derived macrophages (BMDM) and Cardiac fibroblasts (CF) were obtained from WT and M7+/. $[Mg^{2+}]i$ in cardiac tissue, cardiac macrophages and circulating monocytes was significantly reduced (30-50%) in M7+/- vs WT mice. In small arteries studied by intravital microscopy, leukocytes from M7+/- showed reduced velocity (47%), increased adhesion (222%) and transmigration (480%). Expression of vascular pro-inflammatory markers including VCAM-1(33-fold), iNOS (12-fold), and IL-12 (6.8-fold) was increased in M7+/- vs WT. Cardiac galectin-3 (Gal-3) levels (16.6±3.6 vs WT 9.2±1.2 cells/field), collagen area (6.7% vs WT 2.6%), infiltration of CD45+ cells (6±0.6% vs WT 4±0.4%) and protein expression of fibronectin (280%), TGFβ (125%), and p-Smad3 (66%), were increased in M7+/- mice. BMDM macrophages from M7+/- exhibited increased levels of Gal-3 (2.6±0.05 vs WT 2.1±0.09ng/mL), IL-10 (807±92 vs WT 305±37 pg/mL) and IL-6 (84±8 vs WT 13±5 pg/mL). A similar profile was demonstrated in resident peritoneal macrophages. CF treated with supernatant of macrophages from M7+/- increased fibronectin (43%) and PCNA (36%) vs WT. To evaluate whether these processes are Mg^{2+}-sensitive, we examined effects of Mg^{2+} treatment and demonstrated that Mg^{2+} ameliorated pro-fibrotic and pro-inflammatory signalling evident in TRPM7+/- mice. In conclusion, TRPM7-kinase deficiency is associated with cardiac and vascular inflammation and fibrosis, processes associated with cellular Mg^{2+} deficiency. Our findings highlight an important cardiovascular protective role of TRPM7 and Mg^{2+}.

Disclosures: F.J. Rios: None. **Z. Zhi-guo:** None. **L.L. Camargo:** None. **A.P. Harvey:** None. **S. Lacchini:** None. **P. Anyfanti:** None. **S. McGrath:** None. **C.S. Goodyear:** None. **A.C. Montezano:** None. **R.M. Touyz:** None.

Funding: No

Funding Component:

130

ATP Release Drives the Immune Responses Associated With Hypertension

Authors: Tuantuan Zhao, Dept of Physiology, Zhejiang Univ Sch of Med, Hangzhou, China; Qian Zhu, Yu Li, Dept of Physiology, Zhejiang Univ Sch of Med, Hangzhou, China; Xiaoli Liu, Zhejiang Hosp, Hangzhou, China; Peng Shi, Inst of Translational Med, Zhejiang Univ Sch of Med, Hangzhou, China; Kenneth E Bernstein, Cedars-Sinai Medical Ctr, Los Angeles, CA; Xiao Z Shen, Dept of Physiology, Zhejiang Univ Sch of Med, Hangzhou, China

Inflammation is critical in progression of hypertension. However, how an elevated blood pressure (BP) initiates inflammation is unknown, as are the precise effects of high BP on the immune responses. To study this, we first challenged mice with antigen ovalbumin (OVA). Two-fold more OVA-specific $CD8^+$ T cells were present in the blood (2.7 ± 0.23% vs. 1.2 ± 0.47%) and spleen (2.62 ± 0.31% vs. 1.31 ± 0.17%) of angiotensin (Ang) II induced hypertensive mice as compared to those of normotensive controls. To address whether the over-activation of T cell-mediated immune responses is pathogenic, two models of autoimmune disease were studied. RIP-mOVA is a transgenic mouse line that

expresses OVA in pancreatic islet β cells. When OVA-specific OT-I T cells were infused into Rip-mOVA mice, the animals with hypertension induced by either Ang II or L-NAME developed more severe diabetes (blood glucose (mg/dl): Ang II 331, L-NAME 315 *vs.* Control 168). Concanavalin A-induced hepatitis was also significantly worsened by hypertension as compared to the pathology observed in normotensive mice. After examining the characteristics of T cells and antigen presenting cells (APCs), we found that the most distinguishable difference in hypertensive mice was the upregulation of CD86 on APCs. Blocking CD86 by antibody completely abrogated the elevated immune responses in hypertensive mice. To understand what causes CD86 upregulation in APCs of hypertensive mice, we studied damage-associated molecular patterns and found that plasma ATP levels rose as early as 3 days after the induction of hypertension and reached from about 1μM of baseline to a peak level of 3 μM after 2 weeks of hypertension, which exactly parallels the kinetics of CD86 elevation on APCs. Hydrolyzing ATP or blocking its $P2X_7$ receptor normalized APC CD86 expression and eliminated hypertension-induced T cell over-activation. Further, untreated human hypertensive patients have substantially elevated plasma ATP levels (2.21 ± 0.99 μM, *n* = 27) compared to treated hypertensive patients (0.94 ± 0.34 μM, *n* = 17) or normotensive controls (0.63 ± 0.38 μM, *n* =30). A linear trend was observed between ATP levels and BP among all subjects. These studies indicate ATP is critical in initiating hypertension-associated inflammation.

Disclosures: T. Zhao: None. **Q. Zhu:** None. **Y. Li:** None. **X. Liu:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; 2013ZA009, 2014KYB006, 2015C33135. **P. Shi:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NSFC31771266, NSFC81700365. **K.E. Bernstein:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH P01HL129941-01. **X.Z. Shen:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NSFC81670378.

Funding: No

Funding Component:

131

Microbiota-Derived Metabolite Propionate Protects From Hypertensive Cardiovascular Damage

Authors: Nicola Wilck, Hendrik Bartolomaeus, András Balogh, ECRC, Berlin, Germany; Mina Yakoub, Dept of Nephrology, Univ Hosp Düsseldorf, Heinrich-Heine-Univ Düsseldorf, Düsseldorf, Germany; Susanne Homann, Inst of Pharmacology and Clinical Pharmacology, Univ Hosp Düsseldorf, Heinrich-Heine-Univ Düsseldorf, Düsseldorf, Germany; Lajos Markó, ECRC, Berlin, Germany; Sascha Höges, Dept of Nephrology, Univ Hosp Düsseldorf, Heinrich-Heine-Univ Düsseldorf, Düsseldorf, Germany; Dmitry Tsvetkov, Sebastian Wundersitz, Nadine Haase, ECRC, Berlin, Germany; Lydia Hering, Dept of Nephrology, Univ Hosp Düsseldorf, Heinrich-Heine-Univ Düsseldorf, Düsseldorf, Germany; Martina Maase, Kristina Kusche-Vihrog, Inst of Physiology II, Univ of Münster, Münster, Germany; Maria Grandoch, Inst of Pharmacology and Clinical Pharmacology, Univ Hosp Düsseldorf, Heinrich-Heine-Univ Düsseldorf, Düsseldorf, Germany; Jens Fielitz, ECRC, Berlin, Germany; Stefan Kempa, MDC Berlin, Berlin, Germany; Sofia K. Forslund, ECRC, Berlin, Germany; Almagul Kushugulova, Natl Lab Astana Nazarbayev Univ, Astana, Kazakhstan; Ralf Dechend, ECRC, Berlin, Germany; Kai-Uwe Eckardt, Charité Univ Med, Div of Nephrology and Internal Intensive Care Med, Berlin, Germany; Lars C Rump, Dept of Nephrology, Univ Hosp Düsseldorf, Heinrich-Heine-Univ Düsseldorf, Düsseldorf, Germany; Dominik N Müller, ECRC, Berlin, Germany; Johannes Stegbauer, Dept of Nephrology, Univ Hosp Düsseldorf, Heinrich-Heine-Univ Düsseldorf, Düsseldorf, Germany

Objective: Inflammation drives cardiovascular disease, anti-inflammatory approaches may be beneficial. Short-chain fatty acids (SCFA) are bacterial metabolites with anti-inflammatory properties affecting host immune homeostasis including regulatory T cells (Treg). We investigated effects of the SCFA propionate (administered in drinking water, NaCl as control) in two mouse models, namely hypertensive heart disease (wild-type NMRI (WT), angiotensin (Ang)II infusion 1.44 mg/kg/d s.c. for 14 days) and atherosclerosis (Apolipoprotein E knockout (ApoE), AngII infusion 0.72 mg/kg/d s.c.

for 28 days), respectively. **Results:** Propionate attenuated cardiac hypertrophy and fibrosis in both models significantly. Susceptibility to cardiac ventricular arrhythmias was significantly reduced in propionate-treated WT mice. Aortic atherosclerotic lesion area was significantly reduced in propionate-treated ApoE (27.6±8 vs. 7.9±2.4%). Treatment reduced splenic effector memory (CD4+ CD44+ CD62L-) T cell frequencies (WT: 30.5±4.6 vs. 19.1±1.6; ApoE: 41.1±3.1 vs. 32.7±1.4%) and splenic Th17 cells (WT: 1.0±0.2 vs. 0.6±0.1; ApoE: 1.3±0.1 vs. 0.9±0.1%) in both models, indicating beneficial effects on systemic inflammation. Similarly, propionate reduced cardiac immune cell infiltration (CD4+, CD8+, F4/80+) in WT mice. Propionate improved vascular dysfunction and moderately reduced blood pressure in both models. Organ-protective actions of propionate (cardiac inflammation and fibrosis) were abrogated in Treg-depleted (antiCD25-treated) AngII-infused WT mice, suggesting a central role for Treg. To verify our findings in a human cohort, we re-analyzed clinical and metagenomic data from a recent randomized controlled trial investigating the effect of a 90-day synbiotic intervention in 84 subjects with metabolic syndrome including healthy controls. Interestingly, in synbiotic-treated subjects an increased capacity for SFCA production was significantly correlated to blood pressure reduction. **Conclusion:** Data underscore the importance of SCFA for cardiovascular health and suggest that lifestyle modifications leading to augmented SCFA production could be a beneficial non-pharmacological add-on strategy for cardiovascular disease.

Disclosures: N. Wilck: None. **H. Bartolomaeus:** None. **A. Balogh:** None. **M. Yakoub:** None. **S. Homann:** None. **L. Markó:** None. **S. Höges:** None. **D. Tsvetkov:** None. **S. Wundersitz:** None. **N. Haase:** None. **L. Hering:** None. **M. Maase:** None. **K. Kusche-Vihrog:** None. **M. Grandoch:** None. **J. Fielitz:** None. **S. Kempa:** None. **S.K. Forslund:** None. **A. Kushugulova:** None. **R. Dechend:** None. **K. Eckardt:** None. **L.C. Rump:** None. **D.N. Müller:** None. **J. Stegbauer:** None.

Funding: No

Funding Component:

132

Obesity-Related Hypertension Develops Through Splenic Sympathetic Overactivity and is Mediated by Immune-Modulating Functions of Placental Growth Factor

Authors: Daniela Carnevale, Sapienzauniversity/Irccs Neuromed, Pozzilli, Italy; Andrea Lori, Lorenzo Carnevale, IRCCS Neuromed, Pozzilli, Italy; Marialuisa Perrotta, Sapienza Univ of Rome, Pozzilli, Italy; Fabio Pallante, Raimondo Carnevale, IRCCS Neuromed, Pozzilli, Italy; Daniele Iodice, IRCCS NEUROMED, Pozzilli, Italy; Giuseppe Lembo, Sapienza Univ of Rome and Irccs Neuromed, Pozzilli, Italy

Obesity-related hypertension (HTN) is an epidemic health problem and a major risk factor for the development of cardiovascular disease. A consistent amount of research on the pathophysiological basis of obesity has implicated a crucial role of sympathetic overdrive. Interestingly, an overactivation of the sympathetic nervous system (SNS) accompanies HTN as well. Recent data highlighted that increased SNS activation in HTN is important in modulating immune responses, besides controlling typical cardiovascular functions. Whether neuroimmune mechanisms are relevant in obesity-induced HTN is still unknown. To test this hypothesis we subjected 6 week-old C57Bl/6J mice to high fat diet (HFD), as compared to low fat diet (LFD) and monitored blood pressure (BP), which started to rise after 2 months and steadily increased up to 4 months. We measured circulating noradrenalin, finding it significantly elevated 2 months after HFD. To gain insights in the potential SNS modulation of immunity, we measured by microneurography SNS activation on the splenic nerve. Firing frequency (FF, spikes in 10') of splenic nerve was increased in C57Bl/6J mice on HFD (FF:432±81) as compared to LFD (FF:86±10, $p<0.01$). Then, we surgically removed splenic innervation by left celiac ganglionectomy (CGX) and fed mice with HFD for 4 months. Despite CGX-mice become obese similarly to sham, as shown by body weight and microCT-measured fat pad, they were protected from HTN (SBP_{mmHg}=CGX:105±4; sham:125±5, $p<0.001$), suggesting that the splenic sympathetic overdrive influences BP responses but not metabolic alterations. To look for molecular determinants, we analyzed the expression of Placental Growth Factor (PlGF),

previously identified as a neuroimmune mediator, and found it significantly increased in the spleen upon HFD, but only with intact SNS innervation, since CGX-mice did not increase it. In the end, PlGF KO mice subjected to HFD, although becoming obese as much as WT, were protected from HTN (SBP_{mmHg}=PlGF KO:103±4; WT:123±6, p<0.001). Interestingly, both CGX and PlGF KO mice displayed a reduced egression of activated T cells from the spleen upon HFD feeding, suggesting that the protection observed could be related to reduced infiltration of activated lymphocytes in target organs.

Disclosures: D. Carnevale: None. **A. Lori:** None. **L. Carnevale:** None. **M. Perrotta:** None. **F. Pallante:** None. **R. Carnevale:** None. **D. Iodice:** None. **G. Lembo:** None.

Funding: No

Funding Component:

133

Endothelial-Specific Interference With PPARγ Increases the Susceptibility to Angiotensin II-Induced Endothelial Dysfunction in Adult Offspring Born from AVP-Infused Pregnancies

Authors: 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

Mutations in the ligand-activated transcription factor 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 risk to offspring. PE is also associated with vascular dysfunction. We 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 infusion of vasopressin (AVP, 24 ng/hr sc) throughout gestation. Phenotypes of PE were first assessed in pregnant dams and then in adult offspring. 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 were phenotyped in adulthood. Data were stratified to sex, genotype, and exposure to AVP or SAL. At 20 weeks of age, SBP and vasorelaxation responses to acetylcholine were similar in offspring exposed to PE compared to mice born from SAL pregnancies. Adult offspring were next exposed to a sub-pressor dose of Angiotensin II (ANG; 120ng/kg/hr) for 14 days. Adult male ANG-treated E-DN born to PE pregnancies, but not NT mice exhibited significant impairment in ACh-induced relaxation in carotid artery (% relaxation: 62±10 vs 86±11, p<0.05). Adult female ANG-treated E-DN exposed to PE also exhibited a trend for impaired endothelial function compared to NT controls (% relaxation: 63±9 vs 72±10). Endothelial dysfunction in male ANG-treated E-DN born to PE pregnancies was attenuated by tempol (relaxation improved from 56±9% vs 81±12%, p<0.05), indicative of oxidative stress, and by a Rho-kinase inhibitor (relaxation improved from 73±11% vs 89±12%, p<0.05). This data suggests that interference with endothelial PPARγ in pups born from PE pregnancies increases the risk for endothelial dysfunction upon exposure to a cardiovascular stressor in adulthood. Thus, conditions which impair PPARγ activity, such as obesity and diabetes, may predispose adult offspring born from PE pregnancy to cardiovascular disease.

Disclosures: A.R. Nair: None. **M. Mukohda:** None. **L.N. Agbor:** None. **K. Lu:** None. **J. Wu:** None. **J.A. Sandgren:** None. **J.L. Grobe:** None. **C.D. Sigmund:** None.

Funding: Yes

Funding Component: National Center

134

The Effect of Low Birth Weight on the Microcirculation in the 1St Year of Life

Authors: Muti E Goloba, Rajendra Raghuraman, Uzma Khan, Amelia Brown, Monique Klein, Nansi Botros, St George's Univ, London, United Kingdom; Donovan Duffy, Neonatal Unit, St George's Univ Hosp NHS Fndn Trust, London, United Kingdom; Duolao Wang, Dept of Clinical Sciences, Liverpool Sch of Tropical Med, Liverpool, United Kingdom; Isaac Manyonda, Obstetrics & Gynaecology Dept, St George's Univ Hosp NHS Fndn Trust, London, United Kingdom; Tarek F Antonios, St George's Univ and St George's Univ Hosp NHS Fndn Trust, London, United Kingdom

Introduction: Capillary rarefaction, defined as a reduction in capillary density, is an established hallmark of essential hypertension. Low birth weight (LBW) infants, known to have an increased risk of developing hypertension as adults, were unexpectedly found to have a significantly higher capillary density at birth when compared to normal birth weight (NBW) infants. We therefore hypothesised that there is a microcirculatory window in the 1st year of life of LBW infants, during which a process of extensive capillary loss, known as "hyperpruning", occurs.
Methods: The George's Capillary Rarefaction Offspring Study (G-CROS) is a longitudinal, multi-centre study of which 284 infants were NBW, born at term, and 77 were LBW. Intravital microscopy was used to measure the functional (also known as basal) capillary density (BCD), and the structural (also known as maximal) capillary density (MCD) at birth, 3 months, 6 months and 12 months.
Results: LBW infants had a significantly higher capillary density at birth when compared to NBW infants ($p < 0.0001$). NBW infants showed a gradual reduction in capillary density between birth and 12 months, with their greatest reduction occurring between birth and 3 months (BCD mean difference = -27.62 cap/field, p <0.0001 and MCD mean difference = -31.49 cap/field, p <0.0001). Similarly, the most significant reduction in BCD and MCD, in the LBW cohort, was between birth and 3 months (BCD mean difference = -47.01 cap/field, $p < 0.0001$ and MCD mean difference = -48.01 cap/field, $p < 0.0001$). However, within this same 3-month interval, LBW infants demonstrated a significantly higher percentage reduction in BCD (mean difference = 7.81%, $p = 0.0194$) and MCD (mean difference = 8.29%, $p = 0.0361$) when compared to NBW infants.
Conclusions: Once again, LBW infants were shown to have a higher capillary density than NBW infants at birth. However, this study has shown for the first time that there appears to be a microcirculatory window in the first 3 months of life during which LBW infants undergo capillary "hyperpruning". Further follow-up studies are required to investigate the role of early capillary rarefaction in the pathogenesis of hypertension, as well as the mechanisms orchestrating these early microcirculatory changes.

Disclosures: M.E. Goloba: None. **R. Raghuraman:** None. **U. Khan:** None. **A. Brown:** None. **M. Klein:** None. **N. Botros:** None. **D. Duffy:** None. **D. Wang:** None. **I. Manyonda:** None. **T.F. Antonios:** None.

Funding: No

Funding Component:

135

Impaired Autonomic Function in Young Adults Born Preterm With Very Low Birth Weight is Associated With Elevated Serum Uric Acid Levels

Authors: Hossam A Shaltout, Wake Forest Sch of Med, Winston Salem, NC; Patricia A Nixon, Wake Forest Univ, Winston Salem, NC; Mark C Chappell, Debra I Diz, Andrew M South, Elizabeth T Jensen, Lisa K Washburn, Wake Forest Sch of Med, Winston Salem, NC

Preterm birth increases the risk of cardiometabolic disease. We showed that prematurity induces programming effects that lead to elevated blood pressure (BP), higher serum uric acid, and impaired autonomic control characterized by

reduced heart rate variability (HRV), baroreflex sensitivity (BRS), and elevated ratio of low frequency to high frequency power (LF/HF), a marker of sympathovagal balance. However, the relationships among these factors in subjects born preterm are undefined. Since uric acid has been shown to correlate with HRV in adult hypertensive subjects, we hypothesized that higher uric acid is associated with impaired autonomic function in subjects born preterm. Methods:A cohort of 131 young adults born preterm was compared to a cohort of 26 born term (19.6 years). Serum uric acid was measured. BP and ECG were recorded continuously for analysis of autonomic function reflected in HRV and BRS. We used generalized linear models to estimate the association between uric acid and measures of HRV and BRS, adjusting for race, age at follow up, and BMI. The potential interaction between uric acid and preterm birth was tested by introducing an interaction term and, if suggestive of an interaction ($p \leq 0.1$), estimates were calculated within strata (preterm & term).Results: Compared to term, preterm participants had higher serum uric acid levels (5.2 mg/dL, IQR: 4.2, 6.2 vs 4.8 mg/dL, IQR: 3.9, 6.0) and lower HRV as measured by standard deviation of normal to normal RR intervals (71 ms, IQR: 54, 97 vs 80 ms, IQR: 55, 101). Across term and preterm groups combined, uric acid was inversely associated with BRS measured as HF alpha (β: -0.12, 95% CI -0.20 to -0.03, p for interaction = 0.06). Assessment of interaction showed this association was significant in preterms only (β: -0.15, 95% CI -0.24 to -0.06 vs 0.07, -0.17 to 0.30). Uric acid was positively associated with LF/HF (β: 0.13, 95% CI 0.01 to 0.25, p for interaction=0.01). Conclusions: Young adults born preterm had dampened parasympathetic control of heart rate and BP, and higher serum uric acid was associated with sympathovagal imbalance. The data suggest that alterations of uric acid metabolism associated with preterm birth may increase the risk of early cardiovascular disease in part by impairing autonomic control

Disclosures: H.A. Shaltout: None. **P.A. Nixon:** None. **M.C. Chappell:** None. **D.I. Diz:** None. **A.M. South:** None. **E.T. Jensen:** None. **L.K. Washburn:** None.

Funding: No

Funding Component:

136

Attenuation of Increased Blood Pressure by Chronic Estrogen Supplementation in Female IUGR Offspring at 12 months of Age is Associated With a Shift Towards ACE2

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

Low birth weight (**LBW**) women exhibit an earlier age at menopause and a two-fold greater prevalence of hypertension in later life. In a model of LBW induced via placental insufficiency in the rat, female LBW rats develop an increase in blood pressure (**BP**) by 12 months of age. This is associated with persistent estrous that occurs 6 months prior to controls, indicative of early reproductive senescence. Although the underlying mechanisms are unknown, a shift in the hormonal milieu in addition to dysregulation of the renin angiotensin system (**RAS**) are potential contributors to the increase in BP that occurs in women after menopause. The vasoconstrictor arm of the RAS is implicated in increased BP; ACE2 counterbalances this shift towards increased BP. We previously reported that renal ACE2 expression is elevated in female LBW rats that are normotensive in young adulthood; an increase that is not present in female LBW rats in later life. Thus, this study tested the hypothesis that chronic 17β-estradiol (**E2**) supplementation would reduce BP and shift the balance of the RAS in female LBW rats. Placebo or E2 pellets (1.5mg 17β-estradiol, 60-day release) were implanted at 12 months of age for 6 weeks followed by measurement of BP in conscious, chronically catheterized animals. Kidneys were collected for molecular analysis. BP was significantly increased in placebo-treated LBW relative to placebo-treated Control (134 vs 122 mmHg, respectively, $p<0.05$); an increase that was abolished by chronic E2 in LBW (116 mmHg, $p<0.05$). Using Real-Time PCR, cortical ACE2 mRNA expression did not differ between Control or LBW rats. Medullary ACE2 mRNA expression was significantly increased in placebo-treated LBW compared to placebo-treated Controls (1-fold

increase; $p<0.05$). However, following chronic E2, medullary ACE2 mRNA expression was increased to a greater degree in chronic E2-treated LBW rats, almost two-fold higher, relative to chronic E2-treated Control. Therefore, these results suggest that E2 supplementation attenuates increased BP in female LBW rats in a manner that may involve a shift in the RAS towards ACE2 abundance suggesting that ACE2 via chronic E2 may be beneficial in the treatment of increased BP that occurs after reproductive senescence in LBW.

Disclosures: G.K. Davis: None. **A.D. Newsome:** None. **N.B. Ojeda:** None. **B.T. Alexander:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

137

Progesterone Induced Blocking Factor Improves Clinical Characteristics of Preeclampsia in Rupp Rats

Authors: Lorena M Amaral, Mark W. Cunningham Jr, Jesse N. Cottrell, Denise C Cornelius, Tarek Ibrahim, Babbette LaMarca, Medical Ctr- UMMC, Jackson, MS

Preeclampsia (PE) is characterized by new onset hypertension in association with elevated natural killer (NK) cells and inflammatory cytokines which are likely culprits for decreased fetal weight during PE pregnancies. Progesterone induced blocking factor (PIBF) increases during normal pregnancy and has been shown to decrease inflammation and cytolytic NK cells, both of which are increased during PE. Currently, there is no effective treatment for PE except for early delivery of the fetal placental unit, making PE the leading cause for premature births worldwide. We have previously shown that progesterone supplementation with 17-OHPC improves inflammation, fetal weight and blood pressure in the preclinical RUPP rat model of PE. However the mechanism where by progesterone improves the pathophysiology of PE has never been determined. This study was designed to test the hypothesis that PIBF reduces inflammation while improving hypertension in response to placental ischemia. To test this hypothesis, PIBF (2.0 µg/mL) was administered intraperitoneally on gestation day 15 to RUPP or normal pregnant (NP) rats and on day 18 carotid catheters were inserted and on GD 19 blood pressure and samples were collected. MAP in NP rats (n=9) was 101$\pm$ 3 and 110 $\pm$ 3 in NP+PIBF (n=5), 122$\pm$ 2 in RUPP rats (n=7), which improved to 110$\pm$3 mmHg in RUPP+PIBF (n=9), $p<0.05$. Pup weight was 2.4$\pm$ 0.1 g in NP, 2.5$\pm$ 0.1 in NP+PIBF, 1.9$\pm$0.1 in RUPP and improved to 2.2$\pm$0.1 in RUPP+PIBF. Neither placental weight nor litter size was affected by PIBF. Fetal reabsorption was lower in RUPP+PIBF compared to RUPP rats. Total placental NK cells were 31$\pm$ 9 in NP (n=4), 31$\pm$5 in NP+PIBF (n=4), 42 $\pm$8 in RUPP rats (n=4) and reduced to 26$\pm$ 2 in RUPP+PIBF (n=6). Placental cytolytic NK cells were 0.8$\pm$0.1 in NP, 0.2$\pm$0.1 in NP+PIBF, and 1.4$\pm$0.1 in RUPP rats, which decreased to 0.4+0.1 in RUPP+PIBF. $CD4^+$ T cells were decreased from 21$\pm$6 in RUPP rats (n=4) to 8$\pm$2 gate in RUPP+PIBF (n=7). Collectively, our findings demonstrate that PIBF, a produce to progesterone receptor stimulation by progesterone, could be a mechanism to improve fetal growth restriction, inflammation as indicated by CD4+ T cells and cytolytic NK cells while normalizing blood pressure in response to placental ischemia during pregnancy.

Disclosures: L.M. Amaral: None. **M.W. Cunningham Jr:** None. **J.N. Cottrell:** None. **D.C. Cornelius:** None. **T. Ibrahim:** None. **B. LaMarca:** None.

Funding: No

Funding Component:

138

White AdiposeTissue in the Preeclamptic-Like BPH/5 Mouse Has a Unique Inflammatory Molecular Signature During Early Pregnancy

Authors: Jennifer L Sones, Dorien Reijnders, Kelsey Olson, Veterinary Clinical Sciences, Sch of Veterinary Med, Louisiana State Univ,, Baton Rouge, LA; Leanne Redman, Pennington Biomedical Res Ctr, Baton Rouge, LA

Introduction: Preeclampsia (PE) is a hypertensive disease of pregnancy that affects an estimated 8% of women worldwide. The underlying mechanism is unknown; however, maternal obesity increases the risk of PE by 30% and may contribute to increased inflammation seen in preeclamptic mothers. **Hypothesis:** To better understand the role of white adipose tissue (WAT) signaling on adverse pregnancy outcomes, we used the obese PE-like BPH/5 mouse to test the hypothesis that visceral WAT has an inflammatory phenotype prior to the presentation of the maternal hypertensive syndrome **Methods:** Visceral WAT adjacent to the reproductive tract ("reproductive WAT") was harvested from ad libitum fed control C57 and BPH/5 female mice (8 weeks of age) at embryonic day (e) 7.5. RNA was isolated and subjected to Next Generation Sequencing (NGS) using an Illumina platform. DAVID pathway analysis was performed to annotate differentially expressed genes. BPH/5 female mice were calorie restricted by 25% from e0.5 until e7.5 and reproductive WAT was collected for RNA isolation and quantitative real-time PCR. The calorie restriction paradigm has been shown to significantly reduce reproductive WAT accumulation throughout gestation in BPH/5 mice. **Results:** NGS of e7.5 reproductive WAT revealed 899 genes that were significantly dysregulated in BPH/5 vs C57. DAVID showed upregulation of lipid metabolic processes in both C57 and BPH/5 pregnant reproductive WAT, while 18 inflammation-related pathways were significantly upregulated in reproductive WAT of pregnant BPH/5 mice compared to pregnant C57 (FDR<=5%, adjP<=0.1, FC >=1.5). Quantitative real-time PCR confirmed upregulation of PE- related inflammatory genes, interleukin (IL)-6, IL-15 and Ptgs2, in e7.5 BPH/5 reproductive WAT vs C57 (n=6, $p<0.05$). Seven days 25% calorie restriction during pregnancy significantly reduced this elevated IL-6 and Ptgs2 mRNA expression in reproductive WAT (n=6, $p<0.05$) **Conclusions:** These data show that obese PE-like BPH/5 mice have elevated inflammatory gene expression in reproductive WAT before placenta formation. Further investigations are needed to confirm if the reduction in IL-6 and Ptgs2 in reproductive WAT by calorie restriction improves fetoplacental and maternal outcomes in BPH/5 mice

Disclosures: J.L. Sones: None. **D. Reijnders:** None. **K. Olson:** None. **L. Redman:** None.

Funding: No

Funding Component:

P102

Ablation of MMP-9 and H_2S Treatment Mitigates Ocular Hypertension and Retinal Remodeling

Authors: Akash K. George, Mahavir Singh, Rubens P. Homme, Naira Metreveli, **Suresh C. Tyagi**, UNIV OF LOUISVILLE MEDICAL SCHOOL, Louisville, KY

ABLATION OF MMP-9 AND H_2S TREATMENT MITIGATES OCULAR HYPERTENSION AND RETINAL REMODELINGAkash K George, Mahavir Singh, Rubens P Homme, Naira Metreveli, Suresh C TyagiDepartment of Physiology, University of Louisville, Louisville, KY 40202, USAFVB/NJ mouse is homozygous for retinal degeneration 1 allele of *Pde6b*^{rd1} and as a result it is functionally blind because of retinal degeneration. Further, *Mmp9* knockout (KO) mouse is bred on FVB background and null mice for *Mmp9* show altered repair of injury in skin, cornea, CNS, bone marrow reconstitution and altered inflammatory responses. We employed *Mmp9* KO line for studying various aspects of visual physiology and compared them with that of parental strain FVB/NJ along with wild type C57BL/6J (C57). Since our group has previously demonstrated that increased activity of MMP-9 leads to degradation of ECM, we thus hypothesized that MMP-9 plays crucial role in retinal remodeling followed by leakage of plasma contents into ocular compartment thereby

compromising retinal architecture and intraocular pressure. To test this hypothesis we subjected *Mmp9* KO, FVB and C57 mice individually for monitoring their intraocular pressure (IOP) and also performed biochemical, fluorescence imaging, visually guided behavioral studies and histological analyses on their retinae. IOP as measured by tonometry revealed a significant increase in eye pressure especially in FVB strain in comparison to *Mmp9* KO and C57 mice. Retinal blood vessels' leakage was measured using FITC-BSA angiography. Our earlier study revealed a decrease in MMP-9 activity by hydrogen sulfide (H_2S). Here, we also treated FVB mice with H_2S donor and again measured IOP. Results suggested that higher constitutive MMP-9 activity in FVB mice while there was no detectable activity in *Mmp9* KO mice. There was more leakage in FVB as compared to *Mmp9* KO. FVB treated with H_2S showed decreased MMP-9 activity and relatively less leakage of their retinal blood vessels. Ocular as well as systemic blood pressures were higher in FVB as compared to other groups. Visually guided tests revealed improved navigation in FVB after treatment with H_2S. Together the overall results suggest that MMP-9 is associated with altered vision and that treatment with H_2S could halt vision loss.

Disclosures: A.K. George: None. **M. Singh:** None. **R.P. Homme:** None. **N. Metreveli:** None. **S.C. Tyagi:** None.

Funding:

Funding Component:

P103

Hepatocyte Depolarization Induces a Pressor Response

Authors: Yao Xiao, **Benjamin J Renquist**, UNIVERSITY OF ARIZONA, Tucson, AZ

Obesity and the resulting hepatic lipid accumulation increase the incidence of hypertension. In obese adults, the prevalence of excessive hepatic lipid accumulation exceeds 80%. Hepatic lipid accumulation is linked to both the incidence and severity of hypertension. Severing the hepatic vagal nerve eliminates obesity induced hypertension in mice, suggesting that the lipid loaded hepatocyte can affect hepatic vagal nerve activity to increase blood pressure. To understand the role of obesity induced hepatocyte depolarization in the control of blood pressure, we administered an adeno-associated virus designed to induce hepatocyte specific expression of a genetically-engineered, ligand-gated, selective ion channel. In mice that expressed the channel we were then able to acutely depolarize hepatocytes by administering ligand and assess the response. Using electrophysiology, we established that ligand administration depolarized the hepatocyte and decreased hepatic vagal afferent nerve activity by more than 20% (78 out of 100; $P < 0.05$). Using telemetry, we showed that ligand induced hepatocyte depolarization significantly increased blood pressure from 25-40 minutes after ligand administration without affecting heart rate ($P < 0.05$; maximal increase at 35 minutes). The response was maximal 35 minutes after ligand administration when systolic, diastolic, and mean blood pressure were increased by 33.65 ± 7.59, 29.5 ± 7.14, and 31.33 ± 7.26 mmHg ($P < 0.05$; $n = 8$). Hepatic vagotomy completely eliminated the response to ligand. In mice that did not express the channel there was no response to ligand. We have established that the depolarization caused by hepatic lipid accumulation can depress activity of the vagus nerve and modulate blood pressure. Therapeutics that prevent hepatocyte depolarization may be beneficial in preventing obesity induced hypertension.

Disclosures: Y. Xiao: None. **B.J. Renquist:** None.

Funding:

Funding Component:

P104

Urban And Rural Differences In Exposure And Effects Of Micro Air Particles On Blood Pressure Parameters.

Authors: Benedicta N Nkeh-Chungag, WALTER SISULU UNIVERSITY, Mthatha, South Africa; Anye Chungag, Univ of Fort Hare, Alice, South Africa; Constance R Sewani-Rusike, WALTER SISULU UNIVERSITY, Mthatha, South Africa

Several studies have shown a relationship between indoor particulate matter and cardiovascular diseases in adults though this relationship is yet to be clarified in children and adolescents. This study purposed to investigate the relationship between indoor air particulate matter and blood pressure in 10-14 year old rural and urban children in Eastern Cape, South Africa. A cross sectional study of 10-14 year old children was carried out in selected rural and urban schools of the Eastern Cape. Anthropometry was performed and blood pressure measured. Indoor air particle counts were determine. Blood pressure parameters and body mass index (BMI) were converted to percentiles for height, sex and age. Prevalence of overweight/obesity were 10.9%/14.0% and hypertension/prehypertension were 12%/20%. Hypertension/prehypertension were more prevalent in rural compared to urban adolescents. All blood parameters were higher in rural adolescents and higher in rural girls compared to boys. In hypertensive/prehypertensive adolescents, systolic blood pressure (SBP) showed a strong correlation with BMI, waist, hip and mid upper arm circumferences (0.50, 0.51, 0.52, 0.53, 0.49; $p<0.05$ respectively) though SBP was only modestly associated with these variable in normotensive adolescents. Indoor particle counts were higher in rural schools. Blood pressure did not correlate with indoor air particulate matter though there was a weak correlation between PM2.5, 5, 10 and heart rate in rural adolescents. Indoor air particle failed to show any relationship with blood pressure parameters in urban adolescents. Rural adolescents had higher blood pressure and heart rates. Indoor particulate matter may be associated with blood raised pressure via sympathetic activation as suggested by higher heart rates.

Disclosures: B.N. Nkeh-Chungag: None. **A. Chungag:** None. **C.R. Sewani-Rusike:** None.

Funding:

Funding Component:

P105

An Exploration Of The Relationship Between Endothelial Function, Blood Pressure And Anthropometric Measurements In 6-8 Year Old Rural Versus Urban Children In The Eastern Cape Province Of South Africa.

Authors: Prescilla B Letwalo, Edna N Matjuda, Constance R Sewani-Rusike, Benedicta N Nkeh-Chungag, WALTER SISULU UNIVERSITY, Mthatha, South Africa

Arterial stiffness is an independent predictor of cardiovascular disease. Although mostly detected in adults evidence in children is conflicting. Many more children are being diagnosed with hypertension indicating that these children might have some level of endothelial dysfunction. The current study set out to investigate the relationship between endothelial function as measured by the Vicorder and anthropometric measurements as well as blood pressure in 6-8 year old children. A total of 202 children were recruited from a rural and an urban community were recruited into a cross sectional study between March and November 2017. Anthropometry was performed followed by measurement of blood pressure parameters and flow mediated slowing (FMS) using the Vicorder. Urine samples were collected for creatinine/albumin ratio. Body mass index (BMI), systolic and diastolic blood pressure (SBP, DBP) were converted to percentiles for age, height and sex. Stata ® was used for all data analysis. Mean PWV and FMS% were 5.0 m/s and 25.7% respectively. PWV and heart rate (HR) were different by sex and location ($p<0.01$ and $p<0.0001$ respectively) while FMS% was similar in all groups. FMS% correlated negatively with most anthropometric and blood pressure variables. PWV was significantly higher in hypertensive children (5.9 vs 4.7; $p<0.05$) and correlated modestly with Neck circumference ($r= 0.46$; $p< 0.05$), SBP ($r= 0.35$; $p<0.05$), DBP ($r=0.046$; $p< 0.05$) while in rural males it correlated with SBP

and DBP (r=0.50, r= 0.44; p, 0.05) and it urban males it correlated with creatinine/albumin ratio (0.52; p< 0.05). There was no correlation in urban females. PWV was higher in 6-8 year old children who had either hypertension or pre-hypertension and was predicted by DBP in rural girls. Correlation of PVW with creatinine/albumin ration may be indicative of compromised endothelial function.

Disclosures: P.B. Letwalo: None. **E.N. Matjuda:** None. **C.R. Sewani-Rusike:** None. **B.N. Nkeh-Chungag:** None.

Funding:

Funding Component:

P106

Association Of Pulse Wave Velocity With Hypertension And Anthropometric Measurements In 6-8 Years Old Children In Mthatha, South Africa

Authors: Edna N Matjuda, Prescilla B Letswalo, Constance R Sewani-Rusike, Benedicta N Nkeh-Chungag, WALTER SISULU UNIVERSITY, Mthatha, South Africa

Hypertension is becoming a major health problem in children worldwide. There is evidence suggesting a complex association between hypertension, endothelial function and other cardiovascular disease risk factors in the adult population. Very few studies have investigated the relationship between hypertension and endothelial function in sub-Saharan children. The aim of this study was to explore the relationship between blood pressure parameters and pulse wave velocity (PWV) in 6-8 years old children in the Eastern Cape of South Africa. A cross-sectional study of 202 children (103 girls and 99 boys) aged 6-8 years old was conducted between March and November 2017. General anthropometric measurements were measured and blood pressure measurements were recorded. Blood pressure measurements were converted to blood pressure percentiles for height, age and sex. Flow mediated slowing was used to measure endothelial function. The prevalence of hypertension and prehypertension was higher in girls compared to boys (7.9% and 28.7% vs 7.1% and 20.2% respectively). Systolic and diastolic pressure (SBP, DBP) and pulse pressures were similar in boys and girls. No sex related difference in PWV was noted thought the strength of correlation was different. PWV correlated modestly with age, SBP, DBP and mean arterial BP in females (r= 0.24, 0.30, 0.40, 0.49 p<0.05) and in males (r= 0.38, 0.36, 0.34, 0.39; p<0.05). Blood pressure and PWV correlated modestly with anthropometric measurements in females but not in males. This study in a sub-Saharan African population of 6-8 year old children showed that even though there was no sex difference in blood pressure, the prevalence of hypertension and pre-hypertension were different. PWV correlated differently with blood pressure parameters and anthropometric measurements in males and females.

Disclosures: E.N. Matjuda: None. **P.B. Letswalo:** None. **C.R. Sewani-Rusike:** None. **B.N. Nkeh-Chungag:** None.

Funding:

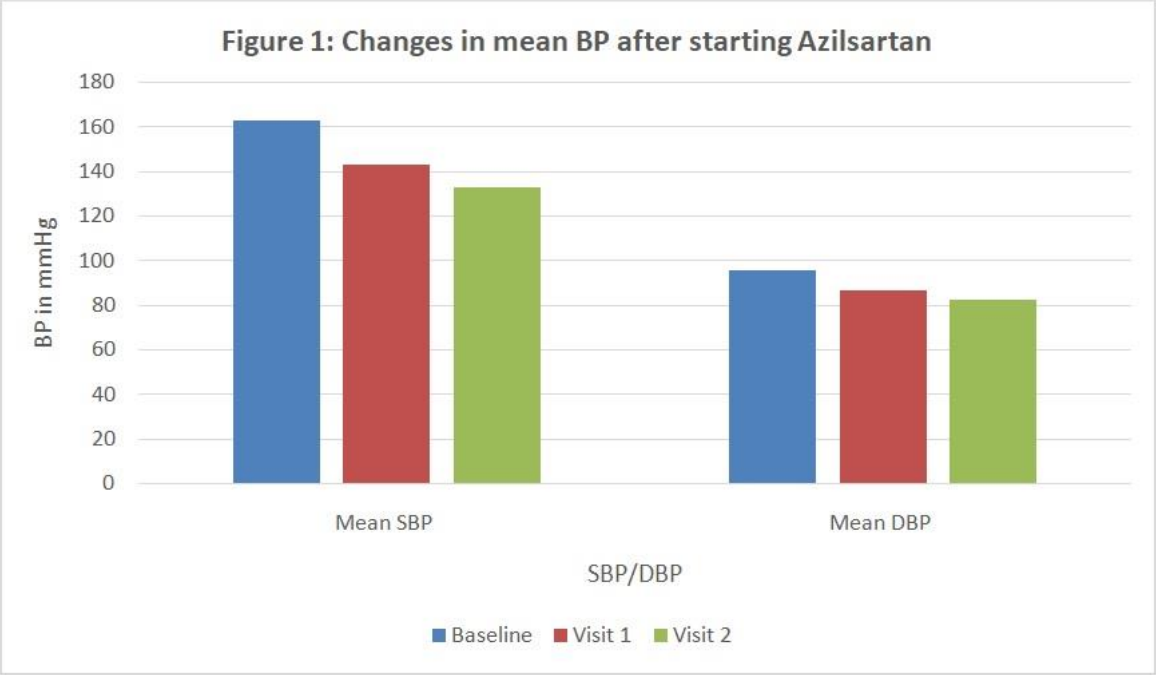
Funding Component:

P107

Real-World Azilsartan efficacy Assessment Study in Indian Hypertensive patients (RAAS-India)

Authors: Uday Jadhav, MGM NEW BOMBAY HOSPITAL, NEW MUMBAI, India; JAMSHED DALAL, KOKILABEN AMBANI HOSPITAL, MUMBAI, India

Background: Azilsartan, a potent angiotensin type 1 receptor blocker (ARB) has limited data on the efficacy and safety in hypertensive subjects in India. **Methods:**Retrospective audit based on data from the prescribing physicians on efficacy of Azilsartan monotherapy or in combination with other antihypertensive drugs. Office blood pressure (BP) measurement prior to initiation of Azilsartan, at 1st and 2nd follow-up visits were recorded. Two-tailed paired *t*-test and nonparametric Wilcoxon signed-rank test were used for statistical analysis. **Results:**Data of 1397 patients was collected of which 844 patient were eligible for analysis at baseline. Mean age was 54.7 years. Azilsartan was started as monotherapy in 64.6% patients and as an add-on to antihypertensive therapy in 35.4% patients. Significant reduction of 19.6 mm Hg in mean Systolic BP (baseline: 162.6 ± 15.6 mm Hg to visit 1: 143 ± 13.7 mm Hg, p<0.001) and 30.2mm Hg in mean Systolic BP (baseline: 162.6 ± 15.6 mm Hg to visit 2: 132.4 ± 10.7, p<0.001) was observed. Significant reduction of 9.2 mmHg in mean diastolic BP (baseline: 95.4 ± 10 mmHg to visit 1 at mean day 36 : 86.2 ± 7.1 p<0.001) and 13.5mmHg in mean diastolic BP (baseline: 95.4 ± 10 mmHg to visit 2 at mean day 71: 81.9 ± 5.7 p<0.001) was observed. Azilsartan in the prescribed dose was well tolerated. Most common adverse event reported were Nausea 10 (1.2 %), metallic taste 10 (1.2 %) and giddiness 8 (0.9 %). No serious adverse events were reported. **Conclusion:** Azilsartan alone or with other antihypertensive drugs significantly reduced BP in Indian hypertensive patients in a real world setting. Azilsartan was well tolerated without any major or serious adverse events.



Disclosures: **U. Jadhav:** D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; GLENMARK PHARMACEUTICALS. **J. Dalal:** D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; GLENMARK PHARMACEUTICALS.

Funding:

Funding Component:

P109

Collecting Duct Renin and Aldosterone Regulate Potassium Homeostasis

Authors: Chuanming Xu, Univ of Utah, slc, UT; Yanting Chen, Sun Yat-sen Univ, Guangzhou, China; Changjiang Zou, Nirupama Ramkumar, Shiying Xie, Fei Wang, Renfei Luo, Tianxin Yang, Univ of Utah, slc, UT

The kalliuric action of the renin-angiotensin-aldosterone system (RAAS) is well established as highlighted by hyperkaliemia side effect of RAAS inhibitors but such action is usually ascribed to systemic RAAS. The present study attempted to address the involvement of intrarenal RAAS in K^+ homeostasis with emphasis on locally generated renin and aldosterone (Aldo) within the collecting duct (CD). In normal C57BL/6 mice, a 1-wk high K^+ (HK) intake (5% KCl in diet) induced parallel increases in renal prorenin (cortex: 298.8%; medulla: 323.8%) assessed by immunoblotting, and urinary prorenin/renin content (URC) (2.5-fold) assessed by ELISA, and urinary renin activity (URA) (1.5-fold), contrasting to suppressed plasma prorenin/renin concentration (by 25.6%) and renin activity (by 61.6%). Following 1-wk HK loading, mice lacking renin in the CD (CD renin KO) had decreased urinary K^+ excretion (by 20.2%) and elevated plasma K^+ level (KO+HK: 4.35 ± 0.14 *vs.* Floxed+HK: 3.89 ± 0.04 mM, $P<0.01$), accompanied with a reduction of URC (by 40%), URA (by 58.5%), urinary free and total Aldo excretion (by 50.6% and 36.6%, respectively), and kidney cortical and medullary Aldo (by 33.5% and 49.9%, respectively), without affecting plasma Aldo or renin levels. HK upregulated renal protein expression of Aldo synthase CYP11B2 (cortical: 133.4%, medullary: 221.5%), renal outer medullary K^+ channel (ROMK) (146.4%), calcium-activated potassium channel subunit alpha-1 (α-BK) (160.9%), α-Na^+-K^+-ATPase (155.8%), β-ENaC (165.1%), and cleaved-γ-ENaC (1800%), all of which were significantly blunted in CD *renin* KO mice (by 25-75%). We have developed an inducible renal tubule-wide *CYP11B2* KO using the Pax8/LC1 transgenes (termed as RT *CYP11B2* KO). While the homozygous deletion is lethal, the heterozygous mice exhibited normal development and were subjected to 3-day HK. K^+-loaded RT *CYP11B2* KO mice had decreased urinary K^+ excretion (by 30.5%) and hyperkalemia (KO+HK: 4.63 ± 0.05 *vs*. Floxed+HK: 3.98 ± 0.09 mM, $P<0.01$), accompanied with a reduction of urinary free and total Aldo excretion (by 20.4% and 34.4%, respectively) without affecting plasma Aldo levels. Taken together, these results support a local action of CD renin and Aldo in regulation of K^+ homeostasis.

Disclosures: C. Xu: None. **Y. Chen:** None. **C. Zou:** None. **N. Ramkumar:** None. **S. Xie:** None. **F. Wang:** None. **R. Luo:** None. **T. Yang:** None.

Funding: No

Funding Component:

P110

Renal Angiotensin Generation Depends on Hepatic Angiotensinogen: Evidence From a Preclinical Study With RNAi Therapeutics Targeting Liver Angiotensinogen

Authors: Katrina M Mirabito Colafella, Monash Univ, Melbourne, Australia; Estrellita Uijl, Richard van Veghel, René de Vries, Ingrid M Garrelds, Ewout J Hoorn, Erasmus MC, Rotterdam, Netherlands; Marko Poglitsch, Attoquant Diagnostics, Vienna, Austria; Jae Kim, Don Foster, Alnylam Pharmaceuticals, Cambridge, MA; A.H. Jan Danser, Erasmus MC, Rotterdam, Netherlands

Angiotensinogen (AGT) synthesis is proposed to occur not only in the liver, but also in kidney, brain and adipocytes. Selectively deleting hepatic AGT might therefore not affect renal angiotensin (Ang) levels. Here we investigated whether arterial pressure lowering with liver-targeted AGT siRNA versus renin-Ang system (RAS) blockers affects renal function and Ang levels. Arterial pressure was measured via radiotelemetry in spontaneously hypertensive rats during vehicle, valsartan, captopril (both p.o.), siRNA (s.c. fortnightly injection), siRNA+valsartan or captopril+valsartan treatment for 4 weeks. Transcutaneous measurement of glomerular filtration rate (GFR) and 24h urinary excretory function was assessed at 2 and 4 weeks. Renal Ang levels were measured by mass spectrometry. Dual RAS blockade synergistically lowered arterial pressure compared to monotherapy. Valsartan and captopril increased AngI (1096±205 and 939±128 fmol/g, respectively versus 413±46 fmol/g in vehicle-treated; both $P<0.01$ vs. vehicle), while captopril+valsartan did not affect AngI (260±42 fmol/g). siRNA and siRNA+valsartan lowered AngI (59±6 and 12±3 fmol/g, respectively; $P<0.0001$ versus vehicle). Conversely, neither siRNA nor valsartan lowered AngII (228±15 and 338±39 fmol/g versus 431±48 fmol/g in vehicle-treated). Captopril modestly lowered AngII while dual RAS blockade with siRNA+valsartan or

captopril+valsartan greatly reduced AngII (115±20, 15±5 and 9±3 fmol/g, respectively; all P<0.0001). The renal AngII/I ratio was increased 4-fold after siRNA (P<0.001), reduced by >70% after valsartan, captopril and captopril+valsartan (all P<0.001) and unaltered after siRNA+valsartan. No treatment affected GFR, natriuresis or albuminuria. In conclusion, the lowering of renal AngI after liver-targeted AGT siRNA suggests that hepatic rather than renal AGT determines renal Ang generation. Upregulation of ACE and/or AT_1R may allow renal AngII to remain intact after AGT siRNA, and only dual RAS blockade significantly reduces renal AngII. AGT siRNA synergistically lowers arterial pressure when combined with existing RAS blockers and may be a novel treatment for hypertension without apparent negative effects on renal function.

Disclosures: K.M. Mirabito Colafella: None. **E. Uijl:** None. **R. van Veghel:** None. **R. de Vries:** None. **I.M. Garrelds:** None. **E.J. Hoorn:** None. **M. Poglitsch:** None. **J. Kim:** A. Employment; Modest; Alnylam Pharmaceuticals. **D. Foster:** A. Employment; Modest; Alnylam Pharmaceuticals. **A. Danser:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Alnylam Pharmaceuticals.

Funding: No

Funding Component:

P111

ELABELA Antagonizes Intrarenal Renin to Lower Blood Pressure and Protect Against Renal Injury

Authors: Chuanming Xu, Fei Wang, Univ of Utah, slc, UT; Yanting Chen, Sun Yat-sen Univ, Guangzhou, China; Shiying Xie, Renfei Luo, Univ of Utah, slc, UT; Danielle Sng, Bruno Reversade, Inst of Medical Biology, A*STAR, 8A Biomedical Grove, Immunos, Singapore; Tianxin Yang, Univ of Utah, slc, UT

Emerging evidence has demonstrated that (pro)renin receptor (PRR)-mediated activation of intrarenal renin-angiotensin system (RAS) plays an essential role in renal handling of Na^+ and water balance and blood pressure. The present study tested the possibility that the intrarenal RAS served as a molecular target for the protective action of ELABELA (ELA, also known as Toddler/Apela), a novel endogenous ligand of APJ receptor (also known as APLNR) in the distal nephron. By RNAscope and immunofluorescence, mRNA and protein expression of endogenous ELA was consistently localized to the collecting duct (CD). In cultured CD-derived M1 cells, exogenous ELA induced parallel decreases of fPRR (by 31.6%), soluble PRR (sPRR) (by 46.5%), and prorenin (by 45.7%) protein expression as assessed by immunoblotting, and medium sPRR (by 66.7%) and prorenin/renin (by 60%) levels by ELISA. Conversely, deletion of PRR in the CD in mice elevated ELA mRNA levels (1.5-fold), and urinary ELA excretion (2.0-fold), supporting the antagonistic relationship between the two systems. Administration of exogenous ELA (5 mg/kg/day, minipump) to high salt (HS)-loaded Dahl salt-sensitive (SS) rats significantly lowered mean arterial pressure (113.0 ± 1.7 *vs*. 133.8 ± 1.5 mmHg, *P*<0.001), systolic blood pressure (BP) (122.5 ± 1.8 *vs*. 142.9 ± 2.6 mmHg, *P*<0.01), diastolic BP (105.8 ± 2.3 *vs*. 127.4 ± 0.9 mmHg, *P*<0.001), heart rate (347.4 ± 4.9 *vs*. 394.1 ± 5.3 bpm, *P*<0.001), and albuminuria (11.4 ± 1.0 *vs*. 5.1 ± 1.1 mg/24h, *P*<0.01), accompanied with a reduction of urinary sPRR (by 53.3%), Ang II (by 54.6%), prorenin/renin (by 68.7%), neutrophil gelatinase-associated lipocalin (by 51.9%), and prostaglandin E2 (by 44.9%) excretion. HS upregulated renal medullary protein expression of fPRR (218.7%) and sPRR (172.4%) in Dahl SS rats, all of which were significantly blunted by exogenous ELA infusion (by 30-55%). Together, these results support the antagonist interaction between ELA and intrarenal RAS in the distal nephron that appears to exert a major impact on blood pressure regulation.

Disclosures: C. Xu: None. **F. Wang:** None. **Y. Chen:** None. **S. Xie:** None. **R. Luo:** None. **D. Sng:** None. **B. Reversade:** None. **T. Yang:** None.

Funding: No

Funding Component:

P112

Site-1 Protease Regulates Potassium Homeostasis via Intrarenal Renin-Angiotensin-Aldosterone System

Authors: Yanting Chen, Sun Yat-sen Univ, Guangzhou, China; Chuanming Xu, Shiying Xie, Fei Wang, Renfei Luo, Tianxin Yang, Univ of Utah, slc, UT

We previously reported that (pro)renin receptor is activated by K^+ loading and is responsible for local generation of aldosterone (Aldo), contributing to the kaliuretic response. Furthermore, site-1 protease (S1P) but not furin or ADMA19 is the predominant PPR cleavage enzyme, and a recombinant sPRR, sPRR-His, stimulated Aldo synthase CYP11B2 expression and released Aldo in primary rat IMCD cells. The present study examined a potential role of renal S1P during high K^+ (HK) loading. In normal C57BL/6 mice, a 3-day HK intake (5% KCl in diet) induced renal protein expression of S1P (Cortical: 172.5%, Medullary: 380.5%). Administration of a S1P inhibitor PF429242 (PF) via mini pump infusion at 25 mg/kg/day for 3 days, to K^+-loaded animals elevated plasma K^+ level (HK+vehicle: 4.12 ± 0.13 vs. HK+PF: 4.52± 0.23 mM, $P<0.01$) and decreased urinary K^+ excretion (by 33%), accompanied with a reduction of urinary prorenin/renin content (by 63.2%), urinary renin activity (by 63.8%), urinary free and total Aldo excretion (by 53.6% and 58.6%, respectively), and kidney cortical and medullary Aldo (by 36.5% and 59.1%, respectively), without affecting plasma Aldo or renin levels. HK upregulated renal protein expression of sPRR (Cortical: 145.5%, Medullary: 211.6%), prorenin (Cortical: 184%, Medullary: 325.8%), CYP11B2 (cortical: 184.9%, medullary: 331.5%), renal outer medullary K^+ channel (ROMK) (139.1%), calcium-activated potassium channel subunit alpha-1 (α-BK) (147.8%), α-Na^+-K^+-ATPase (151.6%), β-ENaC (150%), and cleaved-γ-ENaC (622.3%), and downregulated total NCC and phosphorylated NCC (T53) protein expression (by 33.1% and 35.1%, respectively) as well as *in vivo* NCC activity response to hydrochlorothiazide (by 37.3% decrease in 6-h urine volume and UNaV), all of which were significantly blunted by PF (by 30-70%). In cultured CD-derived M1 cells, exposure to 10 mM KCl for 24 h augmented sPRR (155.6%) and Prorenin (154.4%) protein expression, which were both attenuated by PF (by 45.7% and 86.8%, respectively). Additionally, sPRR-His (10 nM) stimulated prorenin protein expression (165.2%). Together, these results suggest that S1P-derived sPRR contributes to K^+ secretion through activation of intrarenal RAAS.

Disclosures: Y. Chen: None. **C. Xu:** None. **S. Xie:** None. **F. Wang:** None. **R. Luo:** None. **T. Yang:** None.

Funding: No

Funding Component:

P113

Early Life Stress-Induced Increases in Urinary Aldosterone Precedes Renal Dysfunction and Hypertension in Male Mice Fed a High Fat Diet

Authors: Jacqueline R Leachman, Carolina Dalmasso, Xiu Xu, Jason Backus, Lisa Cassis, Analia S Loria, Univ of Kentucky, Lexington, KY

We have previously shown that mice subjected to maternal separation and early weaning (MSEW), a model of early life stress, display exacerbated angiotensin II-dependent obesity-induced hypertension and reduced glomerular filtration rate (GFR) after 16 weeks of high fat diet (HF). In this study, we hypothesized that renin-angiotensin aldosterone system (RAAS) activation in MSEW mice fed a HF for only 12 weeks will precede a decline in GFR and heightened hypertension. MSEW was performed by separating the pups from their mother for 4 to 8 hours from postnatal days (PD) 2 to 16. Mice were weaned at PD 17. Control mice remained undisturbed and were weaned at PD 21. We used 16 MSEW and 14 control litters. Eight-week-old mice were fed on a low fat diet (LF) or HF (10 or 60 % fat Kcal) for 12 weeks. Each litter was represented by one male randomly assigned to each diet. After 11 weeks, 24-hr urine was collected to measure proteins, creatinine, aldosterone and electrolytes by Duo ICAP-OES. At week 12, transcutaneous GFR and blood pressure

were measured in all mice. Male MSEW and control mice fed a HF displayed similar blood pressure and metabolic parameters, including water intake (3.4±0.3 vs. 3.4±0.2 ml/day), diuresis (1.1±0.1 vs. 1.0±0.2 ml/day), natriuresis (0.10±0.01 vs. 0.09±0.01 mmol/day), proteinuria (3.5±0.7 vs. 2.9±0.5 mg/day) and GFR (0.97±0.04 vs. 0.96±0.03 ml/min/100g BW). However, MSEW increased kaliuresis (0.41±0.07 vs. 0.23±0.02 mmol/day, $p<0.05$) and urinary aldosterone (25±4 vs. 11±1 ng/g crea, $p<0.05$). In addition, MSEW mice displayed increased plasma aldosterone (158±32 vs. 87±17 pg/ml, $p=0.056$) and lower plasma renin concentration compared to controls (2.3±0.4 vs. 3.7±0.4 ng/mL/30', $p<0.05$), suggesting elevated circulating angiotensin II. Only MSEW mice showed increased adiposity (36±1 vs. 24±4, %) and a positive correlation between urinary aldosterone and fat mass (2.6±0.9 vs. -0.1± 0.1, $p<0.05$). These data indicate that MSEW-induced RAAS overactivation precedes the increases in blood pressure and decline in GFR found in HF-fed mice later in life. Thus, increased angiotensin II, most likely due to elevated fat angiotensinogen, may stimulate adipocyte and/or adrenal-derived aldosterone production exacerbating obesity-hypertension in MSEW mice.

Disclosures: J.R. Leachman: None. **C. Dalmasso:** None. **X. Xu:** None. **J. Backus:** None. **L. Cassis:** None. **A.S. Loria:** None.

Funding: No

Funding Component:

P114

Predominance of Formation Over Degradation as Mechanism of Angiotensin-II(1-8) Regulation in Aminopeptidase a deficient Kidneys

Authors: Benedikt Marahrens, Charité Univ Med Berlin, Berlin, Germany; Jan Wysocki, Northwestern Univ / Feinberg Sch of Med, Chicago, IL; Juan-Carlos Velez, Dept of Nephrology, Ochsner Clinic Fndn, New Orleans, LA; Micheal Bader, Max Delbrück Ctr for Molecular Med, Berlin, Germany; Daniel Batlle, Northwestern Univ / Feinberg Sch of Med, Chicago, IL

Aminopeptidase A (APA), Angiotensin Converting Enzyme (ACE)-2, Neprilysin (NEP) and other enzymes degrade Angiotensin(Ang)-II, whereas ACE is the main AngII(1-8) forming enzyme. Our objective was to determine the state of the enzymes that form and degrade AngII(1-8) within the kidney when APA, a main degrading enzyme, is absent. We used a mouse model of APA deficiency on Balb/c genetic background. AngII(1-8) levels measured by ELISA in kidneys from APA deficient mice were higher than in control (0.3±0.01 vs 1.1±0.15 fmol/mg protein, $p=0.001$). In APAKO kidneys, ACE activity was markedly reduced to approximately 50% of the activity levels in WT mice (28229±4105 vs 14275±1895 RFU/ug total protein, $p=0.006$). In concordance with the activity assay the relative abundance of ACE protein in APAKO was reduced by Western Blot (1.2±0.14 vs 0.1±0.06, $p<0.001$). mRNA levels were also markedly reduced (0.94±0.12 vs 0.45±0.05, $p=0.006$). Surprisingly, enzyme activities of ACE2 (30±3.7 vs 16±3.1 RFU/ug total protein/hr, $p=0.009$) and NEP (2.4±0.4 vs 1.1±0.3 RFU/ug total protein/hr, $p=0.013$) were lower in APAKO ($n=10$) as compared to WT kidneys ($n=11$). In conclusion, in kidneys from APAKO mice there is an unexpected decrease in AngII(1-8) degrading enzymes. Rather a marked transcriptional downregulation of ACE occurs as the main compensatory mechanism to reduce AngII(1-8) formation. We conclude that elevated AngII(1-8) levels in the kidney lead to a downregulation of ACE transcription as a negative feedback to attenuate AngII(1-8) accumulation.

Disclosures: B. Marahrens: None. **J. Wysocki:** None. **J. Velez:** None. **M. Bader:** None. **D. Batlle:** None.

Funding: No

Funding Component:

P115

Urinary Angiotensinogen is Increased in Type 1 Diabetes With Mild Albuminuria: Gender Differences

Authors: Alejandro Sanchez Nadales, Sheeba Habeeb Ba Aqeel, Minghao Ye, Jan Wysocki, Alfred Rademaker, Mark Molitch, Daniel Batlle, Northwestern Univ Feinberg Sch of Med, Chicago, IL

Urinary angiotensinogen (uAOG) can be increased in patients with type 2 diabetes and in hypertension. We wanted to examine uAOG in patients with type 1 diabetes as a possible biomarker of enhanced Renin-Angiotensin system activity within the kidney at an early stage of the disease and study any potential gender differences **Methods** Urine samples from the Diabetes Control and Complications Trial (DCCT) that were available at the earliest study visit were obtained from NIDDK repository. Cases (N=103) were participants with microalbuminuria at study initiation (30.2-286.5mg/24hrs). Controls (N=103) were patients with type 1 diabetes with normoalbuminuria (<30mg/24hrs). Controls were matched based on age, gender and insulin arm allocation (intensive vs conventional) when the urine was available at the earliest visit (range 0-8). Wilcoxon Rank Sum test was used for non-parametric variables. Gender differences were examined using Mann Whitney test. **Results** At the study visit there were no significant differences in age, gender, Insulin modality and disease duration. Systolic blood pressure was slightly but significantly higher in cases than in controls (median 116, range 92-180 vs 112, range 90-150mmHg, P=0.005). HbA1c was slightly but significantly higher in cases than controls (median 8.0, range 5.2-14.2 vs 7.8, 5-12.6%, P=0.03). The eGFR was normal in both groups but albumin excretion rate (AER) was increased in the cases as compared to the controls. There was a highly significant difference between cases and controls in terms of uAOG (median 6.6, range 1.0-1070 vs 4.0, range 0.1-65.3ng/mg, P<0.001). This difference persisted after adjusting for GFR, systolic blood pressure and HbA1c (P<0.001). Gender analysis revealed that uAOG was higher in females than males (median 11.7, range 1.0-393 vs 5.4, range 1.26-1070ng/mg, P=0.001) **Conclusion** We conclude that uAOG is increased in people with type 1 diabetes with normal GFR and microalbuminuria. This increase in uAOG persists after adjustment for blood pressure, GFR and HbA1c. In addition, uAOG is higher in females than males. Gender differences are important for the interpretation of uAOG as a marker of kidney disease in people with type 1 diabetes.

Disclosures: A. Sanchez Nadales: None. **S. Ba Aqeel:** None. **M. Ye:** None. **J. Wysocki:** None. **A. Rademaker:** None. **M. Molitch:** None. **D. Batlle:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; R01DK104785.

Funding: No

Funding Component:

P116

Induction of Human Endothelin-1 Overexpression for 3 Months Increases Plasma Aldosterone

Authors: Olga Berillo, Suellen C Coelho, Nada Mahjoub, Nathanne Ferreira, Lady Davis Inst for Medical Res, Montreal, QC, Canada; Stefan Offermanns, Max-Planck-Inst for Heart and Lung Res, Bad Nauheim, Germany; Pierre Paradis, Ernesto L Schiffrin, Lady Davis Inst for Medical Res, Montreal, QC, Canada

Background: The mechanisms of blood pressure (BP) regulation by endothelin (ET)-1 produced by endothelial cells are complex and remain unclear. Long-term exposure to endothelial human ET-1 overexpression causes sustained blood pressure elevation via ETA receptors. ET-1 has been shown to stimulate the release of aldosterone from the adrenal cortex. Whether aldosterone plays a role in ET-1 endothelium overexpression-induced BP elevation is still unknown. **Methods and results:** Nine to 12-week-old male ieET-1 mice and control ieCre mice expressing a tamoxifen-inducible Cre recombinase ($CreER^{T2}$) under the control of EC-specific *Tie2* promoter, were treated with tamoxifen (1 mg/kg/day, SC) for 5 days and studied 3 months later. Plasma aldosterone level measured by ELISA was higher in ieET-1 compared

with ieCre mice (1.21±0.14 vs. 0.70±0.09 ng/mL, *P*<0.05). Sodium and water excretion determined by saline challenge tests done every 2 weeks for 3 months were unaffected by ET-1 overexpression. Reverse transcription-quantitative PCR (RT-qPCR) did not reveal changes in mRNA expression of renin, mineralocorticoid receptor (MR), epithelial sodium channel 1 alpha subunit (*Scnn1a*), TSC22 domain family member 3 (*Rsc22d3*), serum- and glucocorticoid-induced kinase 1 (*Sgk1*), and ET type A and B receptors in the kidney or adrenal glands. The mRNA expression of aldosterone synthase (*Cyp11b2*, fold change: 0.52±0.06 vs 1.00±0.16, *P*<0.01), but not the hydroxy-delta-5-steroid dehydrogenase 3 beta- and steroid delta-isomerase 1 (*Hsd3b1)* and 6 *(Hsd3b6)* or steroidogenic acute regulatory protein (*Star*), were decreased in the adrenal cortex of ieET-1 compared with ieCre mice. CYP11B2 and HSD3B1 protein levels measured by Western Blotting were unchanged. Treatment of ieET-1 mice with MR antagonist eplerenone (100 mg/kg per day) during the last 2 weeks decreased systolic BP more during the day (128±2 vs. 134±3 mm Hg) than during the night (137±2 vs. 140±2 mm Hg) compared to untreated ieET-1 mice. **Conclusions:** These results showed that aldosterone contributes at least in part to the BP elevation caused by endothelial human ET-1 overexpression.

Disclosures: O. Berillo: None. **S.C. Coelho:** None. **N. Mahjoub:** None. **N. Ferreira:** None. **S. Offermanns:** None. **P. Paradis:** None. **E.L. Schiffrin:** None.

Funding: No

Funding Component:

P117

Blood Pressure Outcomes in Patients With Primary Aldosteronism after Adrenalectomy versus Medical Management in Relation to the New 2017 ACC/AHA Blood Pressure Guidelines

Authors: Allexa Hammond, Bryan Wu, Hamza Lodhi, Jeomi Maduka, Poghni Peri-okonny, Danielle Tientcheu, Angela Price, Wanpen Vongpatanasin, Univ of Texas Southwestern Medical Ctr, Dallas, TX

Objective: Investigate blood pressure (BP) outcomes in primary aldosteronism (PA) patients following adrenalectomy or medical therapy in the context of the lower BP target goal and threshold proposed by the 2017 ACC/AHA blood pressure guidelines.
Methods: A retrospective study was conducted in patients with confirmed diagnosis of PA who were referred to Hypertension clinic at the University of Texas Southwestern between January 2009 and August 2017. Presence of PA was confirmed using previously recommended cutoff values of urinary aldosterone greater than 12 mcg/2h for the oral salt loading test and serum aldosterone greater than 10 ng/dL after intravenous saline suppression test. Patients were categorized into adrenalectomy or medical therapy groups. The average BP and number of anti-hypertensives were compared between the two groups at each clinic visit. Hypertension cure rate of PA patients undergoing adrenalectomy was compared using the JNC8 threshold BP of 140/90 mmHg versus the 2017 ACC/AHA threshold BP of 130/80 mmHg.
Results: Forty-nine patients were found to have PA. Twenty-two patients had an adrenalectomy, twenty-seven patients were started on a mineralocorticoid antagonist. The adrenalectomy subgroup required a fewer number of anti-hypertensives at the last follow-up visit (p=0.0004) compared to the medically treated group. Systolic BP reduced similarly from the baseline visit to the last visit in the adrenalectomy group compared to the medical therapy group (from 151.3 +/- 5.7 to 134.3 +/- 4.5 mmHg vs. 149 +/- 4.1 to 134.7 +/- 4.1 mmHg, p < 0.01 for visit and p=0.5 for group). Thirteen percent (3 of 23) of adrenalectomy patients achieved cure based on the previous JNC8 guidelines, whereas only 8.7% (2 of 23) achieved cure based on the current guidelines.
Conclusion: Adrenalectomy is more efficacious than medical management in reducing the number of anti-hypertensives needed for BP control. The percentage of patients who achieved cure following adrenalectomy decreased when defined by the 2017 ACC/AHA guidelines.

Disclosures: A. Hammond: None. **B. Wu:** None. **H. Lodhi:** None. **J. Maduka:** None. **P. Peri-okonny:** None. **D. Tientcheu:** None. **A. Price:** None. **W. Vongpatanasin:** None.

Funding: No

Funding Component:

P118

Tissue Transglutaminase Contributes to Pro-Inflammatory Cytokine LIGHT-Induced Ang II Sensitization

Authors: Chen Liu, Renna Luo, Wei Wang, Zhangzhe Peng, Zhen Zhou, Dianna Milewicz, UTHealth, Houston, TX; Gail Johnson, Univ of Rochester, Rochester, NY; Rodney Kellems, Yang Xia, UTHealth, Houston, TX

Hypertension is a severe cardiovascular complication with elevated inflammation and pressor sensitivity. Activated enzymatically and transcriptionally by inflammatory cytokines, tissue transglutaminase (TG2), a ubiquitous crosslinking enzyme, is known to stabilize the Ang II receptor AT1 and contribute to hypertension and cardio-renal syndrome.We hypothesized that TG2 play an essential role in the inflammation-induced Ang II sensitization in hypertension via its posttranslational modifications. To test this, we found a significant TG2-dependent increase in renal transglutaminase activity in animals infused with inflammatory cytokine LIGHT/TNFSF14 (4ng/day) for 14 days (PBS=0.5±0.04, LIGHT=0.8±0.03, LIGHT+TG2 inhibitor ERW1041E=0.6±0.05, LIGHT+TG2-/-=0.4±0.01mU/mg protein, n=4-5, $p<0.05$). Western blot further determined an ~9 fold increase in renal TG2 and ~4 fold in AT1 in animals infused with LIGHT only (n=3, $p<0.05$). Mutagenesis study on AT1 confirmed the requirement of TG2 modification in LIGHT-induced receptor accumulation. Following this, we identified an ~80% pronouncement in Ang II-induced calcium response in luciferase reporter cells co-treated with LIGHT but not LIGHT plus ERW1041E (n=3, $p<0.01$). Taken together, our studies may shed light on the intrinsic role of TG2 as the pressor sensitizer downstream of the cytokines. Our results could also be a proof of concept for the use of TG2 inhibitors as novel antihypertensives.

Disclosures: C. Liu: None. **R. Luo:** None. **W. Wang:** None. **Z. Peng:** None. **Z. Zhou:** None. **D. Milewicz:** None. **G. Johnson:** None. **R. Kellems:** None. **Y. Xia:** None.

Funding: No

Funding Component:

P119

Hypertension and Renal Dysfunction Are Mitigated by Surgical Renal Denervation in an Adult Rat Model of Polycystic Kidney Disease

Authors: Christopher T Banek, Madeline M Gauthier, John W Osborn, Univ of Minnesota, Minneapolis, MN

Polycystic kidney disease (PKD) is the most common form of inherited chronic kidney disease failure. PKD is also associated with hypertension (HTN) and increased peripheral sympathetic activity (SNA). Recent studies have shown ablation of renal nerves may be effective treatment for varying forms of HTN and renal disease; however, the role and mechanism for renal nerves in PKD-associated renal dysfunction and HTN are unclear. Therefore, this study was conducted to test the hypothesis that renal denervation (RDN) would reduce AP, lower peripheral SNA, and improve renal function in the adult PKD rat. To test this hypothesis, 6-month-old male and female PKD rats underwent bilateral surgical RDN or sham surgery (Total n=18; 9/group; 3M/6F). Cardiovascular parameters were monitored by implanted radiotelemeter. Peripheral sympathetic tone was estimated by depressor response to acute ganglionic blockade (GB). Renal function was assessed by measuring glomerular filtration rate (GFR) by FITC-sinistrin clearance. Two weeks

following treatment, rats were euthanized and tissues were collected for further analysis. Data was analyzed by a two-way ANOVA, across sex and treatment groups (α=0.05). Data presented as mean+SEM. At baseline, no differences in mean AP (132±2 vs. 129±3 mmHg), GB response (39±3vs. 42±4 ΔmmHg), nor GFR (0.93±0.06 vs. 0.91±0.04 mL/min/100g) were detected between RDN vs. sham. Two weeks following treatment, AP was lower in RDN (120±2mmHg) vs. sham (128±3mmHg). Similarly, GB was halved in RDN (-16±2 ΔmmHg) compared to sham (-32±3 ΔmmHg). GFR was increased in RDN rats vs. sham (1.18±0.06 vs. 0.84±0.08 mL/min/100g). Lastly, no difference in renal cystic area was detected between RDN and sham (8.27±1.39 vs. 8.74±0.90 %). No effect of sex was detected in any of the reported measurements. Altogether, these data support our initial hypothesis, where RDN effectively lowered AP and peripheral sympathetic tone in the PKD rat. Interestingly, we also observed a substantial increase in GFR in these animals, indicating an improvement in renal function despite no observable effect on cystic area. Of note, RDN was effective in both males and female rats. Further studies using repeated measurements are required to elucidate the order in which these effects occur.

Disclosures: C.T. Banek: None. **M.M. Gauthier:** None. **J.W. Osborn:** None.

Funding: Yes

Funding Component: National Center

P120

Mir-338-3p is Down-regulated in Subcutaneous Small Arteries of Hypertensive Patients With Chronic Kidney Disease

Authors: Nada Mahjoub, Lady Davis Inst, Montreal, QC, Canada

Background: Hypertension (HTN) and chronic kidney disease (CKD) are among the most prevalent global health conditions that cause millions of deaths per year. They are associated with vascular damage characterized by vascular remodeling, stiffening and endothelial dysfunction. miRNAs (miRs) are a class of small non-coding RNA that regulate gene expression by binding to their target messenger RNAs (mRNAs), thereby leading to mRNA degradation or translational repression. 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 gather insight into pathophysiological molecular mechanisms in these conditions. **Methods and results:** Normotensive, HTN [systolic blood pressure (BP) >135 mmHg or diastolic BP of 85-115 mmHg with BpTRU] and CKD subjects (eGFR<60mL/min/m^2) (n=15-16) were studied. Small arteries were isolated from subcutaneous gluteal biopsies and RNA extracted for small and total RNA sequencing using Illumina HiSeq-2500. EdgeR identified DE miRs (P<0.05) uniquely associated with HTN (3 up and 6 down) or CKD (42 up and 39 down) or with both groups (2 down). Reverse transcription-quantitative PCR (RT-qPCR) was used to confirm miRNA differential expression. Correlation between RNA sequencing and RT-qPCR data was demonstrated for 3 miRs from 14 tested: mir-146a-5p (r=0.56, P<10^{-4}), miR-338-3p (r=0.91, P<10^{-16}) and mir-374a-3p (r=0.57, P<10^{-4}). The best correlated DE miRNA, miR-338-3p was down-regulated by 76% (P<10^{-4}) and uniquely associated with CKD. TargetScan predicted that the up-regulated mRNA encoding glutathione peroxidase 3 (*GPX3*, 1.57 fold, P<0.001) to be a miR-338-3p target. Both miR-338-3p and *GPX3* were found to be highly expressed in human aortic endothelial cells (HAECs) by RT-qPCR. Target prediction was validated by showing using RT-qPCR that *GPX3* expression was up-regulated 1.73±0.28 fold in HAECs transfected with anti-miR-338-3p (P<0.05). **Conclusion:** miR-338-3p down-regulation was found in small arteries, uniquely associated with CKD. *GPX3*, which may be important for preservation of endothelial function, is a miR-338-3p potential target. miR-338-3p and associated target may play a role in vascular remodeling in CKD.

Disclosures: N. Mahjoub: None.

Funding: No

Funding Component:

P121

The Effect of Intensive Blood Pressure Targets on Mortality in Chronic Kidney Disease Patients: An Individual Patient Data Meta-Analysis of Randomized Controlled Trials

Authors: Rahul Aggarwal, Nicholas Chiu, Benjamin Petrie, Haares Mirzan, Jackson Steinkamp, Boston Univ, Boston, MA

Introduction:
Current evidence is conflicting on whether intensive blood pressure (BP) management, specifically with targets of <130/90 mmHg, produces a mortality benefit in non-diabetic chronic kidney disease (CKD) patients. MDRD, AASK, and SPRINT are the largest randomized controlled trials available investigating intensive targets in CKD patients. The MDRD and AASK recruited exclusively CKD patients, while SPRINT had a large CKD subpopulation within its cohort. MDRD assigned patients to a standard mean arterial pressure (MAP) target of 107 mmHg vs. an intensive target of 92 mmHg (achieved BPs of 134/81 mmHg vs 126/77 mmHg, respectively). AASK assigned African American patients to a MAP of 102-107 mmHg vs. <92 mmHg (achieved BPs of 141/85 mmHg vs. 128/78 mmHg, respectively). SPRINT compared a systolic target of 140 mmHg vs. 120 mmHg (achieved BPs of 137/74 vs. 123/67 in the CKD subpopulation). Our study pools individual patient data (IPD) from these three trials.
Methods:
IPD for all patients in MDRD, AASK, and SPRINT were obtained and screened for non-diabetic CKD patients. The resulting 4537 patients were assigned to their original randomized intervention group – 2258 patients to the standard group and 2279 to the intensive group. The primary outcome was mortality. Statistical analysis for primary outcome was performed with Cox proportional hazards models stratified by clinical site/center. Interactions between outcome and trial enrollment were also assessed.
Results:
There was no statistically significant difference in mortality rate between intensive and standard BP control (HR: 0.84; 95% CI: 0.66, 1.07; p = 0.148). 126 patients (5.5%) in the intensive group and 149 patients (6.6%) in the standard group expired. No interaction was present between treatment and trial enrollment (MDRD, AASK, SPRINT) (p-value of interaction: 0.080).
Conclusion:
Intensive BP targets do not improve mortality for non-diabetic patients with CKD. Further investigation needs to be performed to determine if this lack of benefit is consistent across subpopulations.

Disclosures: R. Aggarwal: None. **N. Chiu:** None. **B. Petrie:** None. **H. Mirzan:** None. **J. Steinkamp:** None.

Funding: No

Funding Component:

P122

Early Renal Allograft Function and Hypertension after Kidney Transplantation

Authors: Ekamol Tantisattamo, Div of Nephrology and Hypertension, Dept of Med, Univ of California, Irvine Sch of Med, Orange, CA; Siroj Dejhansathit, Possawat Vutthikraivit, Phramongkutklao Coll of Med, Mahidol Univ, Bangkok, Thailand; Haritha Mopuru, Multi-Organ Transplant Ctr, William Beaumont Hosp, Royal Oak, MI; Praveen Ratanasrimetha, Texas Tech Univ Health Sciences Ctr, Lubbock, TX; Rungwasee Rattanavich, Div of Nephrology, Dept of Med, Washington Univ school of Med, St. Louis, MO; Raghavesh Pullalarevu, Multi-Organ Transplant Ctr, Div of Nephrology, Dept of Internal Med, William Beaumont Hosp, Oakland Univ William Beaumont Sch of Med, Royal Oak, MI

Background: Poor renal allograft function is associated with post-transplant hypertension (HTN). The association between allograft function at an early post-transplant period and HTN is unclear. ***Method:*** Of 70 transplant recipients, the difference between serum creatinine (SCr) at the discharge date from kidney transplantation (KT) and those at pre-transplantation (ΔCr) divides the study population into Group 1 (ΔCr ≥80%) and Group 2 (ΔCr <80%). Cox proportional hazard regression is performed to find the association between different ΔCr and post-transplant systolic HTN (SHTN) and diastolic HTN (DHTN) defined by SBP ≥130 and DBP ≥80 mmHg, respectively during a 96-week follow-up period. ***Results:*** Mean age±SEM is 52.66±1.43 years and 58.6% is male. Mean length of stay is 8.50±1.35 days. Thirty patients (43%) are in the Groups 1. Mean absolute ΔCr is greater in the Group 1 (ΔCr -7.41±0.43 vs. -3.49±0.42, p <0.001). Compared to Group 1, Group 2 has a 17.1% increased risk of SHTN (HR 1.171, 95%CI 0.715 to 1.918, p 0.530, Fig1A), but has a 24.5% decreased risk of DHTN (HR 0.755, 95%CI 0.417 to 1.367, p 0.353, Fig1B). After adjusting for age, gender, type of induction immunosuppressants, type of KT, and BMI, Group 2 has an 80.2% greater risk for SHTN (HR 1.802, 95%CI 0.976 to 3.325, p 0.060, Fig2A), but has a 6.8% lowered risk of DHTN (HR 0.932, 95%CI 0.467 to 1.857, p 0.840, Fig2B).***Conclusions:*** The degree of change in SCr during immediate post-KT does not predict post-transplant SHTN and DHTN. However, patients with better renal allograft function appear to become less SHTN but more DHTN. The pattern of association between long-term allograft function and SHTN and DHTN needs further studies.

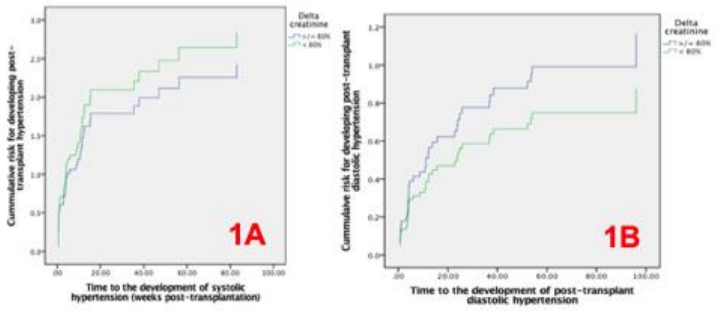


Figure 1: Simple Cox proportional hazard regression of time development of post-transplant systolic (A) and diastolic (B) hypertension compared difference between serum creatinine at the discharge date from kidney transplantation and those at pre-transplantation (Delta (Δ)Cr) ≥ 80% (Group 1) and <80% (Group 2).

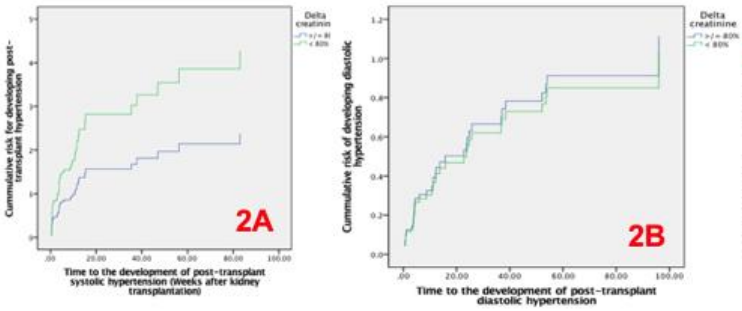


Figure 2: Multiple Cox proportional hazard regression of time development of post-transplant systolic (A) and diastolic (B) hypertension compared difference between serum creatinine at the discharge date from kidney transplantation and those at pre-transplantation (Delta (Δ)Cr) ≥ 80% (Group 1) and <80% (Group 2).

Disclosures: **E. Tantisattamo:** G. Consultant/Advisory Board; Modest; Kidney in Cardiovascular Disease (KCVD) Communications committee, the American Heart Association (AHA). **S. Dejhansathit:** None. **P. Vutthikraivit:** None. **H. Mopuru:** None. **P. Ratanasrimetha:** None. **R. Rattanavich:** None. **R. Pullalarevu:** None.

Funding: No

Funding Component:

P123

Plasma Endocan Level as a Predictor of Cardiovascular Composite Outcomes in Patients With End-Stage Renal Disease

Authors: Ji Yung Lee, Hyung Seok Ihm, Hyeon Seok Hwang, Kyung Hwan Jeong, Chun Gyoo Ihm, Sang Ho Lee, Tae Won Lee, Yang Gyun Kim, Ju Young Moon, Kyung Hee Univ Sch of Med, Seoul, Korea, Republic of

Background: Endocan, a proteoglycan which is a potential biomarker of endothelial dysfunction, has been shown to be associated with increased cardiovascular risk. We investigated plasma levels of endocan in patients with end-stage renal

disease (ESRD) on hemodialysis to predict the risk of cardiovascular diseases. **Methods:** A total of 400 adult patients with ESRD undergoing hemodialysis were prospectively enrolled in 4 tertiary hospitals of South Korea from June 2016 to May 2018. They were observed for development of the cardiovascular composite outcomes. We compared clinical characteristics and the plasma levels of endocan between 47 patients with cardiovascular composite outcomes and 353 control patients without cardiovascular composite outcomes, and developed the predictive markers of cardiovascular diseases using Cox proportional-hazard analysis. **Results:** Previous histories of diabetes, acute coronary syndrome, arrhythmia and congestive heart failure were higher in ESRD patients with the cardiovascular composite outcomes than control patients. Patients with cardiovascular composite outcomes showed lower levels of hemoglobin, albumin, and high-density lipoproteins compared with control patients. Higher level of plasma endocan and white blood cells were associated with patients with cardiovascular composite outcomes. Cox proportional-hazard analysis showed that previous histories of diabetes and plasma level endocan were significantly associated with cardiovascular composite outcomes: hazard ratios were 2.1 (95% confidential interval (CI), 1.1 to 4.3) (p = 0.03) for diabetes and 15.4 (95% CI, 3.2 to 75.2) (p = 0.001) for endocan (log pg/mL). The patients with level of endocan of 2.96 log pg per mL or more showed significantly higher cumulative incidence of the cardiovascular composite outcomes in Kaplan-Meier curve (p = 0.03). **Conclusions:** Plasma Endocan level can a useful biomarker for prediction of cardiovascular diseases in patients with ESRD on hemodialysis.

Disclosures: J. Lee: None. **H. Ihm:** None. **H. Hwang:** None. **K. Jeong:** None. **C. Ihm:** None. **S. Lee:** None. **T. Lee:** None. **Y. Kim:** None. **J. Moon:** None.

Funding: No

Funding Component:

Funding Component:

P125

Role of Circulating Endothelial Progenitor Cell Levels Predict Cardiovascular Events in Patients on Maintenance Hemodialysis

Authors: Hyung-seok Ihm, Chun-gyoo Ihm, Tae-won Lee, Kyung-hwan Jeong, Hyeon-seok Hwang, Sang-ho Lee, Woo-shik Kim, Ji yung Lee, Kyung Hee Univ Sch of Med, Seoul, Korea, Republic of

Background: The number of circulating endothelial progenitor cells (EPCs) has been identified as a surrogate biologic marker for vascular function and cumulative cardiovascular (CV) risk in the general population. Patients with end-stage renal disease (ESRD) on hemodialysis (HD) have markedly decreased EPC counts and function. We hypothesized that the number of circulating EPCs predicts death from all causes and CV events in patients with ESRD on HD. Methods: We quantified the EPCs in blood samples from 70 patients with ESRD on HD. Circulating EPCs were counted by flow cytometry as the number of CD45 low CD34 + VEGFR2 + cells. Death from all causes and CV events served as outcome variables over a median follow-up period of 20 months. Results: It has been postulated that the number of circulating EPCs at baseline ranged from 1 to 350 cells per 200 µl, with a mean of ± standard deviation (SD) of 26.0 ± 48.2 cells per 200 µl. The median, lowest and highest tertiles of EPC counts were 11.0, 9.0, and 17.0 cells/200 µl, respectively. Patients with the lowest tertile EPC counts had significantly higher rates of CV events, but mortality was similar between the two groups. After adjusting for these risk factors, HbA1c and the lowest tertile EPC count remained as independent predictors of CV events. A cutoff value of 9.5 cells per 200 µl maximized the power of the EPC count to predict future CV events as determined by ROC curve analysis. Conclusions: Reduced circulating EPC counts independently predicted CV events in 70 patients with ESRD on maintenance HD. Circulating EPCs may play a role in vascular repair, thereby affecting the clinical course of CV events.

Disclosures: **H. Ihm:** None. **C. Ihm:** None. **T. Lee:** None. **K. Jeong:** None. **H. Hwang:** None. **S. Lee:** None. **W. Kim:** None. **J. Lee:** None.

Funding: No

Funding Component:

P126

Site-1 Protease-Derived Soluble (Pro)Renin Receptor Targets Renal V2 Vasopressin Receptor/AQP2 to Enhance Urine Concentrating Capability

Authors: Fei Wang, Kexin Peng, Chuanming Xu, Shiying Xie, Renfei Luo, Chang-jiang Zou, Tianxin Yang, Univ of Utah, Salt Lake Cty, UT

Our previous study demonstrates that exogenous soluble (pro)renin receptor (sPRR-His) exerts a biological function in regulation of AQP2 expression and urine concentrating capability. The site-1 protease (S1P) has recently been identified as a predominant protease responsible for generation of sPRR. In the present study, we employed pharmacologic and conditional gene knockout studies to test the potential role of S1P-derived sPRR in regulation of fluid homeostasis. In C57/BL6 mice, treatment with a S1P inhibitor PF429242 (PF at 20 mg/kg/d via minipump for 4 days) induced polyuria (urine volume: PF: 1.6 ± 0.4 vs. Vehicle: 0.7 ± 0.2 ml, $p<0.05$) accompanied with 50% reduction of urine osmolality and reduced AVP sensitivity (PF: 22.6% ± 4.0% vs. Vehicle: 74.2% ± 5.0%, $p<0.01$), all of which were nearly completely reversed by sPRR-His treatment via intravenous infusion at 30 µg/kg/d. AVP sensitivity was determined by measurement of urinary osmolality response to acute AVP injection. Immunoblotting demonstrated that PF infusion induced a parallel reduction of protein abundance of V2R (70% off) and AQP2 (80% off). Interestingly, sPRR-His infusion restored the expression of V2R and AQP2. We have developed an inducible renal tubule-wide deletion of site-1 protease using the Pax8/LC1 transgenes (termed as RT S1P KO). RT S1P KO mice exhibited similar polyuria (urine volume: KO: 1.8 ± 0.2 vs. Floxed: 1.1 ± 0.2 ml/day, $p<0.01$) associated with suppressed renal expression of AQP2 and V2R, as well as impaired AVP sensitivity (KO: 64.5% ± 6.0% vs. Vehicle: 150.2% ± 21.0%, $p<0.01$). Administration of sPRR-His to RT S1P KO mice reversed polyuria (urine volume: KO+sPRR-His: 1.0 ± 0.2 vs. KO: 1.8 ± 0.2 ml/day, $p<0.01$) accompanied with restored renal expression of AQP2 and V2R, and AVP sensitivity. These results suggested that S1P acts via its PRR cleaving property to control the activity of AVP/V2R/AQP pathway and thus urine concentrating capability.

Disclosures: **F. Wang:** None. **K. Peng:** None. **C. Xu:** None. **S. Xie:** None. **R. Luo:** None. **C. Zou:** None. **T. Yang:** None.

Funding: No

Funding Component:

P127

Pericyte Detachment is Involved in Renal Congestion in a Novel Rat Model

Authors: Satoshi Shimada, Tohoku Univ, Sendai, Japan; Takuo Hirose, Chika Takahashi, Tohoku Medical and Pharmaceutical Univ, Sendai, Japan; Emiko Sato, Tohoku Univ, Sendai, Japan; Satoshi Kinugasa, Tohoku Medical and Pharmaceutical Univ, Sendai, Japan; Sadayoshi Ito, Tohoku Univ, Sendai, Japan; Takefumi Mori, Tohoku Medical and Pharmaceutical Univ, Sendai, Japan

Increased central venous pressure in congestive heart failure is responsible for renal dysfunction; however, the underlying mechanisms are unclear. We hypothesized that renal interstitial hydrostatic pressure (RIHP) and expansion pressures of the vasa recta are responsible for pericyte detachment resulting renal congestion-mediated fibrosis. We

created a novel rat renal congestion model and investigated the effect of renal congestion on hemodynamics and its molecular mechanisms. The inferior vena cava (IVC) between the renal veins was ligated by suture in male Sprague-Dawley rats to increase upstream IVC pressure and induce congestion in the left kidney only. Left kidney congestion reduced renal blood flow in cortex (33.6%, 28.3 to 16.0 mL/min) and in medulla (41.8%, 11.9 to 6.9 mL/min) and glomerular filtration rate (1.16 to 0.20 mL/min/kg BW), and increased RIHP (12.6 to 17.6 mm Hg). Hypoxia was observed in the medullary thick ascending limb of Henle, only in the congestive kidneys. Tubulointerstitial injury, podocyte injury, albuminuria, and reduced creatinine clearance were observed in the congestive kidneys. Molecules related to extracellular matrix expansion, tubular injury, and focal adhesion were upregulated in microarray analysis. Renal decapsulation ameliorated the tubulointerstitial injury (mRNA expression of *Kim1* 15.3 to 4.3 A.U., *αSma* 5.3 to 2.8 A.U.). Electron microscopy captured pericyte detachment in the congestive kidneys. Transgelin and platelet-derived growth factor receptors (PDGFRs), as indicators of pericyte-myofibroblast transition, were upregulated in the pericytes and the adjacent interstitium. Imatinib, a PDGFR inhibitor, ameliorated the interstitial injury. Our results reveal a novel mechanism of worsening renal function associated with congestive heart failure, and provide a new therapeutic strategy based on a better understanding of the pericyte-myofibroblast transition.

Disclosures: S. Shimada: None. **T. Hirose:** None. **C. Takahashi:** None. **E. Sato:** None. **S. Kinugasa:** None. **S. Ito:** None. **T. Mori:** None.

Funding: No

Funding Component:

P128

Intensive Blood Pressure Treatment and Renal Outcomes in Diabetic Patients

Authors: Ling Wang, Supratik Rayamajhi, Donna Wang, Michigan State Univ, East Lasing, MI

Background There is no certain evidence to support a strategy of lowering systolic blood pressure in persons with type 2 diabetes. We conducted a post-hoc analysis on the data set from Action to Control Cardiovascular Risk in Diabetes blood pressure trial (ACCORD-BP) to test the hypothesis that intensive blood pressure treatment prevents or alleviates adverse renal outcomes among diabetic patients. **Methods** Patients were randomly assigned to a systolic blood pressure (SBP) treatment target of <120 (intensive treatment) or <140 (standard treatment). To rule out the effect of glycemic treatment, we only included patients with standard glycemic control (glycated hemoglobin of 7.0-7.9%) that resulted in 1,184 patients on intensive arm and 1,178 patients on standard arm. Four renal outcomes at the end of the study are considered: 1) Doubling of serum creatinine (SCr) or >20 mL/min decrease in eGFR; 2) UAlb>=300; 3) Renal failure or End-Stage Renal Disease (dialysis) or SCr>3.3; 4) UAlb>=30. Generalized Estimating Equation method is employed. **Results** After 1 year, the mean SBP was 120.8 in intensive group and 134.6 in standard group. SCr doubling or >20 mL/min decrease in eGFR attributed to intensive treatment occurred in 701 of the 1184 participants in the intensive-therapy group (57.0%) and 529 of the 1178 participants in the standard-therapy group (44.9%) (OR=1.83, 95% CI= 1.76-1.90). We further categorized BP over time into three tiers, by low (SBP<120 and DBP <70), medium (SBP=120-140 and DBP=70-85) and high (SBP>140 and DBP>85). Both high SBP (>140) and medium SBP (120-140) as compared to low SBP (<120) level, significantly reduced the chance of serum creatinine doubling or >20 ml/min decrease in eGFR (OR=0.79, p<0.0001). There are no significant differences in other three renal outcomes between intensive and standard group patients. **Conclusion** In patients with type 2 diabetes, targeting a systolic blood pressure of less than 120, as compared with less than 140, increased the chance of having Scr doubling or >20 mL/min decrease in eGFR. These results,

consistent with our previous study using SPRINT data from non-diabetic patients, provide evidence against the notion that intensive blood pressure treatment leads to beneficial renal outcomes in diabetic patients.

Disclosures: L. Wang: None. **S. Rayamajhi:** None. **D. Wang:** None.

Funding: No

Funding Component:

P129

Renal Necrotic Tissue Induces Hypertension

Authors: Jin Wei, Jie Zhang, Lei Wang, Univ of South Florida, Tampa, FL; Ruisheng Liu, Univ of South Florida, tampa, FL

The effect of renal necrotic tissue on blood pressure remain unclear. We hypothesized that infarcted renal tissue induces hypertension by stimulating inflammatory response. Partial renal infarction models were developed by ligating either upper branch (UL group) or lower branch (LL group) in the left renal artery. Mean arterial pressure (MAP) measured with telemetry quickly increased from 3 days after surgery, then gradually declined and reached a plateau within 7 days after surgery, which was 120.4±4.6 mmHg (increased by 23.8±2.5%) in UL group and 114.3±5.6 mmHg (increased by 19.0±1.1%) in LL group (n=8, $p<0.01$ vs baseline). Moreover, resection of the renal necrotic zone at day 14^{th} after ligation surgery normalized the MAP in partial renal infarction groups at day 28^{th} (UL+R: 121.1±5.9 to 98.3±5.1 mmHg; LL+R: 118.6±4.7 to 101.5±3.6 mmHg). The protein levels of TNFα and IL-6 in the left kidney homogenate significantly increased in UL group (11.5±3.4-fold; 3.8±1.2-fold) and LL group (8.4±2.7-fold; 2.7±0.83-fold) compared with the sham group at day 28 after ligation surgery (n=5, $p<0.01$ vs sham). Plasma concentration of TNFα and IL-6 also elevated in UL group (3.5±1.2-fold; 2.4±0.4-fold) and LL group (3.8±0.9-fold; 2.9±0.7-fold) at day 28 (n=7, $p<0.01$ vs sham). Inflammatory cell infiltration was significantly enhanced in UL (337±45 Inflammatory cells/HPF) and LL groups (314±27 Inflammatory cells/HPF) compared with sham group (22±14 Inflammatory cells/HPF) (n=7, $p<0.01$ vs sham). Moreover, resection of the renal necrotic zone at day 14^{th} after ligation surgery normalized the inflammatory response at day 28^{th} in UL+R and LL+R groups, compared with UL and LL groups. We concluded that renal necrotic tissue induces hypertension in C57BL/6 mice, possibly by stimulating of the inflammatory response.

Disclosures: J. Wei: None. **J. Zhang:** None. **L. Wang:** None. **R. Liu:** None.

Funding: No

Funding Component:

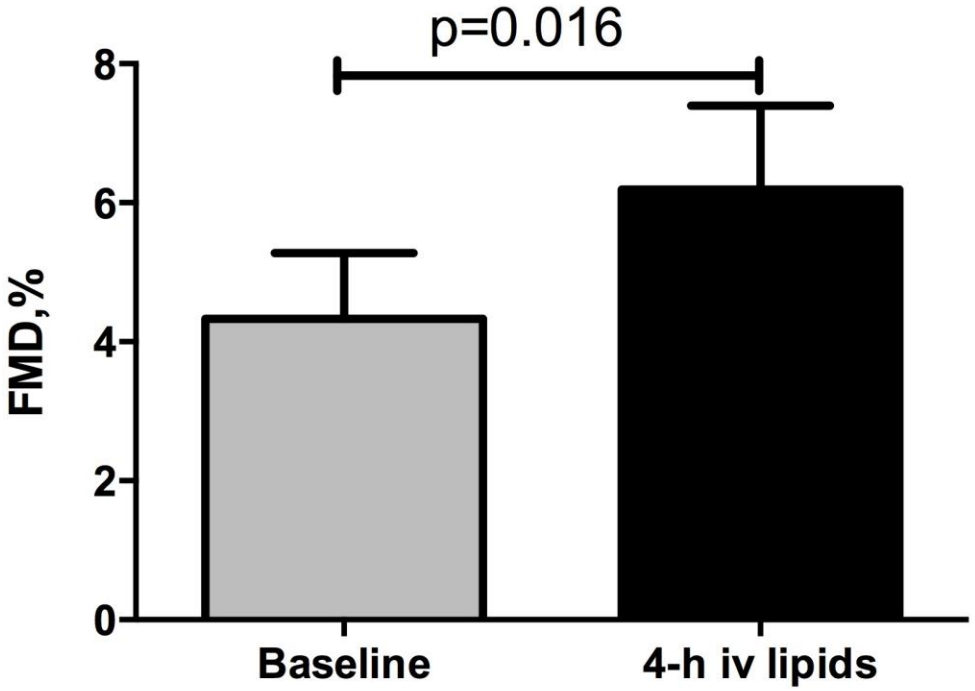
P130

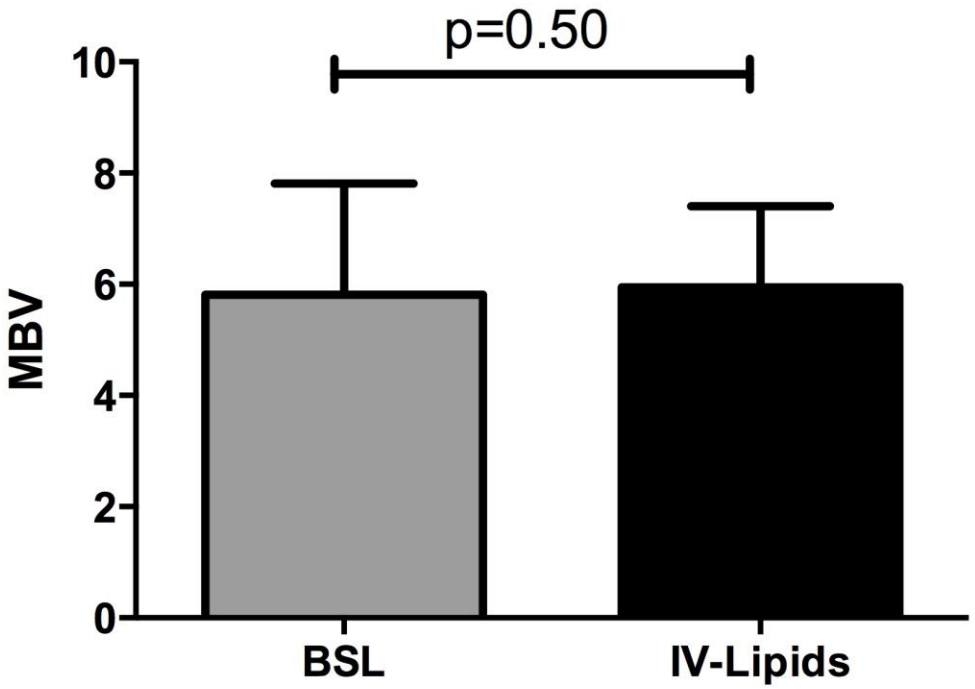
Acute Hyperlipidemia Increases Vasodilation in Conduit Arteries but Has No Effect on Microvascular Recruitment in African American Women

Authors: Jorge E Celedonio, Shahram Mehr, JoAnn Gottlieb, Italo Biaggioni, Cyndya Shibao, Vanderbilt Univ Medical Ctr, Nashville, TN

African Americans have one of the highest prevalence of hypertension in the United States. Impaired endothelial function has been implicated in the pathophysiology of hypertension in this group. We previously reported that brachial artery diameter increases from 3.9±0.14 to 4.1± 0.14 at 4 hours after ingestion of a high fat meal, suggesting vasodilation of conduit arteries. Considering that oral fat ingestion stimulates the release of gastrointestinal peptides with vasoactive properties, we aimed to isolate the vascular effect of fat on conduit artery and microvasculature by

inducing an acute elevation of triglycerides and free fatty acids with an infusion of 20% intralipid® and heparin. To assess endothelial function we measured changes in the diameter of the brachial artery to hyperemia with a continuous edge detection and wall tracking software. Effect on microvasculature was obtained with contrast-enhanced ultrasonography (CEU) of the brachioradialis muscle with lipid-perflutren microbubbles infusion. We studied 14 obese healthy African American women, age 39± 10, BMI 38.7± 3.4 kg/m.^{2} Triglycerides increased from 60.7±30.5 to 523.3±231 mg/dL at the end of the 4 h infusion. Brachial artery diameters were 3.6±0.16 and 3.9±0.18 mm at baseline, and 4-h, P=0.109, respectively. Flow mediated dilation improved at the end of the 4-h infusion period (4.3±0.95 to 6.2±1.21%, P=0.016), **figure**. Microvascular blood volume did not change with the intervention 5.8±4.9 vs. 5.9±3.6, P=0.55. In conclusion, IV acute hyperlipidemia did not increase brachial artery diameter nor microvascular circulation, however it potentiates vascular reactivity in conduit arteries.





Disclosures: **J.E. Celedonio:** None. **S. Mehr:** None. **J. Gottlieb:** None. **I. Biaggioni:** None. **C. Shibao:** None.

Funding: No

Funding Component:

P131

Transdermal Detection of Low Concentrations of Hydrogen Sulfide

Authors: Elani Fourie Wiest, Lynnette Rios, Univ of New Mexico, Albuquerque, NM; Debra Friedrichsen, Grayson Silaski, Exhalix, LLC, Albuquerque, NM; Curtis Mowry, Sandia Natl Labs, Albuquerque, NM; Reza Shekarriz, Exhalix, LLC, Albuquerque, NM; Nancy Kanagy, Univ of New Mexico, Albuquerque, NM

We report here a novel approach to measure circulating hydrogen sulfide (H_2S) non-invasively as a potential way to diagnose and monitor endothelial dysfunction and peripheral artery disease (PAD). PAD is a life-threatening condition caused by arterial constriction and obstruction of blood flow leading to limb ischemia. Current methods to diagnose and monitor PAD lack sensitivity, are expensive, and technically difficult. Recent studies indicate that decreased H_2S production is an underlying cause of PAD. Also, reduced plasma H_2S correlates with endothelial dysfunction in individuals with untreated hypertension, diabetes, sleep apnea and other cardiovascular diseases. The TAGS device was designed to measure transdermal H_2S to test the hypothesis that the diffusion rate (and therefore gas phase concentration) of H_2S is directly proportional to dermal blood flow. Healthy volunteers between the ages of 21-65 were recruited. Exclusion criteria included subjects currently treated for hypertension, hyperlipidemia, and diabetes. Smokers and pregnant women were also excluded. We demonstrate that H_2S can be detected in healthy volunteers (n=11) at 10.8 part per billion (ppb). Interestingly, there is a positive correlation between age and TAGS readings in male volunteers (r=0.614; n= 6) and a negative correlation in female volunteers (r=0.482, n=5). The TAGS device has the potential, therefore, to serve as a more sensitive, economical, and easy to use diagnostic tool to detect H_2S and predict dermal blood flow.

Disclosures: E.F. Wiest: None. **L. Rios:** None. **D. Friedrichsen:** A. Employment; Modest; Dr. Friedrichsen is an engineering consultant for Exhalix, the company who is developing the device we use in this study. **G. Silaski:** A. Employment; Modest; Dr. Silaski is an engineering consultant for Exhalix, the company who is developing the device we use in this study.. **C. Mowry:** None. **R. Shekarriz:** A. Employment; Modest; Dr. Shekarriz is an CEO of Exhalix, the company who is developing the device we use in this study. **N. Kanagy:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; This study was supported by grants from the NIH, NSF and Exhalix.

Funding: No

Funding Component:

P132

Everolimus, a Targeted Cancer Therapy, Improves Endothelium Dependent Relaxation in Spontaneously Hypertensive Rat Mesenteric Arteries

Authors: Patricia Martinez Quinones, Cameron G McCarthy, Camilla F Wenceslau, R Clinton Webb, Augusta Univ, Augusta, GA

Cardiovascular disease is the leading cause of death in cancer survivors. The short-term and long-term cardiotoxic effects of targeted chemotherapeutics is not well known. Everolimus, an inhibitor to mammalian target of rapamycin (mTOR) complex 1, is a targeted therapy approved for the treatment of metastatic breast, colon, renal cell and pancreatic cancer. Clinical retrospective reviews suggest heart failure and hypertension as cardiotoxic effects of everolimus. Our lab previously showed that everolimus exposure on normotensive vessels increased sensitivity to adrenergic stimuli in a time and concentration dependent manner. We hypothesized that everolimus leads to enhanced contractility and impaired relaxation in hypertensive vessels. We studied the effects of everolimus on the contractility and relaxation of mesenteric resistance arteries (MRA) of male Wistar rats (12-15 weeks old, n=4) and spontaneously

hypertensive rats (SHR) (15-18 weeks old, n=2) on a wire myograph. Two concentrations of everolimus were evaluated, 0.1μM and 0.1nM, during a one-hour incubation. Normotensive MRA exposed to everolimus (0.1μM) showed a decreased contractile response to phenylephrine (LogEC50±SEM, Ctrl:-5.627±0.07 vs Drug: -5.390±0.10) but no difference when exposed to 0.1nM. SHR MRA showed no difference in PE-induced contraction at 0.1μM (LogEC50±SEM, Ctrl:-5.638±0.08 vs Drug:-6.286±0.36) or 0.1nM (LogEC50±SEM, Ctrl:-5.638±0.08 vs Drug:-5.332±0.16). No differences were observed in endothelium dependent relaxation response to acetylcholine (ACh) for either drug concentration in the normotensive vessels; however, SHR MRA exhibited enhanced sensitivity to ACh with everolimus exposure (LogEC50±SEM, Ctrl: -6.851±0.15 vs Drug:-9.233±0.37 at 0.1nM; LogEC50±SEM, Ctrl: -6.851±0.15 vs Drug:-8.244±0.34 at 0.1μM). Our data show that everolimus affects contractility in normotensive vessels, while affecting the dilatory response in SHR vessels with an improvement in endothelium-dependent relaxation. Everolimus may be a good therapeutic agent for hypertensive cancer patients. However, long term treatments with this targeted therapy need to be evaluated for vascular dysfunction induction, which could be associated with cardiotoxicity.

Disclosures: P. Martinez Quinones: None. **C.G. McCarthy:** None. **C.F. Wenceslau:** None. **R. Webb:** None.

Funding: No

Funding Component:

P133

Obstructive Sleep Apnea May Exacerbate Carotid Artery Stiffness in Individuals With Chronic Obstructive Pulmonary Disease

Authors: Rachel E Luehrs, The Univ of Iowa, Iowa City, IA; Kerrie L Moreau, The Univ of Colorado, Aurora, CO; Mark Aloia, Howard D Weinberger, Barry Make, Russell Bowler, James D Crapo, Frederick Wamboldt, Natl Jewish Health, Denver, CO; Karin F Hoth, Gary L Pierce, The Univ of Iowa, Iowa City, IA

Background. Chronic obstructive pulmonary disease (COPD) and obstructive sleep apnea (OSA) are two of the most prevalent respiratory disorders in adults and are both independently associated with increased cardiovascular disease (CVD) risk. The coexistence of OSA in individuals with COPD is termed “overlap syndrome” and occurs in up to 65% of all COPD patients. Individuals with overlap syndrome have more frequent COPD exacerbations, poorer quality of life and increased CVD morbidity than patients with either COPD or OSA alone. COPD and OSA are both independently associated with increased carotid artery stiffness, a predictor of CVD events. However, the interaction of COPD and OSA on carotid artery stiffness remains unknown. We hypothesized that a) carotid artery stiffness would be greater in patients with overlap syndrome than COPD patients without OSA and non-COPD controls and b) overlap syndrome patients that reported using continuous positive airway pressure (CPAP) would have lower carotid artery stiffness than overlap patients not using a CPAP.
Methods. Ninety-nine former smokers (n=62 with COPD and n=37 non-COPD controls; age 70 ± 7 yrs; 60M/39F) completed measurements of pulmonary function and common carotid artery stiffness and were retrospectively stratified by self-report of previous clinical diagnosis with OSA.
Results. Participants with overlap syndrome had significantly greater carotid β-stiffness than COPD patients without OSA, non-COPD controls with OSA and non-COPD controls without OSA (overlap 5.7 ± 0.71 vs COPD only 3.9 ± 0.20, OSA only 3.6 ± 0.31, controls 3.7 ± 0.23 U; $F=6.245$, $p<0.01$) and this difference remained significant after adjustment for body mass index ($p=0.01$). There were no differences in blood pressure, heart rate, resting oxygen saturation (SpO_2) or nocturnal mean SpO_2 in those with overlap syndrome vs. the other groups. Furthermore, there was no difference in carotid β-stiffness between overlap syndrome patients that reported using vs. not using a CPAP (5.98 ± 0.7 vs 5.53 ± 1.9 U, $p=0.80$).
Discussion. These data suggest that OSA exacerbates carotid artery stiffening in COPD patients and that carotid artery

stiffness may be one mechanism that mediates the increased CVD risk observed in COPD patients with overlap syndrome.

Disclosures: R.E. Luehrs: None. **K.L. Moreau:** None. **M. Aloia:** None. **H.D. Weinberger:** None. **B. Make:** None. **R. Bowler:** None. **J.D. Crapo:** None. **F. Wamboldt:** None. **K.F. Hoth:** None. **G.L. Pierce:** None.

Funding: No

Funding Component:

P134

Sex Differences in the Effects of Chronic Muscadine Grape Extract Treatment on Cardiac Function Parameters and Aortic Pulse Wave Velocity (PWV) in Hypertensive Transgenic (mRen2)27 Rats

Authors: Fatima Ryalat, Nildris Cruz-Diaz, Jasmina Varagic, Patricia Gallagher, Ann Tallant, Debra Diz, Liliya Yamaleyeva, Wake Forest Univ, Winston Salem, NC

Muscadine grape extract (MGE) polyphenolics have potential beneficial cardiovascular effects. Whether the effects are sex-specific during progression of hypertension is unknown. We measured aortic arch PWV and cardiac function parameters using transthoracic echocardiography (Vevo LAZR 2100) after 15 weeks of MGE-treatment (0.2 mg polyphenolics /mL in drinking water) in transgenic hypertensive (mRen2)27 rats (n = 4 each, control male and female, MGE-treated female; n = 3 MGE-treated male) starting at 15 weeks of age. As expected, systolic blood pressure (SBP) measured via tail cuff after acclimation was higher in males than females at 15 weeks of age (179 ± 3 vs. 169 ± 3 mm Hg, $p = 0.04$). By 30 weeks of age, SBP did not differ between male and female rats (171 ± 4 vs. 171 ± 3 mm Hg). Body weight was higher in male than female rats (589 ± 8 vs. 326 ± 5 g, $p < 0.0001$); thus, cardiac output (CO), stroke volume (SV) and left ventricular mass (cLVM) were normalized to body weight. Systolic cardiac function was lower in male compared with female rats: SV (0.34 ± 0.04 vs. 0.59 ± 0.03 μL/g, $p < 0.001$); CO (0.13 ± 0.001 vs. 0.26 ± 0.02 mL/min/g, $p < 0.001$); ejection fraction (46 ± 4 vs. 74 ± 5 %, $p < 0.05$); fractional shortening (24 ± 3 vs. 45 ± 4 %, $p < 0.05$). In contrast, there was no difference between male and female rats in cLVM (2.35 ± 0.20 vs. 2.85 ± 0.14); heart rate (367 ± 19 vs. 432 ± 22 bpm); E/e' (12 ± 2 vs. 14 ± 0.4), or PWV (2.74 ± 0.21 vs. 2.85 ± 0.09 m/s). Sex differences in systolic cardiac function remained after 15 weeks of MGE treatment and MGE did not alter SBP or diastolic function (E/e'). However, aortic PWV was 36% lower in MGE treated males compared to control males (1.75 ± 0.13 vs. 2.74 ± 0.21 m/s, $p < 0.005$), which was also significantly lower than MGE treated females (2.40 ± 0.05 m/s, $p < 0.05$). Aortic PWV was 16% lower in MGE-treated females than control females, which did not reach significance (2.40 ± 0.05 vs. 2.85 ± 0.09 m/s). In conclusion, MGE intake improves arterial stiffness in male (mRen2)27 rats independent of differences in blood pressure or cardiac function. That aortic PWV in female rats did not derive benefit implies a potentially unique therapeutic profile for MGE in targeting mechanisms important for arterial stiffness in a sex-specific manner.

Disclosures: F. Ryalat: None. **N. Cruz-Diaz:** None. **J. Varagic:** None. **P. Gallagher:** None. **A. Tallant:** None. **D. Diz:** None. **L. Yamaleyeva:** None.

Funding: No

Funding Component:

P135

Bone Mineral Density is Associated With Vascular Endothelial Function and Arterial Stiffness in Apparently Normal Population From China

Authors: Huan Liu, Peking Univ Shougang Hosp, Beijing, China; Yongqiang Hong, Xiuqin Wu, Mindong Hosp Affiliated to Fujian Medical Univ, Fujian, China; Hongyu Wang, Jinbo Liu, Hongwei Zhao, Peking Univ Shougang Hosp, Beijing, China

Objective: To evaluate the relation of vascular parameters with bone mineral density (BMD) in the apparently normal population. Background: Vascular parameters including brachial arterial flow-mediated vasodilatation (FMD), brachial ankle pulse wave velocity (ba-PWV) and ankle brachial index (ABI), are reported associated with bone health. However, prior investigations have been limited to highly selected samples. Methods: We studied 165 individuals without a history of cardiovascular disease in the Chinese Arteriosclerosis Series Evaluation-She Minority Study (CASE-SMS) cohort (mean age 46.94±11.23 years, 59.4% women). BMD was quantified by dual-energy X-ray absorptiometry (DXA). Results: Age-adjusted analysis showed there was no difference in FMD, ba-PWV, ABI, lumbar vertebra BMD and left hip BMD between male, premenopausal female and postmenopausal female (all p>0.05). Multivariable linear analysis showed that FMD (B=0.005, p=0.016) and ba-PWV (B=-0.009, p=0.014) were both associated with lumbar vertebra BMD independently of each other. Multivariable logistic analysis showed FMD and ba-PWV were both related to low BMD in total (FMD OR 0.860, 95% CI 0.802 to 0.922, p<0.001; ba-PWV OR 1.494, 95% CI 1.275 to 1.751, p<0.001), male (FMD OR 0.861, 95% CI 0.757 to 0.979, p=0.022; ba-PWV OR 1.523, 95% CI 1.190 to 1.951, p=0.001), premenopausal female (FMD OR 0.842, 95% CI 0.734 to 0.966, p=0.014; ba-PWV OR 1.421, 95% CI 1.063 to 1.899, p=0.018) and postmenopausal female population (FMD OR 0.825, 95% CI 0.715 to 0.952, p=0.008; ba-PWV OR 1.740, 95% CI 1.187 to 2.551, p=0.004). In all the analyses, ABI was not associated with BMD (all p>0.05). Conclusion: There was no difference in FMD, ba-PWV, ABI, lumbar vertebra BMD and left hip BMD between male, premenopausal female and postmenopausal female after adjusting for age. FMD and ba-PWV were independently associated with lumbar vertebra BMD and low BMD. However, ABI was not associated with BMD.

Disclosures: H. Liu: None. **Y. Hong:** None. **X. Wu:** None. **H. Wang:** None. **J. Liu:** None. **H. Zhao:** None.

Funding: No

Funding Component:

P136

A Case Report of Aortic Dissection With Higher Level of Pulse Wave Velocity

Authors: Jinbo Liu, Peking Univ Shougang Hosp, Beijing, China; Tianrun Li, Peking Univ Third Hosp, Beijing, China; Huan Liu, Hongwei Zhao, Wei Huang, Lichun Wang, Yuejie Song, Xiaolan Yu, Hongyu Wang, Peking Univ Shougang Hosp, Beijing, China

Background: Hypertension was an important risk factor of aortic dissection. Arteriosclerosis evaluated by arterial stiffness was the basic patho-physiological change of hypertension. We reported a case of aortic dissection with higher level of arterial stiffness.
Methods: A patient (women, 62-year old) visited Peking University Shougang Hospital with sudden intense back pain for 3 hours on November 17, 2017. Her medical history was hypertension with treatment of levamlodipine 2.5mg/day, without regular monitoring.
Results: Computed tomography angiography (CTA) indicated a cute Stanford B Type thoracic aortic dissection. The results of white blood cell count, platelet count, electrolytes, urea, creatine, erythrocyte sedimentation rate and liver function tests were all normal with higher level of D-Dimer of 13.5mg/L (reference range <1.0mg/L). Cardiac ultrasound showed no abnormal signs of aortic valve reflux and ventricular wall activity, with normal ejection fraction (59%).

Electrocardiogram showed signs of cardiac ischemia with changes of T waves. Thoracic endovascular aortic repair therapy (TEVAR) was performed on November 18, 2017 and the back pain was relaxed. However, the patient had intermittent back pain on November 21, 2017 with recurrent aorta dissection showed by CTA. TEVAR was performed again immediately on November 21, 2017. Finally, her treatment was nifedipin controlled-release 30mg/day, irbesartan and hydrochlorothiazide 150mg/12.5mg/day, bisoprolol fumarate 3.75mg/day, terazosin hydrochloride 2mg/day, aspirin 100mg/day, with systolic blood pressure 90-100mmHg and heart rate of 55-60 beats per minute. Arterial stiffness showed that left brachial ankle pulse wave velocity (ba-PWV) of the patient was 2138cm/s (reference range <1400cm/s) and the right ba-PWV was 2018cm/s (reference range <1400cm/s).
Conclusions: Our present case report showed that hypertension subject with higher level of pulse wave velocity was more likely to happen aortic dissection, so arterial stiffness should be evaluated during the management of hypertension. If we could find the abnormality of arteriosclerosis earlier, we could carry out some management to reduce arterial stiffness, and aortic dissection might be prevented in future.

Disclosures: J. Liu: None. **T. Li:** None. **H. Liu:** None. **H. Zhao:** None. **W. Huang:** None. **L. Wang:** None. **Y. Song:** None. **X. Yu:** None. **H. Wang:** None.

Funding: No

Funding Component:

P137

Arterial Stiffness in Diabetic Patients on Oral Antidiabetic Agents V/s a Combination of Oral Antidiabetic Agents Plus Insulin

Authors: Joaquin Cigarroa, Ignacio DiCicco, Son Pham, Joan Hecht, Robert Chilton, Rene Oliveros, Univ of Texas Health Science, San Antonio, TX

In a previous publication (Journal of the American Society of Hypertension 2014;8(4):e35-e36 we did a comparison study of arterial stiffness in diabetic pts taking oral antidiabetic agents (ODA) v/s those taking Insulin. The aim of the present study was to compare aortic stiffness determined from resting and exercise pulse pressure (PP) in a group of pts taking ODA v/s a group of pts taking a combination of ODA and insulin.
We studied 86 diabetic pts referred to us for the performance of a maximal treadmill exercise test as part of a workup for chest pain. They were divided into 46 pts taking ODA (group 1) v/s 46 pts taking a combination of ODA plus insulin (group 2). Their age, heart rate(HR) both rest and exercise, PP both rest and exercise, exercise duration in seconds and HbA1C are noted in the table.
There was no significant difference in the HR and PP both at rest and during exercise between both groups. Also, no significant difference was found in their ages and exercise duration although group 2 pts were older, had greater PP both at rest and exercise and had shorter exercise duration. The only significant difference was that goup 2 had a sinificantly higher A1C than group 1($p<0.003$) reflecting the severity of their diabetes.
We conclude from this small study, that the addition of Insulin to oral antidiabetic agents did not affect aortic stiffness as determined by PP which is a surrogate for other measures of aortic stiffness.

Disclosures: J. Cigarroa: None. **I. DiCicco:** None. **S. Pham:** None. **J. Hecht:** None. **R. Chilton:** None. **R. Oliveros:** None.

Funding: No

Funding Component:

P138

The Arterial Wall of the Central Vascular Compartment a Novel Target of Iron-Overload Disorders: A Preliminary Study With Experimental Correlation in Animals

Authors: Mariano Duarte, Analia Aquieri, Javier Coyle, Claudio Yaryour, Esteban Gonzalez Ballerga, Hosp de Clinicas Facultad de Me, Buenos Aires, Argentina; Carlos Reyes Toso, Cátedra de Fisiología Facultad de Medicina UBA, Buenos Aires, Argentina

In Hemochromatosis (H) iron is stored in tissues, causing organ injury and failure. Patients with H have a shorter life expectancy and an increase in CVR. Our group could prove that arterial stiffness was increased and they had added endothelial dysfunction. After that we also were able to demonstrate that both improve by reducing iron levels with phlebotomies. Due to these findings we ask how iron-overload made it (getting inside or more likely inducing inflammation). Anyway either directly or indirectly AW could be a target of iron-overload. To test this hypothesis we did an experimental correlation by using a known model of H in rats and we analize histologically the AW Twelve young rats (3 months old), an average weight of 300 gr, fed as usual, in individual cages with dark light cycles of 12 h and controlled temperature (22±3°C), were ramdonly divided in 2 groups: 1) Iron-overoload group (IOG). They were daily administrated intraperitoneally 10 mg of elemental iron (0,1 ml of dextran iron, Venofer®) 12 injections in total 2) Control group (CG) the same injections scheme but with placebo. The handlings of animals was under the norms of the bioterium and standards designed for that purpose. At the end of experimental period (8 weeks) animals were sacrificed and studied. The histoarchitecture of the proximal aortic wall (fixed in 4 % formalin, paraffin-embedded, sectioned into thin slices, H&E, Masson´s trichrome and VVG staining) showed no significant differences in the histology (H&E) between IOG and CG. No evidence of iron inside the AW. On the other hand inflammatory component was significantly increased in IOG compared to CG, perivascular lymphocyte count 5-12/0 IOG/CG, perivascular macrophages, 3-5/0 IOG/CG. Elastic fibers were not fragmented but become thin and lost their disposition in IOG.
In conclusions we can affirm that AW could represent a novel target in H and could may the nexus to cardiovascular disease. The indirect mechanism (inflammation and elastic fibers) explains why previously improved after phlebotomies. Our findings supports future research including inflammatory mediators and their tissue expression, and extend them to others iron overload disorders.

Disclosures: M. Duarte: None. **A. Aquieri:** None. **J. Coyle:** None. **C. Yaryour:** None. **E. Gonzalez Ballerga:** None. **C. Reyes Toso:** None.

Funding: No

Funding Component:

P139

Endothelial Bbsome is Essential for Vascular Endothelial Function

Authors: Jingwei Jiang, John J Reho, Donald A Morgan, Kamal Rahmouni, Univ of Iowa Carver Coll of Med, Iowa City, IA

The BBSome, a complex containing 8 Bardet-Biedl syndrome (BBS) proteins including BBS1, has merged as an important regulator of various cellular processes including trafficking of receptors to plasma membrane. We hypothesized that the BBSome in endothelial cells contribute to the control of vascular function. We generated mice lacking the BBSome specifically in endothelial cells by crossing $Bbs1^{fl/fl}$ mice with the endothelial-specific $Tie2^{cre}$ mice. The $Bbs1^{fl/fl}/Tie2^{Cre}$ mice developed normally compared to littermate control mice with no difference in body weight (females: 24.3±1.3 vs 22.7±1.0 g in control; males: 31.8±1.7 vs 30.0±0.6 g at end of 16 weeks of age), fat mass or food intake. Circulating glucose, triglycerides and cholesterol were also unaltered in $Bbs1^{fl/fl}/Tie2^{Cre}$ mice. Next, we assessed vascular function *ex vivo* using aortic rings and resistance-sized mesenteric arteries. Strikingly, endothelial cell-specific BBSome deficiency

caused endothelial dysfunction in both male and female mice, with more severe changes observed in females. In aortic rings, relaxation responses to acetylcholine were decreased in female $Bbs1^{fl/fl}/Tie2^{Cre}$ mice compared to controls (Max. relaxation: 37.8±15.2 vs 62.0±10.3%). The male $Bbs1^{fl/fl}/Tie2^{Cre}$ mice displayed a slightly decreased response that was not statistically different (Max relaxation: 38.2±11.7 vs 53.7±12.0%). In the mesenteric arteries, both male and female $Bbs1^{fl/fl}/Tie2^{Cre}$ mice showed significantly decreased relaxation response evoked by acetylcholine (Max. relaxation: male: 43.6±7.6 vs 56.4±6.2% in controls, $P<0.05$; female: 35.8±8.0 vs 75.9±10.0% in controls, $P<0.05$). However, the relaxation responses induced by sodium nitroprusside in both the aorta and mesenteric arteries were not different in male and female $Bbs1^{fl/fl}/Tie2^{Cre}$ mice compared to controls. Thus, endothelium-specific BBSome deficiency leads to endothelial, but not smooth muscle dysfunction. Notably, female $Bbs1^{fl/fl}/Tie2^{Cre}$ mice displayed about 25 mmHg elevation in mean arterial pressure relative to controls. Thus, the BBSome in endothelial cells contribute to vascular endothelial function and blood pressure regulation.

Disclosures: J. Jiang: None. **J.J. Reho:** None. **D.A. Morgan:** None. **K. Rahmouni:** None.

Funding: No

Funding Component:

P140

Leptin Supplementation Restores Endothelial Function and Vascular Adrenergic Tone via Direct Endothelial Mechanisms and Sympato-Activation, Respectively, in a Mouse Model of Congenital Lipodystrophy

Authors: Thiago B Bruder-Nascimento, Jessica Faulkner, Simone Kennard, Weiqin Chen, Eric Belin de Chantemele, Augusta Univ, Augusta, GA

Absence of adipose tissue in congenital generalized lipodystrophy (CGL) leads to metabolic and cardiovascular disease and reduced leptin levels. We tested the hypothesis that leptin supplementation restores endothelial function and reduces vascular adrenergic response respectively via acting directly on endothelial cells (EC) and desensitizing vascular adrenergic response, in CGL mice. Deletion of Berardinelli-Seip gene (Bscl2) was used to mimic the human pathology in mice. Bscl2-/- mice showed reduced adipose mass [% of fat: wild-type (WT): 8.6 ± 0.3 vs Bscl2-/-: 2.4 ± 0.1, $P<0.05$], leptin levels [ng/mL: WT: 4.0 ± 0.3 vs Bscl2-/-: 0.3 ± 0.1, *$P<0.05$], impaired aortic endothelium-dependent relaxation to acetylcholine (Ach): [% relaxation: WT: 71.9 ± 1 vs Bscl2-/-: 49.9 ± 2*, *$P<0.05$], increased vascular contractility to phenylephrine (Phe), associated with upregulation of α-adrenergic receptors, high mean arterial pressure (MAP) and reduced heart rate (HR): [(mmHg: WT: 100.7 ± 5 vs Bscl2-/-: 109.5 ± 6*), (bpm: WT: 592.7 ± 9 vs Bscl2-/-: 542.9 ± 15*) *$P<0.05$]. Moreover, Bscl2-/- mice shown increase in Nox1 gene expression and increases in vascular $^{-}O_2$ and H_2O_2 levels. Both chronic (in vivo) and acute (organ bath) leptin supplementation or NOX 1 inhibition (GKT771, 10μM) restored endothelial function in addition to reducing endothelial Nox1 expression and ROS production, which suggest a direct role for leptin in endothelial cells. EC leptin receptor deficiency induced an endothelial dysfunction in mice, which was restored by Nox1 inhibition, further supporting a direct role of leptin in endothelial cells. In parallel, leptin supplementation reduced vascular adrenergic tone and aortic α-adrenergic receptors and increased in HR without changing MAP. Our data show that leptin replacement restores endothelial function and reduces vascular adrenergic tone in mouse model of congenital lipodystrophy by acting directly in EC and desensitizing vascular adrenergic response, respectively. This highlight the importance of leptin in the maintenance of vascular homeostasis and presents leptin as a potential therapeutic avenue for the treatment of vascular diseases associated with low leptin levels.

Disclosures: T.B. Bruder-Nascimento: None. **J. Faulkner:** None. **S. Kennard:** None. **W. Chen:** None. **E. Belin de Chantemele:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P141

Aging in Mice Enhances Microvascular Reactive Oxygen Species (ROS) That Impair the Anti-Contractile Actions of Perivascular Adipose Tissue (PVAT)

Authors: Tian Li, Christopher S WILCOX, Dan Wang, Georgetown Univ Div of Nephrology, Washington, DC

Background: Reactive oxygen species (ROS) contribute to oxidative stress and aging that predicts cardiovascular disease (CVD). Perivascular adipose tissue (PVAT) enhances endothelial function and reduces contractility of normal vessels. We tested the hypothesis that ROS and age impair PVAT signaling using mesenteric arterioles (MAs) from young and old wild type (Wt) and superoxide dismutase 3 knockout mice (KO). *Methods and results:* MAs were isolated with and without PVAT from young (3 – 4 months; n = 5 – 7) and old (23 – 24 months; n = 5 – 7) wild-type (Wt) and superoxide dismutase 3 knockout (-/-) mice (to create oxidative stress). Contractions were assessed on an isometric myograph and ROS and mitochondrial ROS by fluorescence microscopy in MAs loaded with dihydroethidium or MitoSOX Red. The increase in ROS and mitochondrial ROS signals with 10^{-7} mol·l^{-1} endothelin 1 (ET1) were increased in MAs from Wt old vs young mice (0.21 ± 0.05 vs 0.11 ± 0.04 units; P<0.05 and 0.15 ± 0.04 vs 0.08 ± 0.04 units; P<0.05 respectively). Amongst Wt mice, the effect of PVAT to reduce contractions to 10^{-6} mol·l^{-1} U-46,619 was reduced in old vs young mice (15 ± 1 vs 45 ± 6% reduction; P<0.005) with similarly reduced effects of PVAT in vessels from old mice on contractions to 10^{-7} mol·l^{-1} endothelin 1 (ET1: 15 ± 4 vs 34 ± 3% reduction; P<0.005). Compared to young Wt mice, MAs from young SOD3 -/- mice had reduced protection by PVAT from contractions to U-46,619 (25 ± 3 vs 45 ± 6% reduction; P<0.01) or to ET1 (3 ± 2 vs 34 ± 8% reduction; P<0.005). The effects of oxidative stress to lessen the protective effects of PVAT were lessened in old mice where the reductions in contractions mediated by PVAT to U-46,619 were no longer different in SOD +/+ vs -/- mouse MAs (12 ± 1 vs 15 ± 1% reduction; NS) while contractions to ET1 remained somewhat protected by PVAT (8 ± 2 vs 15 ± 4% reduced; P<0.05). *Conclusions:* Aging enhances ROS generation by mesenteric arterioles that impairs the beneficial effects of PVAT to protect vessels from contractions with thromboxane and endothelin. This discloses a novel mechanism for age-related cardiovascular disease.

Disclosures: T. Li: None. **C.S. Wilcox:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PI. **D. Wang:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PI.

Funding: No

Funding Component:

P142

Aberrant Notch2 Signaling in the Vascular Endothelium Leads to Increased Proliferation and Migration Operant in Pulmonary Arterial Hypertension

Authors: Sanghamitra Sahoo, John C Sembrat, Mauricio Rojas, Elena A Goncharova, Patrick J Pagano, Univ of Pittsburgh, Pittsburgh, PA

Background: Pulmonary arterial hypertension (PAH) develops when pulmonary vascular resistance rises sharply, leading to right heart failure and death. Most studies have focused on attenuating lung vessel constriction; however, little is known about the mechanisms controlling intimal proliferation and luminal obstruction. Numerous studies suggest that the Notch signaling influences vascular cell proliferation and migration, hallmark features of deleterious remodeling. Despite this, a role for Notch2 in PAH is entirely unknown. In this study, we hypothesize that hypoxia-induced repression

of lung endothelial Notch2 results in increased EC proliferation/migration in PAH. **Methods and Results:** In a hypoxia model replicating the PAH phenotype in vitro, HPAECs were subjected to 24 hrs hypoxia (1% O_2) compared to normoxia (21% O_2). Hypoxia attenuated Notch2 mRNA (0.64±0.02-fold vs. normoxia, p<0.0001) but did not affect other endothelial Notch receptors - Notch1 and 4. Furthermore, hypoxia decreased Notch2 protein levels (0.65±0.05-fold vs. normoxia, p<0.0001). q-RTPCR of lysates of pulmonary arteries from PAH patients showed marked reductions in Notch2 mRNA (0.30±0.08-fold vs. control patients, *p*<0.05). Notch2 siRNA under normoxic conditions increased EC migration in a "wound healing-scratch" assay (1.5±0.01-fold vs. scrambled control, p<0.05) and EC proliferation (1.2±0.07-fold vs. scrambled control, *p*=0.05) measured by CyQuant assay. Western blot analysis of signaling intermediaries showed decreased cell cycle inhibitor $p21^{cip}$ (0.54±0.05 vs. scrambled control, p<0.05), and increased levels of phospho-ERK1/2 (1.3±0.04-fold vs. scrambled control, p<0.001). **Conclusions & Discussion:** In the present study, we found that hypoxia suppressed Notch2 expression in the endothelium which appears to derepress ERK1/2 and downregulate $p21^{cip}$ leading to EC proliferation and migration. These data are consistent with a key modulatory role for Notch2 dysfunction in EC proliferation/migration and vascular remodeling in PAH. Data from human patients substantiates this dysfunction. The current study identifies a new signaling axis mediated by endothelial Notch2 that could contribute to pathophysiological changes associated with human PAH.

Disclosures: S. Sahoo: None. **J.C. Sembrat:** None. **M. Rojas:** None. **E.A. Goncharova:** None. **P.J. Pagano:** None.

Funding: No

Funding Component:

P143

Nox1 Elicits Endothelial Cell Proliferation in a CREB- and Ref-1- Dependent Manner

Authors: Daniel de Jesus, Yao Li, Evan DeVallance, Patrick Pagano, Univ of Pittsburgh, Pittsburgh, PA

Pulmonary arterial hypertension (PAH) is a complex and progressive disorder, characterized by an increase in vascular remodeling, a rise in pulmonary arterial pressure and right heart failure. Recently, we and others showed that BMP antagonist Gremlin1 elicits pulmonary endothelial cell (EC) proliferation in response to hypoxia and that Gremlin1 haplodeficiency attenuates PAH. NADPH oxidase (Nox)-derived reactive oxygen species (ROS) seem to play a pivotal role in PAH. However, the mechanisms by which ROS propagates the disease are unclear. Transcription factor CREB, which is activated by hypoxia, ROS, and interaction with Ref-1 (redox factor 1), mediates gene transcription related to proliferation and vascular remodeling. We postulated that Nox1/Ref-1-mediated CREB activation leads to Gremlin1-induced EC proliferation. Human pulmonary arterial EC (HPAECs, Lonza) were subjected to 24 hr hypoxia (1% O_2 vs. normoxia - 21% O_2) to mimic the EC phenotype in PAH. Hypoxia upregulated Nox1 protein level (1.40±0.09-fold, p<0.05) and H_2O_2 production level/mg protein (2.20±0.21-fold, p<0.0001) vs. normoxia. siNox1 (Nox1 gene silencing) abolished hypoxia-induced Nox1, and Nox1 inhibitor, NoxA1ds, decreased hypoxia-promoted H_2O_2. Moreover, siNox1 decreased hypoxia-induced pCREB (1.74±0.22-fold v. normoxia, vs. 1.07±0.08 siNox1+hypoxia, p<0.05), and siCREB decreased hypoxia-induced Gremlin1 (1.83±0.16 hypoxia vs. 0.35±0.01 siCREB+hypoxia, p<0.05). Further, hypoxia augmented nuclear pCREB/Ref-1 interaction (1.812±0.207-fold vs. normoxia, p<0.05) and pCREB association with its DNA binding motif. siRNA for Ref-1 impaired hypoxia-induced pCREB DNA binding (1.96±0.06 hypoxia vs. 0.815±0.219 siRef-1+hypoxia, p<0.01). Moreover, siCREB decreased hypoxia-induced EC proliferation (1.40±0.050 hypoxia vs. 0.96±0.04 siCREB+hypoxia). Finally, preliminary data show pCREB binding to the Gremlin1 promoter. Taken together, our data support a previously unidentified redox signaling pathway by which Nox1-derived ROS promote Gremlin1 expression and EC proliferation in PAH.

Disclosures: D. de Jesus: None. **Y. Li:** None. **E. DeVallance:** None. **P. Pagano:** None.

Funding: No

Funding Component:

P144

Characterization of Vascular Endothelial and Smooth Muscle Exosomes and Their Role in Vascular SignalTransduction

Authors: Michael Boyer, Temple Univ, Philadelphia, PA; Yayoi Kimura, Yokohama City Univ, Yokohama, Japan; Satoru Eguchi, Victor Rizzo, Temple Univ, Philadelphia, PA

Vascular homeostasis is predicated on the integral interaction between a myriad of cell types. Principle to homeostasis is the interaction between the endothelial cells (ECs) of the intima and the vascular smooth muscle cells (VSMCs) of the media. Disruption of the tightly controlled communication between these cell types can lead to development of pathophysiology. In the past decade, much study has been given to a novel means of intercellular communication in exosomes. Exosomes are nanovesicles derived from endosomal compartments capable of carrying functional proteins, small RNAs and lipids into extracellular space. Initially characterized in the field of immunology, little is known on exosomes within vascular biology. Therefore, we present an *in vitro* characterization of rat EC and VSMC exosomes and evidence of EC exosomes as initiators of signal transduction within VSMCs. Primary rat aortic EC and VSMC exosomes isolated in serum-free conditions displayed characteristic size, shape and protein content of exosomes as analyzed through nanoparticle tracking analysis, electron microscopy, and western blot. Interestingly, alpha smooth muscle actin was found to be cell-specific for VSMC exosomes. Proteomics identified differential expression between EC and VSMC exosomes suggesting altered cargo between the two populations. Incubation of EC exosomes with VSMC identified a dose- and time-dependent increase in the expression of vascular cell adhesion molecule 1 in VSMC with a maximum at 10^9 EC exosomes and 6 hours through immunoblot analysis. In addition, EC exosomes stimulated expression of early growth response protein 1 at 1-3 hours, suggesting activation of pro-inflammatory gene expression. EC exosomes stimulated a maximal rate of protein synthesis at 3 hours in VSMC (3.53 fold increase over control, p=0.016) determined through puromycin incorporation showing qualitative changes in cell function. Analysis of cell volume identified a hypertrophic response in VSMC after exosome incubation as well, displaying a role for exosomes in vascular hypertrophy. In conclusion, we present a broad characterization of exosomes in vascular biology and a functional crosstalk mechanism between RAEC and VSMC through exosome transfer.

Disclosures: M. Boyer: None. **Y. Kimura:** None. **S. Eguchi:** None. **V. Rizzo:** None.

Funding: Yes

Funding Component: Great Rivers Affiliate (Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)

P145

The Bacterial Metabolite Trimethylamine (TMA), but Not Trimethylamine N-Oxide (TMAO), Causes Vascular Contraction

Authors: Carolina B Restini, Gregory D Fink, Stephanie W Watts, Michigan State Univ - MSU, East Lansing, MI

Circulating levels of TMAO are elevated in humans with cardiometabolic diseases. The gut microbiota-initiated TMA/flavin-containing monooxygenase 3 (FMO3)/TMAO pathway has been identified as a potential modulator of host cardiometabolic phenotypes. Like TMA/TMAO, FMO3 expression is positively correlated with body mass index and high-fat diet-induced obesity We hypothesized that TMA/TMAO stimulate vascular contraction which could be modified by perivascular adipose tissue (PVAT) expression of FMO3. Our model was the isolated thoracic aorta with (+) or without (-) PVAT, in presence (+) or in absence (+) of endothelium (E) of male Sprague Dawley rat to perform tissue bath studies to

measure isometric tone using a large range of TMA/TMAO concentrations: 1 nM-10^{-1} M. Immunohistochemistry (IHC) studies were done to identify the presence of FMO3 in aorta. TMA and TMAO, at any concentration, did not relax half maximally contracted tissues when compared to vehicle control. TMA and TMAO elicited arterial contraction independent of PVAT or E status. Interestingly, contractions stimulated by TMA were significantly higher (% PE max 123.7±15.6%) than by TMAO (% PE max 38.3±15.5%). IHC studies revealed FMO3 in aortic PVAT, but the FMO3 inhibitor methimazole (100 μM) did not affect aortic contraction stimulated by TMA (+PVAT). The L type Ca2+ channel antagonist nifedipine (100 nM), reduced the TMA-induced contraction (% PE max: +PVAT: 73±9%; -PVAT: 63±6.7%), compared to vehicle (+PVAT: 149.8±18%; -PVAT: 111.8±9%). These results support that TMA can induce arterial contraction with higher potency and efficacy compared to TMAO, independent of the conversion to TMAO. The contractile mechanism is ≈ 50% dependent of Ca^{2+} influx through L-type VOC. Considering the high concentration needed to achieve the contraction it is unlikely that increasing vascular tone is a mechanism that could contribute to CVD but dependence of contraction on Ca^{2+} channel activation suggests specific activity of TMA in the vessel wall that warrants further study.

Disclosures: C.B. Restini: None. **G.D. Fink:** None. **S.W. Watts:** None.

Funding: No

Funding Component:

P146

Cytochrome B5 Reductase (CYB5R3) Function in Smooth Muscle Cells and Blood Pressure Regulation

Authors: Brittany G Durgin, Scott A. Hahn, Megan P. Miller, Adam C. Straub, Univ of Pittsburgh, Pittsburgh, PA

Hypertension (HTN) is a major risk factor for cardiovascular-related morbidity and mortality. Dysregulation of nitric oxide (NO) signaling has been found in both human and mouse studies to impact the renin-angiotensin-aldosterone system (RAAS) and contribute to the vascular dysfunction leading to HTN. NO signals via soluble guanylyl cyclase (sGC) to generate cyclic guanosine monophosphate (cGMP) and downstream activation of protein kinase G (PKG) to induce vasodilation. However, the sGC heme iron needs to be in the reduced (Fe^{2+}) state for NO to bind as oxidized (Fe^{3+}) sGC is insensitive to NO signaling. We found that NADH cytochrome b5 reductase 3 (CYB5R3) is a sGC heme reductase that maintains sGC heme iron in its reduced state. Transient knockdown and pharmacological inhibition of CYB5R3 in smooth muscle cells (SMCs) blocks NO-mediated cGMP production and aortic relaxation. Taken together, we hypothesized that SMC CYB5R3 was important in blood pressure regulation. We created tamoxifen-inducible SMC-specific CYB5R3 knockout mice (SMC CYB5R3 KO) and found that they (n=8) had an ~5 mmHg increase in mean arterial blood pressure at baseline as compared to SMC CYB5R3 WT mice (n=9). In response to Angiotensin II (Ang II)-induced hypertension, SMC CYB5R3 KO mice (n=5) exhibited an ~14 mmHg increase in blood pressure and a significantly reduced heart rate as compared to SMC CYB5R3 WT mice (n=5). Lastly, ex vivo two-pin wire myography of Ang II-induced hypertensive SMC CYB5R3 KO (n= 6) mesenteric arteries treated with NO-independent sGC activator Bay 58-2667 showed increased vasodilation as compared to SMC CYB5R3 WT mice (n=10); Bay 58-2667 treatment at baseline showed no difference between groups. We predict that CYB5R3 regulation of SMC sGC heme iron redox state influences blood pressure in a NO-dependent manner. To test this, we will perform an intervention study where we will measure blood pressure in Ang II-induced hypertensive SMC CYB5R3 WT and SMC CYB5R3 KO mice treated with Bay 41-2272, a NO and heme dependent sGC stimulator, and Bay 58-2667. Also, we will assess the role of SMC CYB5R3 in protection against Ang II-induced vessel fibrosis and SMC dysfunction.

Disclosures: B.G. Durgin: None. **S.A. Hahn:** None. **M.P. Miller:** None. **A.C. Straub:** C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; Bayer Pharmaceuticals.

Funding: No

Funding Component:

P148

Muscarinic Acetylcholine Receptors are Localized to Primary Endothelial Cilia and Involved in Nitric Oxide Signaling

Authors: Hannah C Saternos, Hayley Gibson, Mahmood Meqdad, **Wissam A AbouAlaiwi**, Univ of Toledo, Toledo, OH

Primary cilia are considered sensory hubs housing a variety of mechanosensory proteins, chemosensory receptors, and ion channels to translate extracellular stimuli into an intracellular biochemical signals such as the synthesis and release of nitric oxide (NO). Malformations in cilia structure or defects in cilia function lead to ciliopathies such as Polycystic Kidney Disease (PKD) and hypertension. Muscarinic receptors 1 and 3 play an essential role in regulating cardiovascular function by mediating both dilation and constriction in the vasculature. However, nothing is known about the relationship between primary cilia and muscarinic receptors. Supported by our exciting discovery of the mAChRs in the cilia, we hypothesize that ***primary cilia in the vascular system play important roles in regulating blood pressure through NO biosynthesis.*** Our objective is to unravel the mechanism by which cilia dysfunction contribute to hypertension and to introduce cilia as a novel therapeutic target for PKD. To explore the relationship between mAChRs and primary cilia, the effects of muscarinic modulators on cilia length and function in wildtype, and cilia mutant endothelial cells, *Pkd1*$^{-/-}$ (dysfunctional cilia) and *Tg737*$^{orpk/orpk}$ (cilia-less) were examined. We show that both AChM1R and AChM3R localize to primary endothelial cilia. AChM1R and AChM3R activation lead to a significant increase in cilia length in endothelial cells treated with cdd0102a, an AChM1R and AChM3R agonist (2.84±0.02 vs. 3.47± 0.04 for wildtype, 2.31± 0.03 vs. 2.53± 0.03 for *Pkd1*$^{-/-}$, and 0.024 ± 0.005 vs. 0.3 ± 0.004 for *Tg737*$^{orpk/orpk}$) compared to control cells. Treatment with pirenzepine, an AChM1R antagonist, led to a significant decrease of cilia length (2.66± 0.02 vs. 2.48± 0.03 in wildtype, and 2.12± 0.02 vs. 1.93± 0.02 in *Pkd1*$^{-/-}$) compared to control cells. Treatment with cdd0102a also significantly upregulated the expression of AChM1R, AChM3R and phosphorylated eNOS in wildtype and *Pkd1*$^{-/-}$ cells. More importantly, cdd0102a treatment rescued NO response in *Pkd1*$^{-/-}$ cells in response to fluid shear stress. Our data clearly suggest that modulating cilia sensory function could have a positive influence on nitric oxide generation and blood pressure regulation.

Disclosures: H.C. Saternos: None. **H. Gibson:** None. **M. Meqdad:** None. **W.A. AbouAlaiwi:** None.

Funding: Yes

Funding Component: Great Rivers Affiliate (Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)

P149

VEGF Inhibitor-Induced Hypertension and Renal Injury: Prevention by Aspirin?

Authors: Katrina M Mirabito Colafella, Monash Univ, Melbourne, Australia; Karla B Neves, Univ of Glasgow, Glasgow, United Kingdom; Augusto C Montezano, Uinversity of Glasgow, Glasgow, United Kingdom; Richard van Veghel, Ingrid M Garrelds, Erasmus Medical Ctr, Rotterdam, Netherlands; Hans J Baelde, Leiden Univ Medical Ctr, Leiden, Netherlands; Anton H van den Meiracker, Erasmus Medical Ctr, Rotterdam, Netherlands; Rhian M Touyz, Univ of Glasgow, Glasgow, United Kingdom; AH Jan Danser, Erasmus Medical Ctr, Rotterdan, Netherlands; Jorie Versmissen, Erasmus Medical Ctr, Rotterdam, Netherlands

Vascular endothelial growth factor inhibitor (VEGFi)-induced hypertension and renal injury in cancer patients mimic the clinical phenotype of preeclampsia. ET-1 has been implicated to be involved. Since aspirin can prevent the onset of preeclampsia, we hypothesized that co-treatment of the VEGFi sunitinib (SU) with aspirin might be beneficial and may improve ET-1 status. Male WKY rats were treated with vehicle, 14 mg/kg/day SU, or SU + low or high dose aspirin (5 or 100 mg/kg/day) for 8 days. Mean arterial pressure (MAP) was measured via radiotelemetry. On day 8, 24h urine was

collected to determine albuminuria and prostanoid (PGF2α and TxB2) levels. Plasma endothelin-1 (ET-1) was measured via ELISA. Endothelial dysfunction was assessed in iliac vessels and reactive oxygen species (ROS) was measured via aortic superoxide anion production. Renal cyclooxygenase (COX)-1, COX-2 and ET-1 mRNA expression were determined via PCR. SU induced a rapid and sustained increase in MAP (24±2 versus 1±1 mmHg in vehicle on day 6; P<0.001), which was blunted by both low and high aspirin doses (18±3 and 13±4 mmHg respectively on day 6; P<0.05 versus SU). Plasma ET-1 was increased by SU (2.2±0.3 versus 1.4±0.1 pg/ml in vehicle; P<0.05) and this was not affected by aspirin. SU increased albuminuria (1.2±0.2 versus 0.3±0.1 mg/24h in vehicle; P<0.05) which was prevented by high, but not low dose aspirin (0.5±0.1 and 1.3±0.1 mg/24h, respectively), suggesting that this effect is COX-2 dependent. Although renal mRNA expression levels were unchanged, renal COX-2 and ET-1 mRNA expression correlated positively, suggesting that ET-1 upregulates COX-2. SU increased ROS production 2.5-fold (P<0.05 versus vehicle) and this was prevented by both aspirin doses. SU did not induce endothelial dysfunction, nor did it alter prostanoid levels. However, both aspirin doses reduced TxB2 (P<0.05 versus vehicle). In conclusion, high rather than low dose aspirin was more efficacious for the prevention of VEGFi-induced hypertension and renal injury, suggesting that its beneficial effects largely involve COX-2 inhibition. Aspirin may be a novel intervention to allow cancer patients to gain the full benefit of VEGFi without deleterious cardiovascular and renal side effects.

Disclosures: K.M. Mirabito Colafella: None. **K.B. Neves:** None. **A.C. Montezano:** None. **R. van Veghel:** None. **I.M. Garrelds:** None. **H.J. Baelde:** None. **A.H. van den Meiracker:** None. **R.M. Touyz:** None. **A. Danser:** None. **J. Versmissen:** None.

Funding: No

Funding Component:

P150

Chronic Exercise Provides Antihypertensive Effects With Upregulation of Oxidative Stress-Induced Nitric Oxide Upregulation in The Kidney of High-Fructose Fed Rat

Authors: Gaizun Hu, Lusi Xu, Masahiro Kohzuki, Osamu Ito, Tohoku Univ Graduate Sch of Med, Sendai, Japan

Object The fructose consumption was reported to correlated with increase of oxidative stress, which thereby impair nitric oxide system. Chronic exercise (Ex) can provide antihypertensive effects though nitric oxide upregulation. The mechanisms of Ex in nitric oxide upregulation are various and remains unclarified. **Method** A total of 24 Sprague Dawley rats at 5-week age were allocated to 3 groups as controlled diet group (CON), high-fructose diet group (HFr) and HFr underwent 12-week chronic exercise (HFr-Ex). The systolic blood pressure (SBP) and urinary albumin excretion were taken every 2 weeks. At the final week, after the insulin tolerance test, the rats were euthanized. The plasma and kidneys were obtained. The plasma and renal oxidative stress markers were measured. The nitric oxide related parameters including renal endothelial nitric oxide synthase (eNOS) expression were measured. **Result** The SBP was significantly increased in HFr than CON, which Ex significantly attenuated the SBP elevation (115 vs. 141 mmHg, p<0.01). Ex reversed albuminuria induced by HFr without alteration in creatinine clearance ratio (474 vs. 1446μg/day, p<0.01). Ex significantly lowered uric acid, and attenuated the insulin resistance (uric acid, 1.41 vs. 2.05 mg/dL, p<0.01; HOMA-IR, 0.64 vs. 0.50, p<0.01). Ex significantly inhibited renal cortex xanthine oxidase activity (XO), meanwhile the NADPH oxidase activity (NADPH) was even enhanced (XO, 15.7 vs. 11.8 units/mg protein, p<0.05; NADPH, 897 vs. 1370 c.p.m, p<0.01). The hydrogen peroxide was not changed. Renal eNOS expression was increased in HFr (228%, p<0.01), and Ex significantly enhanced the expression higher than HFr (316%, p<0.01). The phosphorylation of eNOS in serine 1177 were inhibited by HFr and reversed by Ex (87%, p<0.01). AMPK was not changed by HFr, and Ex significantly increased AMPK expression (266%, p<0.01). The phosphorylation of AMPK at threonine 172 were increased by HFr (306%, p<0.01), and Ex even enhanced the expression (473%, p<0.01). **Conclusion** Ex provided antihypertensive effects with nitric oxide

upregulation. The mechanism of nitric oxide upregulation may mediate with the improve of phosphorylation and alteration in oxidases activities.

Disclosures: G. Hu: None. **L. Xu:** None. **M. Kohzuki:** None. **O. Ito:** None.

Funding: No

Funding Component:

P151

Short- and Long-Term Induction of Human Endothelin-1 Overexpression in Mice Up-Regulated the Expression of *Khdrbs3* in Mesenteric Arteries

Authors: Olga Berillo, Suellen C Coelho, Ku-Geng Huo, Sofiane Ouerd, Lady Davis Inst for Medical Res, Montreal, QC, Canada; Chantal Richer, Daniel Sinnett, CHU Ste-Justine, Montreal, QC, Canada; Pierre Paradis, Ernesto L Schiffrin, Lady Davis Inst for Medical Res, Montreal, QC, Canada

Background: Transgenic mouse with tamoxifen-inducible endothelium-restricted human ET-1 overexpression (ieET-1) exhibited blood pressure (BP) elevation three weeks (short-term) and three months after induction (long-term). Vascular injury was observed only after long-term exposure to endothelial ET-1 overexpression. It is unknown whether short or long-term exposure to ET-1 overexpression results in gene dysregulation. We aimed to identify differentially expressed (DE) microRNAs (miRs) and genes in mesenteric arteries of ieET-1 mice after short- and long-term induction of human ET-1 overexpression. **Methods and results:** Ten to 12-week old male ieET-1 mice and control ieCre mice expressing a tamoxifen-inducible Cre recombinase under the control of endothelium-specific *Tie2* promoter, were treated with tamoxifen (1 mg/kg/day, s.c.) for 5 days and sacrificed 16 days or 3 month later. RNA was extracted from mesenteric arteries of ieCre and ieET-1 mice and used for small and total RNA-sequencing using Illumina HiSeq-2500. EdgeR was used for differential expression analysis (false discovery rate <0.05, fold change ≤ or ≥ 1.5). No DE miRs were identified. DE genes were identified in ieET-1 compared to ieCre mice after short-term induction (mRNAs: 1 up and 6 down; non-coding [nc]RNAs: 3 up) and after long-term induction (mRNA: 1 up). *Khdrbs3* (KH domain containing, RNA binding, signal transduction associated 3), which was up-regulated after both short- and long-term exposure to endothelial ET-1 overexpression, was validated by reverse transcription-quantitative PCR (RT-qPCR). We demonstrated a correlation between RNA-sequencing and RT-qPCR data for short- and long-term groups (r=0.8, P<0.0005). The mRNA expression of *Khdrbs3* was 2 times more in fibroblasts than in smooth muscle cells and endothelial cells (2.37±0.28 vs. 1.11±0.17 and 1.12±0.12, P<0.01). **Conclusions:** The results showed that short- and long-term exposure to endothelial ET-1 overexpression up-regulated *Khdrbs3* in mesenteric arteries. *In vitro* study revealed that this gene was expressed to a greater level in fibroblasts than other vascular cells. However, the role of *Khdrbs3* in ET-1-induced vascular injury remains to be determined.

Disclosures: O. Berillo: None. **S.C. Coelho:** None. **K. Huo:** None. **S. Ouerd:** None. **C. Richer:** None. **D. Sinnett:** None. **P. Paradis:** None. **E.L. Schiffrin:** None.

Funding: No

Funding Component:

P152

Exercise Training Upregulates Nrf2 Gene Expression in the RVLM of Mice With Heart failure

Authors: Ahmed M Wafi, Lie Gao, Li Yu, Irving H Zucker, Univ of Nebraska Medical Cent, Omaha, NE

Sympatho-excitation is an important hallmark in the heart failure (HF) state. It is known that HF is associated with global oxidative stress, which contributes to pathogenesis of sympatho-excitation. Central reactive oxygen species (ROS) accumulate within neurons and evoke alterations in protein function, leading to an enhanced neuronal excitability, thereby evoking the higher level of sympathetic activation in HF. On the other hand, exercise training (ExT) is associated with a reduction of oxidative stress, in part by upregulation of antioxidant enzymes. However, the link between ExT and antioxidants in the brain of animals with HF has not been explored. In this study, we hypothesized ExT enhances transcriptional activation of Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), a master transcription factor that modulates a wide array of antioxidant enzyme gene expression, in the rostral ventrolateral medulla (RVLM). Sham and coronary artery ligation-induced HF mice were divided into either sedentary (Sed) or ExT groups. After 6 weeks of treadmill training, maximal exercise tolerance, urinary norepinephrine (NE) and Nrf2 as well as NAD(P)H dehydrogenase [quinone] 1 (NQO1) gene expression were evaluated by real time RT-PCR. We found that: (1) HF mice displayed significantly lower maximal distance than Sham mice, which was improved by ExT (maximal distance: Sham- Sed 255 ± 34 m (n = 6), HF Sed 148 ± 37 (n = 5), HF ExT 304 ± 34 (n = 6), $p<0.05$ HF Sed vs HF ExT); (2) urinary NE concentration was significantly higher in HF Sed group which was attenuated by ExT (urinary NE: Sham Sed 442 ± 38 (n = 6), HF Sed 596 ± 41 (n = 5), HF ExT 465 ± 38 ng/ml (n = 6), $p<0.05$); (3) ExT attenuated HF- induced lower Nrf2 and NQO1 gene expression and also upregulated gene expression of these two proteins in Sham mice (Nrf2: Sham Sed 0.92 ± 0.10 (n = 6), Sham ExT 1.32 ± 0.10 (n = 6), HF Sed 0.60 ± 0.11 (n = 5), HF ExT 0.95 (n = 6), $p<0.05$), (NQO1: Sham Sed 0.98 ± 0.12 (n = 6), sham ExT 1.39 ± 0.12 (n = 6), HF Sed 0.60 ± 0.13 (n = 5), HF ExT 1.07 ± 0.12 (n = 6), $p < 0.05$). These data suggest that upregulation of Nrf2 gene expression could be a mechanism by which ExT exerts its beneficial effects on sympathetic tone in HF. Further studies are required to investigate how ExT-induced Nrf2 upregulation alters sympathetic nerve activity in HF.

Disclosures: A.M. Wafi: None. **L. Gao:** None. **L. Yu:** None. **I.H. Zucker:** None.

Funding: No

Funding Component:

P153

Crosstalk Between Vascular Redox and Calcium Signalling in Hypertension Involves Transient Receptor Potential Melastatin 2 (TRPM2) Channel

Authors: Rhéure Alves-Lopes, Karla B Neves, Aikaterini Anagnostopoulou, Univ of Glasgow, Glasgow, United Kingdom; Silvia Lacchini, Univ of Sao Paulo, Sao Paulo, Brazil; Augusto C Montezano, Rhian M Touyz, Univ of Glasgow, Glasgow, United Kingdom

Mechanisms involved in redox-sensitive calcium signalling in hypertension are elusive. TRPM2 is a cation channel permeable to Ca2+ that is highly sensitive to increased levels of H2O2. TRPM2 also regulates Na+ influx, which may influence the sodium-calcium exchanger (NCX), which in reverse mode, promotes Ca2+ influx. We hypothesise that vascular oxidative stress in hypertension causes TRPM2 activation, increased influx of Ca2+ and vascular dysfunction. Vascular smooth muscle cells (VSMCs) from hypertensive and normotensive subjects and mesenteric arteries from wildtype (WT) and hypertensive LinA3 mice (human renin-expressing mice) were used. H2O2 levels were increased in hypertensive VSMCs (% of control:188.6±36.3), followed by increased activity of TRPM2 modulator PARP1 (% of control: 158.4±18.7), effect reversed by PEG-Catalase (104.1±7.0), suggesting an event regulated by H2O2. Higher calcium influx in hypertensive VSMCs (AUC NT 15710±812.3 vs HT 21440 ± 1685) was normalized by PEG-Catalase, and TRPM2 inhibitors (2-APB, Olaparib and 8-Br-cADPR) and by Na+ depletion and NCX inhibitors (Benzamil, KB-R7943 and YM 244769). Phosphorylation of the pro-contractile signaling protein, MLC20 in VSMCs from hypertensive patients (% of control 186.3±21.5) and agonist-induced vascular responses in LinA3 mice (max response: NT 0.29±6.7 vs HT 9.3±0.5) were attenuated by inhibitors of TRPM2 and NCX. These results demonstrate that in hypertension vascular oxidative

stress influences TRPM2-mediated Ca2+ and Na+ homeostasis, through processes that involve NCX activation. Our findings identify novel signalling pathways linking ROS-TRPM2-Ca2+-Na+, which may play a role in hypercontractile responses in hypertension. We highlight redox-sensitive TRPM2 as a putative vascular target in hypertension.

Disclosures: R. Alves-Lopes: None. **K.B. Neves:** None. **A. Anagnostopoulou:** None. **S. Lacchini:** None. **A.C. Montezano:** None. **R.M. Touyz:** None.

Funding: No

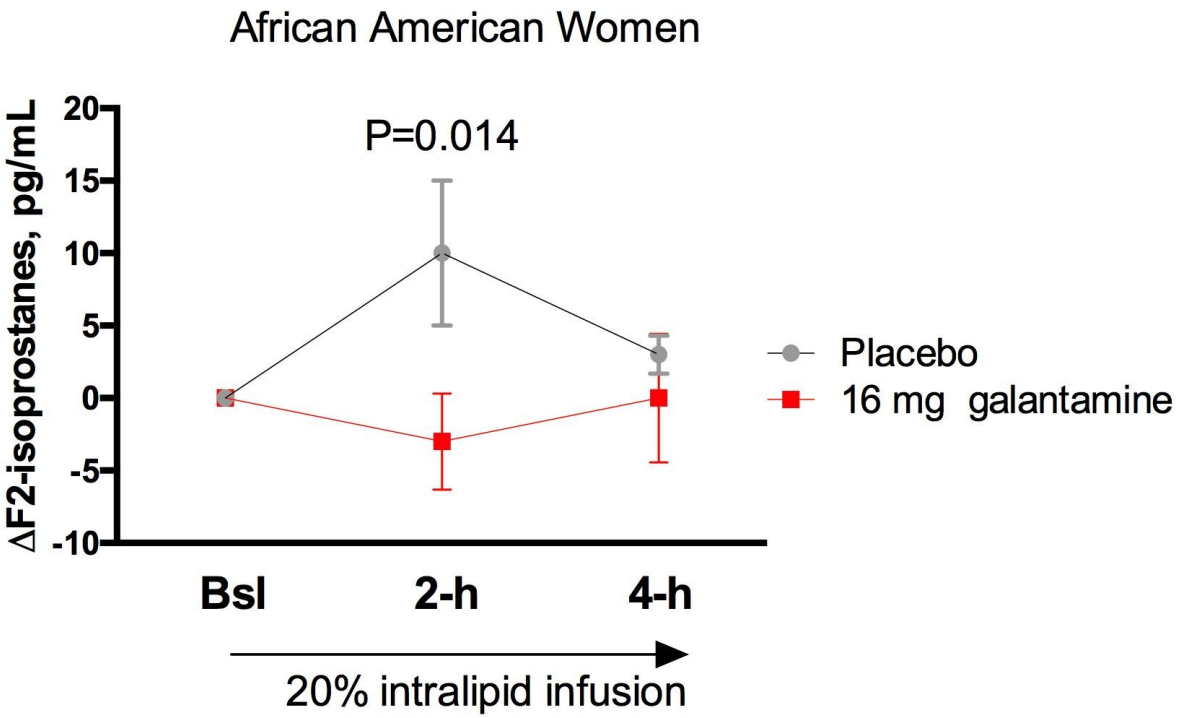
Funding Component:

P154

Central Acetylcholinesterase Inhibitor, Galantamine, Prevents Lipid-Induced Oxidative Stress in African Americans

Authors: Jorge E Celedonio, Shahram E Mehr, Sachin Paranjape, Andre Diedrich, Italo Biaggioni, **Cyndya A Shibao**, VANDERBILT UNIVERSITY MEDICAL CENTER, Nashville, TN

African American women (AAW) have one of the highest prevalence of hypertension in the US. Obese AAW have decreased parasympathetic (PNS) activity compared to white women (WW). Continuous lipid infusion that causes cardiovascular autonomic imbalance (decrease in PNS and increase in sympathetic activity) induces a greater increase in oxidative stress in African Americans than in whites. Considering that PNS protects against oxidation and that central acetylcholinesterase inhibitors have been shown to suppress oxidative stress in animal models; we tested the hypothesis that the central acetylcholinesterase inhibitor, galantamine attenuates oxidation in response to lipid infusion in obese AAW compared to WW. We randomized 14 healthy obese AAW (39.5±10.7 yo, BMI 38.8±3.4) and 10 WW (35.9±8.3 yo, BMI 36.3±2.1). All subjects underwent 4-h infusions of Intralipids and heparin. On separate days subjects received either 16 mg galantamine or placebo in a crossover fashion. Lipid-induced oxidative stress and inflammation were assessed with plasma F2-isoprostanes and cytokines at baseline, 2 and 4-h post-intralipid infusion. In AAW, 16 mg of galantamine significantly suppressed the increase in lipid-induced oxidative stress (-3±12 vs. 10±18 pg/mL with placebo, P=0.014), **figure**. No effect was noted in WW. Galantamine tended to increase IL10 levels (17.3±20.7 vs. 4.8±7.58 pg/mL, P=0.06). We did not observe any effect on blood pressure or heart rate. Conclusion: Increased parasympathetic tone with central acetylcholinesterase inhibitor, galantamine, suppressed lipid-induced oxidative stress in obese African American women.



Disclosures: **J.E. Celedonio:** None. **S.E. Mehr:** None. **S. Paranjape:** None. **A. Diedrich:** None. **I. Biaggioni:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH, FDA. D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Lundbeck. G. Consultant/Advisory Board; Modest; Theravance. **C.A. Shibao:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH, FDA. D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Lundbeck. G. Consultant/Advisory Board; Modest; Lundbeck.

Funding: No

Funding Component:

P155

B Cells Are Not Necessary for the Developmentof Ang II-induced Hypertension and End-Organ Damage

Authors: Yuhan Chen, Vanderbilt Univ Medical Ctr, nashville, TN; Bethany L Dale, Vanderbilt Univ, Nashville, TN; Arvind Kant Pandey, Vanderbilt Univ, nashville, TN; Matthew R Alexander, Fanny Laroumanie, Meena S Madhur, Vanderbilt Univ Medical Ctr, nashville, TN

Chronic inflammation characterized by vascular T cell and macrophage infiltration is associated with angiotensin II (Ang II)-induced hypertension and end-organ damage. However, the role of B cells in the development of hypertension is not well established. Elevated levels of serum immunoglobulins have been observed in experimental and human hypertension. Prior studies demonstrated that B-cell-activating factor receptor-deficient (BAFF-$R^{-/-}$) mice, which lack mature B cells, are protected from Ang II-induced hypertension and vascular remodeling. However, BAFF-R can be expressed on non-B cells as well. Thus, to further investigate the role of B cells in hypertension, we studied mice with targeted disruption of the membrane exon of the immunoglobulin μ heavy chain gene ($\mu MT^{-/-}$) that encodes the constant region of the IgM isotype. In these mice, B cells are arrested at the pre-B cell maturation stage. Serum immunoglobulins were undetectable at a 1:30,000 dilution in $\mu MT^{-/-}$ mice. Interestingly, $\mu MT^{-/-}$ mice exhibited similar blood pressure increase in response to 4 weeks of Ang II infusion compared to age-matched wild type (WT) mice (p=0.675). Flow cytometry of aortae from Ang II-treated $\mu MT^{-/-}$ mice demonstrated fewer CD45+ cells (p=0.047), similar

levels of CD8+ and CD4+ T cells (p=0.631 and p=0.721 respectively), a trend for more F4/80+ monocytes/macrophages (p=0.2567), and virtually no CD19+ B cells (p=0.002) compared to WT mice. Endothelium-dependent vasodilatation to acetylcholine was comparable between $\mu MT^{-/-}$ mice and WT mice after Ang II treatment (p=0.223), however $\mu MT^{-/-}$ mice displayed enhanced endothelium-independent vasodilatation to sodium nitroprusside than WT mice (p=0.024). Urinary albumin/creatinine ratio was similar between Ang II-treated $\mu MT^{-/-}$ mice and WT mice (p=0.488), indicating similar renal damage. Taken together, $\mu MT^{-/-}$ mice are not protected from Ang II-induced hypertension, suggesting that B cells and immunoglobulin production are not critical for hypertension and the associated end-organ damage.

Disclosures: Y. Chen: None. **B.L. Dale:** None. **A.K. Pandey:** None. **M.R. Alexander:** None. **F. Laroumanie:** None. **M.S. Madhur:** None.

Funding: No

Funding Component:

P156

Toll-Like Receptor 2 Deficiency Exacerbates Deoxycorticosterone Acetate-Salt-Induced Hypertension

Authors: Estrellita Uijl, David Severs, A.H. Jan Danser, Robert Zietse, Ewout J. Hoorn, Erasmus MC, Rotterdam, Netherlands

The early stage of hypertension increases so-called damage-associated molecular patterns (DAMPs). In turn, DAMPs can activate Toll-like receptors (TLRs) and thereby exacerbate hypertension. Although various TLRs have been implicated in the pathogenesis of salt-sensitive hypertension, the role of TLR2 and TLR4 remains unclear. Here, we hypothesize that TLR2 or TLR4 deficiency protects against salt-sensitive hypertension. To address this, we treated wild-type (WT, n=3), $TLR2^{-/-}$ (n=5) and $TLR4^{-/-}$ (n=5) mice with deoxycorticosterone acetate and 0.9% NaCl as drinking water (DOCA-salt) for four weeks. Baseline telemetric mean arterial blood pressure (MAP) was slightly but non-significantly higher in $TLR4^{-/-}$ (108±2 mmHg) and $TLR2^{-/-}$ mice (111±3 mm Hg) compared with WT mice (99±5 mmHg). MAP peaked similarly after two weeks in WT and $TLR4^{-/-}$ mice (111±1 vs. 116±3 mmHg; both $P<0.05$ vs. baseline), but reduced after four weeks (108±3 vs. 110±2 mmHg). In contrast, $TLR2^{-/-}$ mice displayed a much greater rise in MAP to DOCA-salt and remained hypertensive after two weeks (134±5 mmHg) and after four weeks (128±4 mmHg; $P<0.0001$ vs. baseline; $P<0.05$ for delta MAP vs. $TLR4^{-/-}$). Kidney sodium transporter analysis showed that the phosphorylated (activated) form of the sodium-chloride cotransporter (NCC) was 2.4-fold higher in $TLR2^{-/-}$ compared with WT mice ($P<0.05$). In wire myographs of iliac arteries, angiotensin II-induced constriction was non-significantly lower in iliac arteries of $TLR4^{-/-}$ mice (53±9%) than in $TLR2^{-/-}$ (84±9%) and WT mice (84±15%). Addition of the nitric oxide synthase blocker L-NAME increased the contractile response to Ang II in WT mice, but not in $TLR2^{-/-}$ mice, indicating NO deficiency in the latter mice. The Ang II type 2 (AT2) receptor blocker PD123319 blunted the effects of Ang II in both WT and $TLR2^{-/-}$ mice, suggesting that DOCA-salt upregulates constrictor AT2 receptors, but not in $TLR4^{-/-}$ mice. In conclusion, TLR2 deficiency unexpectedly exacerbates experimental salt-sensitive hypertension. This effect appears to involve increased renal sodium reabsorption through NCC, decreased vascular availability of nitric oxide and a constrictive rather than a vasodilatory response to AT2 receptor stimulation.

Disclosures: E. Uijl: None. **D. Severs:** None. **A.H.J. Danser:** None. **R. Zietse:** None. **E.J. Hoorn:** None.

Funding: No

Funding Component:

P157

Immunophenotyping Human Hypertension Using Single Cell Mass Cytometry

Authors: Matthew R Alexander, Vanderbilt Univ Medical Ctr, Nashville, TN; Bethany L Dale, Cara E Wogsland, Vanderbilt Univ, Nashville, TN; Fernando Elijovich, Jonathan M Irish, Meena S Madhur, Vanderbilt Univ Medical Ctr, Nashville, TN

Hypertension is the leading risk factor for global morbidity and mortality. Emerging evidence in animal models implicates a variety of innate and adaptive immune cells in hypertension pathogenesis, and limited studies in humans have demonstrated increased circulating memory and senescent T cells in hypertensive individuals. However, the abundance and role of specific immune cells in human hypertension remains unclear. To perform unbiased high dimensional profiling of peripheral immune cells in humans, we developed and validated a novel approach using mass cytometry to detect 31 extracellular markers at the single cell level. Initial results in a normotensive individual using this panel revealed robust identification of major circulating immune cell subsets including $CD4^+$ cells (60% of $CD45^+$ cells), $CD8^+$ cells (21%), γδ T cells (6%), $CD25^+CD127^{lo}$ T regulatory cells (5%), $CD19^+$ B cells (3%), and $CD11c^+$ myeloid cells (3%). Unsupervised viSNE plots using this panel of 31 markers demonstrates grouping of distinct circulating immune cell subsets based on similarity of marker levels **(Figure)**. We are extending this analysis to peripheral blood mononuclear cells collected from 16 additional control and 17 hypertensive individuals matched for age, gender, race, and BMI to determine differences in the abundance of known and potentially novel circulating immune cell subsets. The combination of high dimensional mapping and unsupervised computational analysis will permit rigorous immunophenotyping of human hypertension and potentially identify novel therapeutic targets.

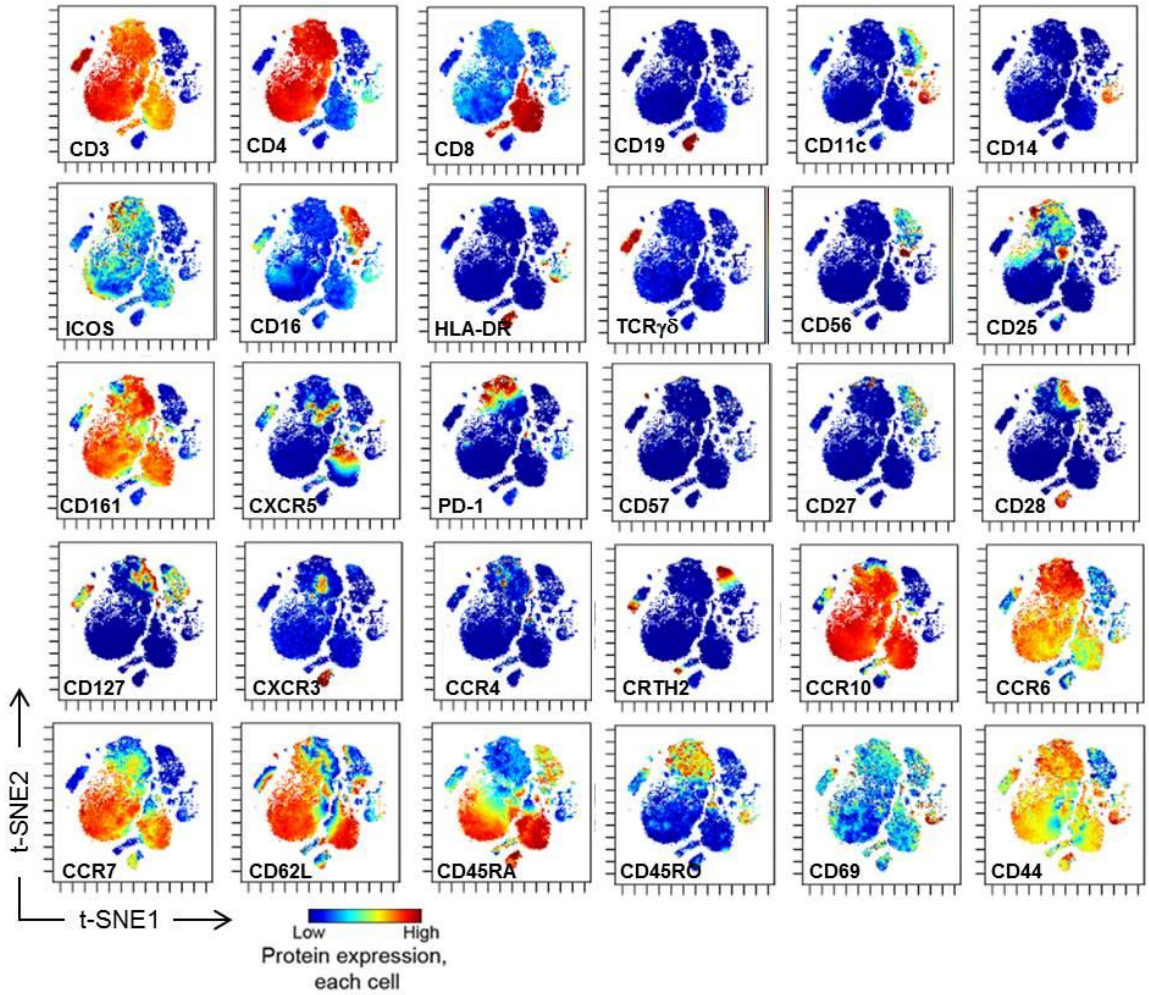


Figure: Immunophenotyping of CD45+ human peripheral blood mononuclear cells from a normotensive individual using mass cytometry. Images represent a viSNE plot resulting from unsupervised grouping of cells based on similarity of protein levels across all 31 markers. Each panel shows protein levels of an individual marker on unit-less t-SNE axes, with red indicating the highest relative levels. CD45+ cells are used for analysis, hence CD45 is not shown.

Disclosures: M.R. Alexander: None. **B.L. Dale:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 17PRE33460032 AHA Predoctoral Award. **C.E. Wogsland:** None. **F. Elijovich:** None. **J.M. Irish:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Cytobank Inc.. D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Fluidigm Corporation. 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; Cytobank Inc.. G. Consultant/Advisory Board; Significant; Cytobank Inc. **M.S. Madhur:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 1DP2 HL137166-01 NIH New Innovator Award NIH Common Fund/NHLBI; 1K08 HL121671 NIH/NHLBI.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P158

Sulforaphane Rich Broccoli Powder Attenuates the Augmented Angiotensin II Induced Renal Inflammation and Injury in GSTM1 Deficient Mice

Authors: Shirin Pourafshar, Sylvia Cechova, Sun-Sang J Sung, Univ of Virginia, Charlottesville, VA; Phillip Ruiz, Univ of Miami Health System, Miami, FL; Guang Yang, Heinrich-Heine Univ of Dusseldorf, Dusseldorf, Germany; Thu Le, Univ of Virginia, Charlottesville, VA

Glutathione-*S*-transferase μ-1 (*GSTM1*) enzyme belongs to the superfamily of phase II antioxidant glutathione-*S*-transferases (GSTs) that are downstream targets of the Nrf2 antioxidant transcription factor. In humans, a common *GSTM1* gene deletion variant, the null allele *GSTM1(0)*, results in decreased or complete absence of GSTM1 enzymatic activity and is associated with higher levels of oxidative stress. We reported that *GSTM1(0)* is associated with accelerated kidney disease progression in the African Americans Study of Kidney Disease (AASK) participants. To understand the direct impact of *GSTM1* deficiency on renal inflammation and oxidative stress, we generated *Gstm1* deficient mice (KO) to determine their response to angiotensin II, delivered @ 1000 ng/kg/min for 4 weeks via mini-osmotic pump. Blood pressure (BP) was measured by radiotelemetery. Kidney histopathology was assessed by a renal pathologist blinded to genotype and experimental conditions. Renal leukocyte populations were analyzed quantitatively by flow cytometry. *Gstm1* KO mice had significantly higher levels of baseline systolic BP (SBP) and ~ 17 mmHg higher SBP after 4 weeks of Ang II-HTN, compared to WT mice. *Gstm1* KO mice have increased renal superoxide levels by nearly 3 folds - independent of activation of Nox2 and Nox4 NADPH oxidases or alteration in superoxide dismutase - and worse kidney injury. These changes were associated with significantly increased renal expression of genes involved in inflammation - chemokine ligand 1 (CXCL-1), monocyte chemotactic protein 1 (MCP-1), Interleukin-1β (IL-1β) and IL-6 ($P<0.05$). By flow cytometry, *Gstm1* KO mice displayed ~ two fold increases in all leukocyte populations in the kidney, with the exception of the numbers of B cells that were not significantly different between groups. This increased renal inflammation was attenuated by dietary administration of broccoli powder that is rich in sulforaphane, a phytochemical that increases Nrf2 expression, independent of BP. In Ang II-hypertension, loss of GSTM1 enzyme may be deleterious by augmenting oxidative stress and inflammation in the kidney. Stimulation of the Nrf2 pathway in hypertension may be a novel therapeutic approach to prevent kidney disease progression in GSTM1 deficiency.

Disclosures: S. Pourafshar: None. **S. Cechova:** None. **S.J. Sung:** None. **P. Ruiz:** None. **G. Yang:** None. **T. Le:** None.

Funding: No

Funding Component:

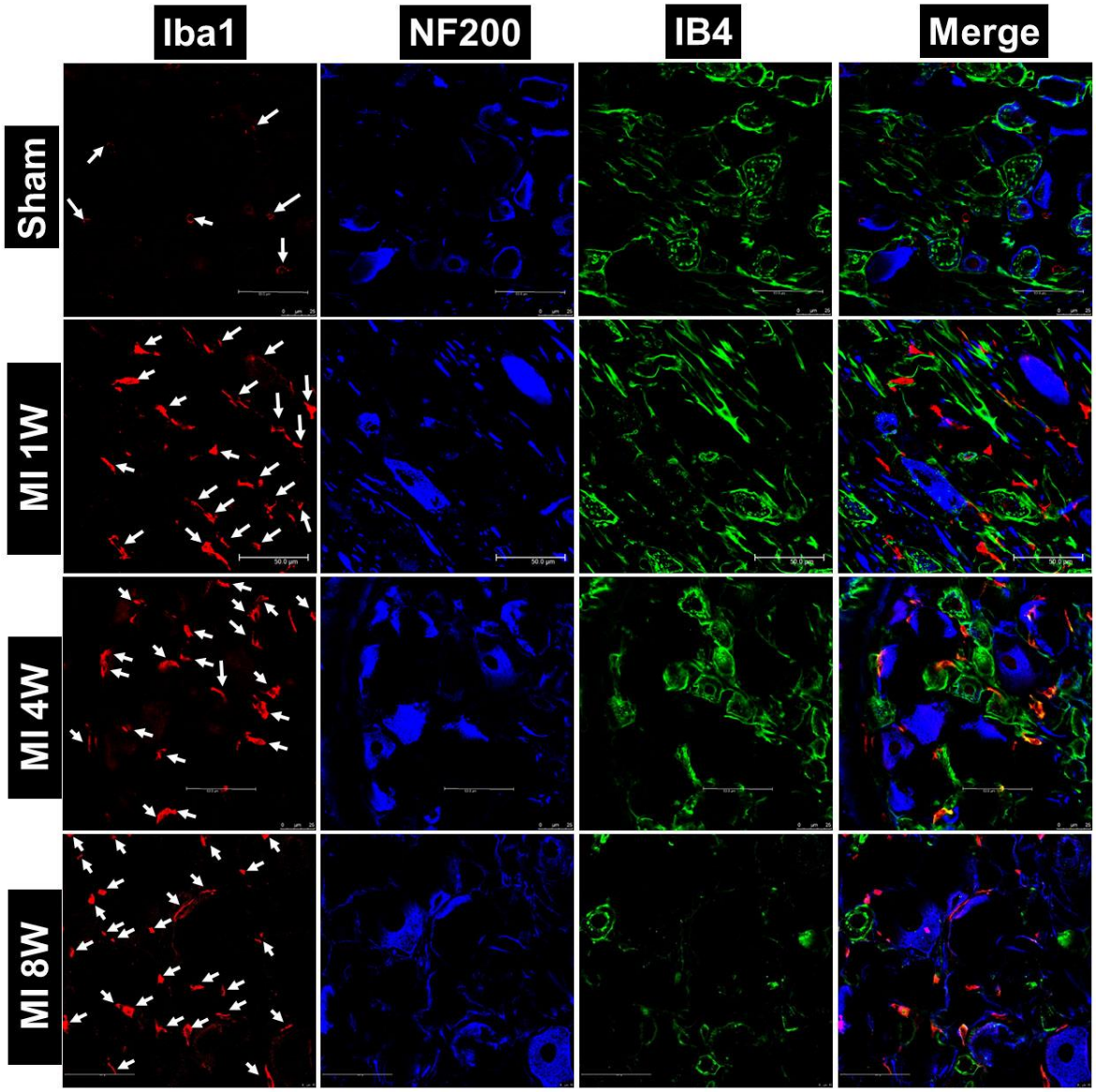
P159

Neural Inflammation Results in Cardiac Sympathetic Afferent Sensitization in Post-Myocardial Infarction Rats

Authors: Juan Hong, UNMC, Omaha, NE

Sympatho-excitation is a hallmark of the chronic heart failure (CHF) state. The enhanced cardiac sympathetic afferent reflex (CSAR) contributes to the elevated sympathetic tone in CHF. However, the mechanisms underlying the CSAR sensitization have not been fully discovered. We hypothesized that myocardial infarction (MI) will trigger a neuro-inflammatory cascade that includes macrophage activation in thoracic dorsal root ganglia (DRG), which results in cardiac spinal afferent sensitization. Our immunofluorescence data (**Figure 1**) demonstrated that, the number of Iba-1-positive (a macrophage marker) cells in T1-T4 DRGs of MI rats was significantly increased at 1 week and lasted at least 8 weeks post MI. RNA-seq analysis data in T1-T4 DRGs of MI rats also showed upregulated mRNA expressions of several macrophage-activation related genes such as IRF7, RGD1309362, slfn4 and Ifit1. In the in vitro experiments, after the 50B11 DRG cells were co-cultured with LPS-pretreated BV2 cells (activated microglia/macrophage), the protein expressions of voltage-gated potassium channel isoforms (Kv1.4, Kv4.2, Kv4.3 and Kv3.4) were largely reduced, which explains how macrophage activation cause DRG neuronal sensitization. Lastly, our data showed that 4-week treatment with minocycline (a macrophage inhibitor, 40 mg/kg/day) via drinking water markedly reduced the enhanced CSAR in the post-MI rats (MAP, 30.2±2.6 vs. 18.0±1.9 mmHg, n=6, $p<0.01$; HR, 43.5±5.5 vs. 12.5±2.0 bpm, n=6, $p<0.01$; RSNA,

169.2±19.5 vs. 77.8±4.6% baseline, n=6, p<0.01). These data suggested that macrophage infiltration in sensory ganglia contributes to the sensitization of the CSAR in the post-MI state.



Disclosures: J. Hong: None.

Funding: No

Funding Component:

P160

Hypertension is Associated With Monocytes Activation in the Blood in Mice and Humans

Authors: Luciana S Carmo, Liang Xiao, Roxana Loperena, Fernando Elijovich, Natalia Rugerri Barbaro, David Harrison, Vanderbilt Univ Medical Ctr, Nashville, TN

Monocytes play a crucial role in immune activation and the development of hypertension. Deletion of monocytes prevents experimental hypertension, and monocyte derived dendritic cells (DCs) and macrophages promote T cell activation and tissue damage. Our group recently found that monocytes exposed to hypertensive mechanical stretch convert to a pro-inflammatory phenotype denoted by expression $CD14^{+}$ $CD16^{+}$, also known as intermediate monocytes.

We aimed to determine whether hypertension has a direct effect on the bone marrow to activate the production and mobilization of monocytes. To study this, we initially analyzed the circulating monocytes from 15 normotensive and 12 hypertensive subjects using flow cytometry and found that hypertensive subjects have an absolute monocytosis compared to normotensives (1014 ± 113 vs. 567.5 ± 97 per uL, $p < 0.05$). This is associated with high numbers in both the classical $CD14^{+}CD16^{-}$ monocytes and the non-classical $CD14^{low}CD16^{+}$ monocytes. To further study mechanisms involved, we analyzed monocytes in the blood and bone marrow from C57BL/6 mice following two weeks of sham or Ang II infusion (490 ng/kg/min, s.c.). Similar to the findings in humans, more total monocytes were found in mice with Ang II-induced hypertension (46.7±10.1 vs 18.7±4.7 per µL, $p<0.05$). Ang II also induced an increase in Ly6C high expressing monocytes (Ang II: 34.1±65.6 vs. Sham: 12.3±3.5 per µL, $p<0.05$), reflecting activation. We also observed an increase in Ly6C high expressing monocytes in the bone marrow. Taken together, our results suggest that hypertension is associated with monocytosis in humans and mice, and this is likely due to increased BM production. This increase in monocytes may prime the immune system for activation and increased tissue inflammation. Moreover, the level of circulating monocytes might prove to be a useful biomarker of inflammation in hypertension.

Disclosures: L.S. Carmo: None. **L. Xiao:** None. **R. Loperena:** None. **F. Elijovich:** None. **N. Rugerri Barbaro:** None. **D. Harrison:** None.

Funding: Yes

Funding Component: National Center

P161

Effect of Rapamycin on the Expression of T Regulatory Cells, Foxp3, and Toll-Like Receptors in the Kidneys of *Npr1* Gene-Knockout Mice

Authors: Venkateswara R Gogulamudi, Indra Mani, Umadevi Subramanian, **Kailash Nath Pandey**, Tulane Som Physiology, New Orleans, LA

The alterations in the inflammatory system contribute to the pathogenesis of hypertension leading to end-organ damage. Guanylyl cyclase/natriuretic peptide receptor-A (GC-A/NPRA) gene (*Npr1*) disruption elevates blood pressure and activates the immunogenic responses in mice. The goal of this study was to determine the effect of rapamycin on the expression of T regulatory (Treg) cells, Foxp3, and Toll-like receptors (TLRs) in *Npr1* gene-disrupted and -duplicated mice. The adult 0-copy ($Npr1^{-/-}$), 2-copy ($Npr1^{+/+}$), and 4-copy ($Npr1^{++/++}$) female mice were pre-treated with rapamycin (1mg/kg/day). Kidneys, spleen, and blood were collected, qRT-PCR, Western blot, immunofluorescence, and flow cytometry analyses were done to assess the expression of Tregs, Foxp3, and TLRs. The disruption of *Npr1* led to significant reductions in $Foxp3^{+}$ mRNA expression in 0-copy (77.5%) and 1-copy (71.5%) mice but not in 2-copy and 4-copy mice. Similarly, $CD25^{+}$ expression was reduced in 0-copy mice by 75% and in 1-copy by 60% compared to 2-copy and 4-copy mice. In contrast, the total $CD4^{+}$ count was up-regulated by 40% in 0-copy and 31% in 1-copy mice. Treatment with rapamycin up-regulated $Foxp3^{+}$ cells in 0-copy (17.38%, $P<0.001$) and 1-copy (8%, $P<0.05$) mice, and $CD25^{+}$ T cell levels were increased by 62.4% ($P<0.001$) in 0-copy mice and 38.6% ($P<0.05$) in 1-copy mice compared to vehicle-treated control mice. In contrast, $CD4^{+}$ cell expression was significantly reduced by 15.4% ($P<0.001$) in 0-copy and 12.2% ($P<0.05$) in 1-copy mice. Interestingly, rapamycin significantly ($P< 0.001$) increased renal $Foxp3^{+}$ protein expression to 85% in 0-copy mice and 68% in 1-copy mice compared to untreated controls. The expression of TLR2/TLR4 was significantly increased by 3.4- to 4.5-fold in 1-copy and 0-copy mice than 2-copy and 4-copy mice; however, rapamycin greatly decreased the expression of both TLRs in both 1-copy and 0-copy mice. In summary, rapamycin treatment upregulated the expression of $Foxp3^{+}$, $CD25^{+}$ cells, and TLRs while suppressing pathogenic $CD4^{+}$ T cell expression in 0-copy and 1-copy mice kidneys. The present results implicate that *Npr1* and immune cell interactions could provide new therapeutic targets for the treatment and prevention of hypertension and kidney dysfunction in humans.

Disclosures: **V.R. Gogulamudi:** None. **I. Mani:** None. **U. Subramanian:** None. **K.N. Pandey:** None.

Funding: No

Funding Component:

P162

Adiposity-Induced Hypertension is Associated With Sex-Specific Macrophage Infiltration of Mesenteric Perivascular Adipose Tissue in Dahl S Rats.

Authors: Ramya K Kumar, Cheryl E Rockwell, Alexandra E Turley, Hannah Garver, Gregory D Fink, Stephanie W Watts, Michigan State Univ, East Lansing, MI

Perivascular adipose tissue (PVAT) may be a key player in mediating adiposity-associated hypertension because of its proximity to blood vessels. Although increased immune cell occupancy in adipose tissue is proposed to couple adiposity to hypertension, little is known about PVAT's immune complement. We hypothesized that an immune complement exists in PVAT that would become pro-inflammatory with adiposity-induced hypertension. Male and female Dahl S rats were fed a regular (10% calories from fat) diet (control) or a high fat (60%) diet (HFD) from weaning through 24 weeks of age. HFD rats demonstrated significantly higher systolic blood pressure [mm Hg; control: 127±7 (M), 145±8 (F); HFD: 168±9 (M), 184±11 (F); tail cuff] and visceral adiposity (micro-CT scans). PVATs from the thoracic aorta (APVAT), mesenteric resistance vessels (MRPVAT), non-PVAT retroperitoneal fat (RP fat) and spleen (positive control) were harvested from each animal. T cells (CD4 and CD8), B cells, macrophages, mast cells and neutrophils in the stromal vascular fraction were quantified using 7-color flow cytometry. The type and number of immune cells are presented as a percentage (%) of total live singlet cells. Each immune cell type is reported as % of total immune cells, and CD4, CD8 cells as % of T cells. The table illustrates significant ($P<0.05$) differences with HFD *vs* respective controls. HFD-induced hypertension in Dahl S rats leads to greater macrophage infiltration of MRPVAT and RP fat in females *vs* males. Further studies are needed to gain mechanistic insights into how macrophage subtypes and their associated cytokines in PVAT may alter vascular tone and blood pressure.

Disclosures: **R.K. Kumar:** None. **C.E. Rockwell:** None. **A.E. Turley:** None. **H. Garver:** None. **G.D. Fink:** None. **S.W. Watts:** None.

Funding: No

Funding Component:

P164

Inflammatory Cell Populations in a Mouse Model of Cardiorenal Syndrome

Authors: Suang Suang Koid, Intl Univ of Health and Welfare, Tokyo, Japan; Francis H Shand, Satoshi Ueha, Kouji Matsushima, The Univ of Tokyo, Tokyo, Japan; Tatsuo Shimosawa, Intl Univ of Health and Welfare, Tokyo, Japan

Objective Renal dysfunction frequently coexists with heart disease, manifesting in a condition called cardiorenal syndrome (CRS). CRS results in accelerated failure of the heart and kidney via a complex combination of hemodynamic, neurohormonal, immunological and biochemical feedback pathways. Preliminary evidence suggests that inflammatory cells such as monocytes and macrophages may contribute to the pathogenesis of CRS. However, animal models of CRS remain limited and the roles of specific inflammatory cell subsets in CRS remains unclear. Here, we describe the

establishment of a mouse model of CRS. ***Methods*** Three-week-old C57BL/6J mice were subjected to sham uninephrectomy and normal-salt (SHAM/NS) diet or left uninephrectomy and high-salt (UNIX/HS) diet. After 4, 6 or 8 weeks, urine was collected for salt and protein analyses. The heart and kidney were collected for histochemical staining and analyses of inflammatory cell distribution by flow cytometry. ***Results*** UNIX/HS mice had a higher kidney weight/body weight ratio and higher water intake than that of SHAM/NS mice. As early as 4 weeks post-surgery, UNIX/HS mice had higher urine creatinine levels (0.22 ± 0.02 mg/day c.f. 0.13 ± 0.02 mg/day) and higher proteinuria (0.46 ± 0.18 mg/day c.f. 5.37 ± 1.19 mg/day) than SHAM/NS mice. These results suggest the development of kidney dysfunction. Histological analysis revealed increased fibrosis in the heart and kidney at 8 weeks after UNIX/HS. Using flow cytometry, we characterized changes in the inflammatory cell populations in the heart and kidney that occur as CRS progresses. A significant increase in $Ly6C^{hi}$ monocyte (0.29 ± 0.06% c.f. 0.15 ± 0.02% of mean 1.0 x 10^6 total live cells) and neutrophil (1.00 ± 0.27% c.f. 0.41 ± 0.09%) proportions in the heart was observed 8 weeks after UNIX/HS. ***Conclusion*** Therapeutic strategies targeting inflammatory signaling pathways and modulating specific inflammatory cell populations may reduce organ damage and improve prognosis in patients with CRS.

Disclosures: S. Koid: None. **F.H.W. Shand:** None. **S. Ueha:** None. **K. Matsushima:** None. **T. Shimosawa:** None.

Funding: No

Funding Component:

P165

Genetic Polymorphisms Linked to Diabetes With Chronic Renal Insufficiency

Authors: Ling Wang, Donna Wang, Michigan State Univ, East Lasing, MI

Chronic kidney disease (CKD) has raised to a global health concern with huge economic burden and detrimental consequences for health. Among the risk factors, diabetes is a leading cause for CKD and renal failure. Multiple studies such as familial aggregation studies have provided evidence for a genetic component to the kidney disease. Heritability estimates of eGFRcrea are reported between 0.41 and 0.75 in individuals with the major CKD risk factors, hypertension and diabetes. However, precise genetic loci predicting CKD with or without diabetes are unknown. Here, we test the hypothesis that genetic polymorphisms are distinct between CKD patients with and without diabetes. We performed a genome-wide association studies using data from the Chronic Renal Insufficiency Cohort (CRIC) study to identify genetic various related to diabetes among CKD patients. CRIC Study is a United States multicenter, prospective study of racially and ethnically diverse patients with CKD. Clean genotype data from 970,342 genotyped or imputed single-nucleotide polymorphisms (SNPs) were available for 3,541 patients (1,953 males and 1,588 females) in CRIC study. Logistic regressions were carried out comparing distribution of SNPs among CKD patients with or without diabetes controlling for confounding factors of gender and race. Among all 3,541 CKD patients in CRIC, 1,724 CKD patients had Diabetes at baseline (Mean of Glycosylated hemoglobin =7.7%). We found that three genes, including *KCNMB4* (rs767397, encoding calcium-activated potassium channel, subfamily M subunit beta 4), *FOXA1* (rs911822, encoding a forkhead transcription factor) and *SIMC1* (rs16831314, encoding SUMO-interacting motif containing protein 1), were putative risk genes for diabetes among CKD patients (P-value<5×10^{-6}). These results are the first demonstrating that distinct genetic polymorphisms present in CKD patients with diabetes, indicating that these genes may be used to assess the risk of CKD with diabetes and that proteins encoded by these genes may contribute to diabetic nephropathy.

Disclosures: L. Wang: None. **D. Wang:** None.

Funding: No

Funding Component:

P166

Identification of *Cyr61* and *Tfgbi* as Hypertensive Renal LEC Genes

Authors: Dakshnapriya Balasubbramanian, Catalina A Lopez-Geslton, Pauline Ighofose, Micaela Saathoff, Joesph M Rutkowski, Brett M Mitchell, Texas A and M Univ HSC, College Station, TX

Increased renal lymphatic density is observed in various experimental models of hypertension. To identify differentially expressed genes in lymphatic endothelial cells (LECs) from hypertensive kidneys, we utilized Gene Expression Omnibus (GEO) which was developed by the NIH NCBI. GEO is an international public repository that archives and distributes high-throughput functional genomic data sets such as microarray and next-generation sequencing data. GEO2R is an interactive web tool that allows users to compare samples across experimental conditions in a GEO Series in order to identify genes that are differentially expressed. Results are presented as a table of genes ordered by significance. Using GEO2R, the search terms “lymphatic” and “hypertensive kidney” were used to identify studies of LEC-associated genes and renal hypertension-associated genes. Among the search results for LEC genes, 4 studies were chosen that were done in mice comparing: a) murine primary LECs vs. murine endothelial SVEC4-10 cell line, b) collecting lymphatic endothelium of mice with 293EBNA xenografts with and without overexpression of VEGFD, c) LECs from afferent and efferent lymphatic vessels, and d) LECs and blood vascular endothelial cells from mouse intestine. For renal hypertensive genes, we chose a study that reported differentially expressed genes in kidneys of mice treated with angiotensin II for 1, 3 or 7 days compared to controls. From all of these lists, the top 250 differentially expressed genes were chosen. The list of renal genes was crossed with a combined list of LEC genes to obtain potential “LEC genes differentially expressed in hypertensive kidneys”. This yielded a list of 40 genes for which mRNA expression was tested in the kidneys of mice from 3 different models of hypertension: nitric oxide inhibition-induced, salt-sensitive, and angiotensin II-induced. Among the differentially expressed genes, *Cyr61* and *Tgfbi* were elevated significantly in all 3 hypertensive models. Cyr61 is a well-known inducer of angiogenesis. Tfgbi is involved in LEC adhesion to extracellular matrix under hypoxic conditions. The upregulation of these two genes likely play a major role in renal LEC function during hypertension.

Disclosures: D. Balasubbramanian: None. **C.A. Lopez-Geslton:** None. **P. Ighofose:** None. **M. Saathoff:** None. **J.M. Rutkowski:** None. **B.M. Mitchell:** None.

Funding: Yes

Funding Component: South Central Affiliate (Arkansas, New Mexico, Oklahoma & Texas)

P167

Altered Gut Microbiome Contributes to Metabolite Biomarkers in Patients With Pulmonary Hypertension

Authors: Seungbum Kim, Jacquelyn M Walejko, Univ of Florida, Gainesville, FL; Katya Rigatto, Univ Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil; Ray Jordan, Brian Shapiro, Mayo Clinic, Jacksonville, FL; Carl J Pepine, Elaine M Richards, Mohan K Raizada, Univ of Florida, Gainesville, FL

Objectives: Pulmonary arterial hypertension (PAH) is a cardiopulmonary disease without effective cure. The objective of this study is to test the hypothesis that PAH patients have a distinct gut microbiome composition acting as a driving force for the increased PAH metabolic biomarkers, acyl carnitines and urate. **Methods:** Fecal samples from 18 type 1 PAH patients (mean pulmonary arterial pressure [mPAP] 57.4±16.7 mmHg) and 13 reference subjects in Brazil were collected for comparison of microbiome using shotgun metagenomics. Plasma samples from 33 type I PAH patients (mPAP 51.7± 13.5 mmHg) and 19 reference subjects in the USA were used for untargeted metabolomic analysis. **Results:** More than 80 bacterial taxa were differentially enriched in the microbiomes of the PAH cohort. Random forest machine-learning algorithm predicted PAH patients with 82.4% accuracy from their gut microbiome composition. The most increased taxa in the PAH microbiome were trimethylamine (TMA) producing bacteria like Collinsella (P=0.0037). The

PAH metabolome also showed increases of gut microbiome-derived short chain acyl carnitines like isubutyrylcarnitine (P=0.010) and valerylcarnitine (P=0.030), which are the precursors of TMA. In addition, increased urate (P=0.0004) and allantoin (P=0.027) in PAH plasma correlated with significantly increased enzymes producing urate in the PAH microbiome (purine metabolism (P=0.017) and xanthine oxidase (P<0.05)). **Conclusion:** These findings suggest that the random forest modeling of the gut microbiome composition can be used for prediction of PAH and that the unique gut microbiome of PAH influences acyl carnitines and urate metabolism to contribute to pathophysiology.

Disclosures: S. Kim: None. **J.M. Walejko:** None. **K. Rigatto:** None. **R. Jordan:** None. **B. Shapiro:** None. **C.J. Pepine:** None. **E.M. Richards:** None. **M.K. Raizada:** None.

Funding: No

Funding Component:

P169

Characterization of the Peak Exercise Blood Pressure Response During Cardiopulmonary Exercise Testing in African American and Caucasian American Men

Authors: Ahmad Sabbahi, Ross Arena, Univ of Illinois at Chicago, Chicago, IL; Leonard Kaminsky, Ball State Univ, Muncie, IN; Jonathan Myers, VA Palo Alto Health Care System, Palo Alto, CA; Bo Fernhall, Shane Phillips, Univ of Illinois at Chicago, Chicago, IL

Objective It has well been established that African American (AA) individuals have higher overall incidence and prevalence of hypertension compared to their Caucasian American (CA) counterparts. However, the maximum BP response of AA to exercise has not been characterized. The objective of this study is to investigate this peak BP response across both ethnic groups.***Patients and Methods*** A total of 9363 apparently healthy men from the FRIEND database who underwent maximum cardiopulmonary exercise tests on a cycle ergometer were included in this analysis. All subjects had a respiratory exchange ratio (RER) of ≥ 1.0 and had no reports of diagnosed disease.***Results*** There were significant differences between age and ethnic groups for peak SBP and peak DBP starting from the 30-39 years age group to the 60-69 years age group (*P*<.001). There were also significant differences in delta SBP and DBP between age and ethnic groups (*P*<.001). ***Conclusion*** These data indicate that the physiologic BP response to exercise in BA men is exaggerated compared to their WA counterparts, irrespective of baseline resting BP values.

Table 2. Cardiovascular and pulmonary responses to maximum exercise on a cycle ergometer for African American and Caucasian men. Values presented as mean ± SD.

	20-29 years		30-39 years		40-49 years		50-59 years		60-69 years	
Characteristic	African-American	Caucasian	African-American	Caucasian	African-American	Caucasian	African-American	Caucasian	African-American	Caucasian
Sample Size (n)	192	436	408	1235	736	2247	720	2296	243	850
Resting SBP (mm Hg)	117 ± 14	116 ± 17	121 ± 15	117 ± 17	124 ± 17	120 ± 17	126 ± 16	123 ± 17	130 ± 16	125 ± 16
Resting DBP (mm Hg)	76 ± 10	75 ± 12	80 ± 10	77 ± 12	82 ± 11	79 ± 12	81 ± 10	79 ± 11	82 ± 11	78 ± 9
Resting HR (bpm)	75 ± 11	80 ± 13	77 ± 12	77 ± 13	76 ± 12	76 ± 12	74 ± 11	75 ± 12	72 ± 12	72 ± 11
Peak SBP (mm Hg)	169 ± 26	168 ± 22	180 ± 28	173 ± 24	188 ± 28	179 ± 24	188 ± 29	182 ± 26	193 ± 29	182 ± 26
Peak DBP (mm Hg)	78 ± 13	76 ± 10	84 ± 15	80 ± 11	88 ± 15	82 ± 11	87 ± 14	83 ± 12	87 ± 13	81 ± 11
Max HR (bpm)	156 ± 16	166 ± 18	154 ± 17	159 ± 17	150 ± 18	152 ± 17	143 ± 18	146 ± 17	137 ± 18	139 ± 18
Delta SBP (mm Hg)	53 ± 22	52 ± 24	59 ± 25	55 ± 23	64 ± 25	58 ± 24	62 ± 26	59 ± 24	63 ± 26	57 ± 21
Delta DBP (mm Hg)	2 ± 10	1 ± 12	4 ± 11	3 ± 12	5 ± 13	3 ± 11	5 ± 12	4 ± 11	6 ± 12	3 ± 8
Max RER	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
Absolute VO_2 (L/min)	2.2 ± 0.5	2.6 ± 0.6	2.1 ± 0.5	2.5 ± 0.6	2.1 ± 0.4	2.4 ± 0.5	1.9 ± 0.4	2.2 ± 0.5	1.8 ± 0.4	2 ± 0.4
Relative VO_2 (ml/kg/min)	26.4 ± 5.5	30.4 ± 7.1	23.9 ± 5.2	27.9 ± 6.4	22.2 ± 4.4	26.3 ± 6.2	21.4 ± 4.5	24.7 ± 5.8	20 ± 4.3	23 ± 5.3

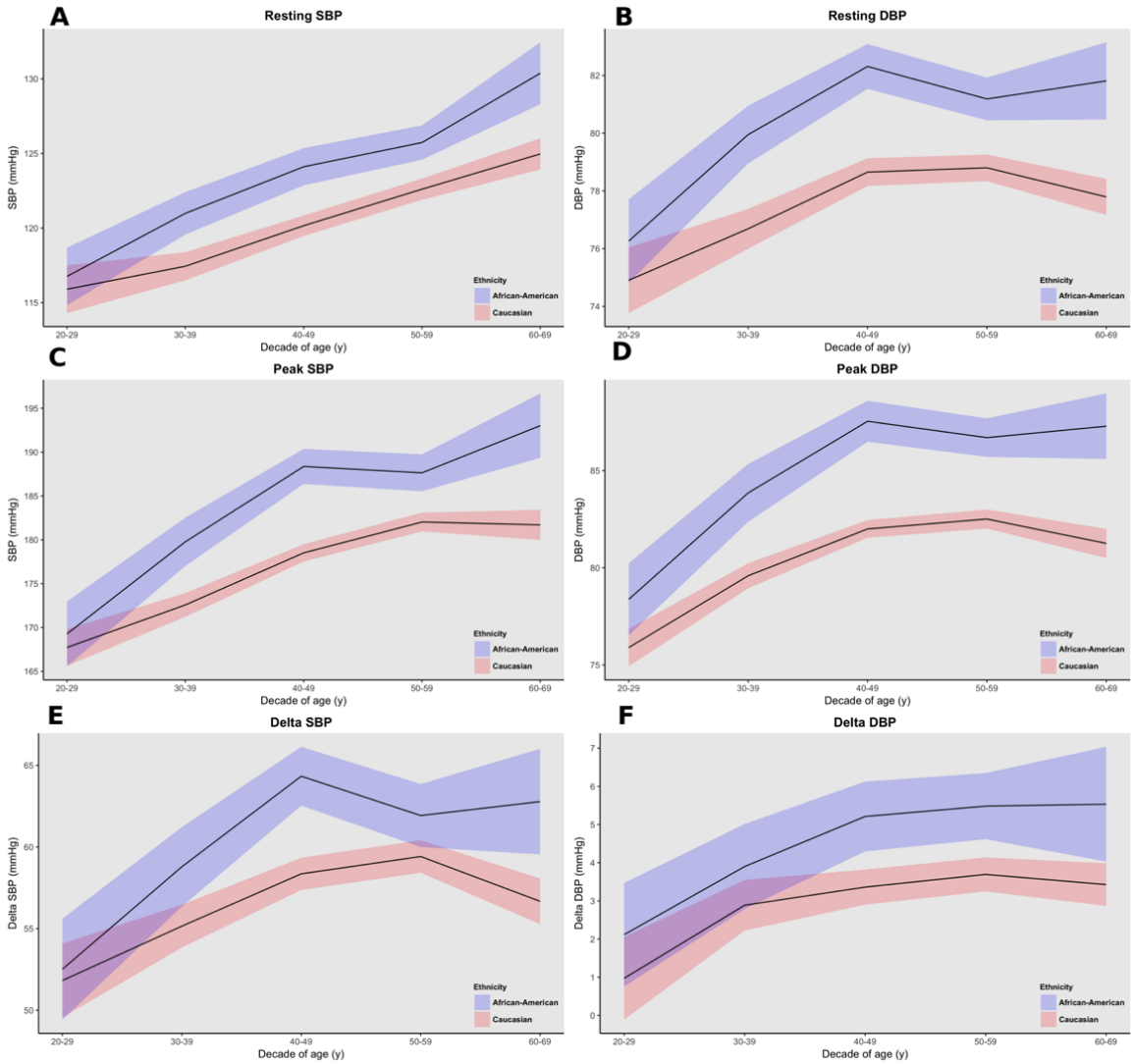


Figure 1. Blood pressure responses at rest and maximum exercise using a cycle ergometer in African American and Caucasian American men. Lines indicate mean values and shaded ribbons indicate 95% confidence intervals. ***A-B****, resting systolic and diastolic blood pressures (SBP, DBP).* ***C-D****, peak exercise systolic and diastolic blood pressures (SBP, DBP).* ***E-F****, delta systolic and diastolic blood pressures defined as (peak value - resting value).*

Disclosures: A. Sabbahi: None. **R. Arena:** None. **L. Kaminsky:** None. **J. Myers:** None. **B. Fernhall:** None. **S. Phillips:** None.

Funding: No

Funding Component:

P170

Morning Surge and Cardiovascular Events in African Americans: The Jackson Heart Study

Authors: John N Booth, Univ of Alabama at Birmi, Birmingham, AL; Marwah Abdalla, Columbia Univ, New York, NY; Mario Sims, Univ of Mississippi Medical Ctr, Jackson, MS; Mark Butler, Tanya Spruill, New York Univ, New York, NY; Paul Muntner, Univ of Alabama at Birmi, Birmingham, AL; Daichi Shimbo, Columbia Univ, N, NY

Introduction: A larger sleep-trough morning surge, defined as the mean two-hour post-awakening blood pressure (BP) minus the lowest asleep BP, has been associated with cardiovascular disease (CVD) events in Asians and Europeans. High asleep BP in African Americans (AA) may preclude a morning surge and an association with CVD due to a smaller BP change upon awakening.

Objective: Determine the association of morning surge with CVD events in AAs.
Methods: We analyzed participants from the Jackson Heart Study, a community-based cohort of AAs, with a complete 24-hour ambulatory BP monitoring recording and self-report asleep and awake times at baseline in 2000-2004 (n=767). Participants were grouped into tertiles of sleep-trough morning surge, two-hour post-awakening BP and lowest sleep BP. CVD events (nonfatal/fatal stroke, nonfatal myocardial infarction or fatal coronary heart disease; n=61) through December 2012 were adjudicated.
Results: Participants' mean age was 59.2 years and 32.1% were male. Multivariable adjusted hazard ratios (95% CI) for CVD events associated with Tertile 2 and 3 versus Tertile 1 of morning systolic BP (SBP) surge were 1.45 (0.71 – 2.97) and 2.03 (0.98 – 4.23), respectively; of post-awakening SBP were 2.01 (0.81 – 4.99) and 4.41 (1.85 – 10.50), respectively; and of lowest asleep SBP were 1.55 (0.68 – 3.52) and 2.22 (0.96 – 5.14), respectively (**Table**). Also, there was a graded increasing risk for CVD associated with Tertile 2 and 3 versus Tertile 1 of post-awakening diastolic BP (DBP), but not morning DBP surge and lowest asleep DBP.
Conclusion: Higher morning SBP surge, post-awakening SBP and lowest asleep SBP and DBP were associated increased CVD risk in AAs.

Disclosures: J.N. Booth: None. **M. Abdalla:** None. **M. Sims:** None. **M. Butler:** None. **T. Spruill:** None. **P. Muntner:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Amgen Inc. **D. Shimbo:** None.

Funding: Yes

Funding Component: National Center

P171

Occupational, Sport and Leisure Physical Activity Have Contrasting Effects on Neural Baroreflex Sensitivity. The Paris Prospective Study III

Authors: Rachel Climie, INSERM, U970, Dept of Epidemiology; Paris Descartes university, Paris, France, Paris, France; Pierre Boutouyrie, INSERM, U970, APHP. Paris Descartes university, Paris, France; Edward Chaussade, APHP, Paris Descartes university, Paris, France; Marie-Cecile Perier, INSERM, U970, Dept of Epidemiology; Paris Descartes university, Paris, France; Mathew Plichart, APHP, Paris Descartes university, Paris, France, Paris, France; Lucile Offrendo, Catherine Guibout, Thomas van Sloten, INSERM, U970, Dept of Epidemiology; Paris Descartes university, Paris, France; Frederique Thomas, Bruno Pannier, Investigations Préventives et cliniques (IPC), Paris, France; James Sharman, Menzies Inst for Medical Res, Hobart, Australia; Stephane Laurent, INSERM, U970, APHP. Paris Descartes university, Paris, France; Xavier Jouven, Jean-Philippe Empana, INSERM, U970, Dept of Epidemiology; Paris Descartes university, Paris, France

Background: Physical activity (PA) is beneficial for baroreflex sensitivity (BRS), but it is unclear whether the type of PA has similar effects on the neural (nBRS) or vascular (carotid stiffness) components of BRS. We sought to determine this in healthy adults from a community- based study via assessment of occupational (OPA), sport (SPA), leisure (LPA) and total PA (TPA). **Methods:** In 8649 adults aged 50 to 75 years, resting nBRS (estimated by low frequency gain, from carotid distension rate and heart rate) and carotid stiffness were measured by high-precision carotid echotracking. PA was self-reported using the Baecke questionnaire, which distinguishes OPA, SPA, LPA and TPA. The associations between PA and nBRS and carotid stiffness were quantified using multivariate linear regression analysis. Analyses were conducted separately in the working and non- working population. **Results:** In working adults (n=5039), OPA was associated with lower nBRS function (p=0.026) and borderline higher carotid stiffness (p=0.08). The associations between OPA and nBRS remained independent after additionally adjusting for SPA (p=0.03) and exaggerated exercise blood pressure (p=0.005), a predictor of future hypertension and cardiovascular events. When examining the type of OPA separately (i.e. lifting heavy loads, standing or walking at work) lifting heavy loads only was associated with impaired nBRS (p=0.048). When stratified by education, this association remained only in those with less than tertiary education. SPA was associated

with higher nBRS (p=0.0005) and borderline lower carotid stiffness (p=0.052). Neither LPA nor TPA was associated with nBRS or carotid stiffness. In non-working adults (n=3610), SPA and TPA were both associated with lower carotid stiffness (p=0.012 and p=0.020), but not nBRS. LPA was not associated with either parameter. **Conclusion:** Occupation-related PA, in particular lifting heavy loads, is associated with lower nBRS function, especially in those with lower education. Higher amounts of sport-related PA are associated with higher nBRS and lower carotid stiffness.

Disclosures: R. Climie: None. **P. Boutouyrie:** None. **E. Chaussade:** None. **M. Perier:** None. **M. Plichart:** None. **L. Offrendo:** None. **C. Guibout:** None. **T. van Sloten:** None. **F. Thomas:** None. **B. Pannier:** None. **J. Sharman:** None. **S. Laurent:** None. **X. Jouven:** None. **J. Empana:** None.

Funding: No

Funding Component:

P172

A Correlative Study of Cuff Hypertension and Anthropometric Variables in Obese and Non Obese Subjects

Authors: Abhishek Saini, Ajoy Krishnamurthy, MVJ Medical college & Res Hosp, Bengaluru, India; Chandrashekar Bohra, Univ of South Florida, Tampa, FL

Introduction According to the National Health And Nutrition Examination Survey (NHANES), 65 million Americans have hypertension and one-quarter of adults have blood pressure in the “pre hypertension” range. In such scenario accurate BP measurement is very important. This study focused on prevalence of hypertension among Obese individuals. Methods 400 patients (200 patients each with < and > BMI 25) were studied in rural tertiary hospital in India. Inclusion criteria - patients aged 20-50 yrs. Obese (defined by BMI > 25) . Exclusion criteria, Subjects on anti- hypertensive medication ,Secondary causes of clinically identifiable hypertension Diabetic subjects who are known hypertensives or with complications of DM, Pregnant women, Known cases of Ischemic heart disease. Enrolled patient were evaluated for Height , weight. Two cuff sizes 12 cm/15 cm used to measure BP. Statistical analysis was done with SPSS package and MS Excel, Student’s t-test and linear correlation coefficient used. p values < 0.05 were considered t significant. SBP - systolic Blood pressure and DBP - Diastolic Blood pressure. Results SBP and DBP increased significantly with increase of BMI in both men and women (p < 0.005). There is statistically significant standard and large cuff differences in SBP with P value 0.003 and 0.01 and DBP with p values were 0.004 and 0.00 for male and female respectively. There was also a statistically significant (p value < 0.05) standard-large cuff difference in both SBP and DBP in W/H ratio categories (<1 and >1) in males Discussion Cuff characteristics, i.e. the cuff-bladder width and length, can bias measurement of blood pressure in obese. Our study explained the potential for overdiagnosis of BP with using a wrong size cuff for people with BMI > 25 and W/H > 1. The prevalence of Systolic Hypertension based on JNC guidelines in the obese population with a regular cuff was 28% and with large appropriate size cuff it was 12%, a difference of 16% and similarly a difference of 12.5 % with diastolic BP. The prevalence of obesity in the US is 37% and this concept of Cuff Hypertension highlighted in our study is very important is such a setting as it can lead to lot of mis-diagnosed hypertension with unnecessary treatment burden and side effects.

Disclosures: A. Saini: None. **A. Krishnamurthy:** None. **C. Bohra:** None.

Funding: No

Funding Component:

P173

Sex Differences in the Diurnal Natriuretic Response to Benzamil in Sprague Dawley Rats

Authors: Reham H Soliman, Jermaine G Johnston, Eman Y Gohar, David M Pollock, Univ of Alabama at Birmingham, Birmingham, AL

Epithelial sodium channel (ENaC) expression follows a circadian pattern, but how this impacts activity is not known. In addition to the well-established sex differences in renal Na handling, recent data show that ENaC expression is higher (Western blot) in female rats. Further, we do not know if there are time of day differences or sex differences in ENaC activity. Therefore, the aim of this study was to determine if the diuretic response to ENaC inhibition (benzamil) is different between sexes at different times of day. SD rats (12-16 wk old) were placed in metabolic cages, where 12-hour urine collections were obtained to measure baseline urine volume and Na excretion as well as water and food intake. On day 3, benzamil was given at a dose of 1mg/kg (i.p.) either at the beginning of their inactive period/lights on (Zeitgeber Time 0, ZT0) or their active period/lights off (ZT12). The natriuretic response to benzamil was significantly greater in male compared to female rats (909 ± 302 vs 523 ± 83 µEq/kg/hr n=8) at ZT0 and (934 ± 94 vs 714 ± 151 µEq/kg/hr n=8) at ZT12. The diuretic response followed natriuresis being more prominent in male than female rats regardless of time of day (4.2 ± 0.7 vs 3.3 ± 0.5 ml/kg/hr n=8) at ZT0 and (3.6 ± 0.6 vs 2.5 ± 0.3 ml/kg/hr n=8) at ZT12. However, the larger response to benzamil given at the beginning of the inactive period (ZT0) compared to active period (ZT12) was not statistically significant (311 ± 98 vs 354 ± 30 µEq/hr and 120 ± 17.75 vs 174 ± 37 µEq/hr n=8) in male and female rats respectively. Given that endothelin-1 (ET-1) is an upstream inhibitor of ENaC, we measured urinary ET-1 levels to assess intrarenal production. ET-1 excretion significantly increased following benzamil administration in both sexes but was significantly greater in females. ET-1 excretion increased from 0.06 ± 0.01 to 0.29 ± 0.05 pg/hr in males (n=8) and from 0.10 ± 0.01 to 0.57 ± 0.28 pg/hr in females (n=8) at ZT0. At ZT12, ET-1 increased from 0.09 ± 0.02 to 0.23 ± 0.06 pg/hr in males (n=8) and from 0.14 ± 0.05 to 0.34 ± 0.06 pg/hr in females (n=8). These results demonstrate that the response to ENaC inhibition is less prominent in females independent of renal ET-1. This suggests less ENaC activity independent of expression in females or differences in non-ENaC related effects of benzamil.

Disclosures: R.H. Soliman: None. **J.G. Johnston:** None. **E.Y. Gohar:** None. **D.M. Pollock:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P174

Loss of Bmal1 in the Collecting Duct Lowers Blood Pressure in Male, but Not Female, Mice

Authors: Dingguo Zhang, Chunhua Jin, Binli Tao, David M. Pollock, Univ of Alabama at Birmingham, Birmingham, AL

Kidney function follows a 24-hour rhythm that is subject to regulation by so-called circadian clock genes. Previous studies suggested a potential role of circadian gene *Bmal1* in regulating renal excretory function and blood pressure, yet the mechanism is not completely clear. Our lab showed that high salt diet induces a phase shift in *Bmal1* expression in the renal inner medulla that is dependent on endothelin B receptor (ET_B) function. In addition, the ET_B receptor-mediated natriuretic effect is time-of-day- and sex-dependent. We recently generated a mouse model that lacks *Bmal1* expression in principal cells of the renal collecting duct, which has the highest concentration of ET_B receptor. The current study was designed to test the hypothesis that Bmal1 in the collecting duct regulates blood pressure in a sex-dependent manner. Male (n=8-9) and female (n=6) collecting duct *Bmal1* knockout (CD*Bmal1*KO) and floxed control (flox) mice were implanted with telemetry transmitters, and fed normal salt (0.49% NaCl) diet (NS) for 6 days, followed by 6 days of high salt (4% NaCl) diet (HS). After this period, mice were treated with an ET_B receptor antagonist (A-192621;

30mg/kg/day) for another 6 days. At the end of the study, we performed cosinor analysis on telemetry data collected throughout the study. Under NS, male CD*Bmal1*KO showed significantly lower 24-hr mean arterial pressure (MAP) compared to flox (105±1 vs 112±1 mmHg, p=0.01). However, we did not observe any significant differences in the MAP of female mice (106±1 vs 108±1 mmHg, p=0.26). Under HS, MAP of male CD*Bmal1*KO was significantly lower compared to flox (107±1 vs 114±1, p=0.01). No significant differences were observed in female mice (109±1 vs 110±1 mmHg, p=0.63). In male CD*Bmal1*KO, the increase in MAP in response to the ET_B receptor antagonist was significantly attenuated compared to flox (124±1 vs 130±1 mmHg, p<0.01). However, the increase in MAP in female mice was not significantly different between CD*Bmal1*KO and flox (130±1 vs 127±2 mmHg, p=0.33) during ET_B blockade. There were no differences in the amplitude of diurnal MAP between genotype in either sex on any diet. These data suggest that collecting duct Bmal1 plays an important role in blood pressure regulation in male, but not female, mice.

Disclosures: D. Zhang: None. **C. Jin:** None. **B. Tao:** None. **D.M. Pollock:** None.

Funding: No

Funding Component:

P175

Influence of Age and Estradiol on Blood Pressure Responses to Exercise

Authors: Megan M Wenner, Univ of Delaware, Newark, DE; Evan L. Matthews, Montclair State Univ, Montclair, NJ; Jasdeep Kaur, Univ of Texas at Arlington, Arlington, TX; Jody L. Greaney, The Pennsylvania State Univ, State College, PA; Paul J. Fadel, Univ of Texas at Arlington, Arlington, TX

An exaggerated pressor response to exercise is associated with increased risk of cardiovascular events. The exercise pressor reflex, comprised of metabolic and mechanically sensitive skeletal muscle afferents, contributes importantly to blood pressure control during exercise. Data in preclinical animal models suggest that estradiol (E2) attenuates the pressor responses to exercise pressor reflex activation, yet it remains unclear if these findings translate to humans. Accordingly, we tested the hypothesis that pressor responses during exercise are augmented in post-menopausal women (PMW) compared to young women (YW), and that E2 administration will attenuate these responses in PMW. **Methods:** Mean arterial pressure (MAP, Finometer) and heart rate (ECG) were continuously measured in 12 PMW (age 59±2 years) and 16 YW (age 22±1 years) during 2-min of isometric handgrip performed at 30% of maximal voluntary contraction (MVC). Handgrip was immediately followed by 3-min of post-exercise ischemia (PEI), which isolates the muscle metaboreflex. Separately, BP responses during isometric handgrip at 35% MVC and PEI were measured in 6 PMW (age 53±1 years) before and after 1 month of transdermal E2 administration (100 μg/day). **Results:** Resting MAP was similar between PMW (77±3 mmHg) and YW (80±3 mmHg; *P*>0.05). During handgrip, the increase in MAP was greater in PMW (Δ20±2mmHg) compared to YW (Δ12±1 mmHg; *P*<0.05), and this was maintained during PEI (Δ14±1 mmHg PMW vs. Δ9±1 mmHg YW; *P*<0.05). Estradiol administration decreased resting BP in PMW (MAP 93±3 mmHg vs. 89±3 mmHg; *P*<0.05). Moreover, the increase in MAP during exercise in PMW was attenuated following estradiol administration (Δ31±8 mmHg vs. Δ20±6 mmHg; *P*<0.05). There was also a tendency for this reduction in MAP during PEI (Δ22±6 mmHg vs. Δ18±7 mmHg; *P*=0.08). **Conclusions:** These preliminary data suggest that PMW exhibit an exaggerated BP response to isometric exercise, due in part to heightened metaboreflex activation, and that estradiol administration can attenuate such responses.

Disclosures: M.M. Wenner: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; 16SDG30700015. **E.L. Matthews:** None. **J. Kaur:** None. **J.L. Greaney:** None. **P.J. Fadel:** None.

Funding: No

Funding Component:

P176

Hypertension is Mediated by an Angiotensin II-Dependent Mechanism in Adipocyte Prorenin Receptor KO Female Mice and by the Para-Sympathetic Nervous System in Adipose Prorenin Receptor KO Male Mice

Authors: Eva Gatineau, Ming Gong, Frederique Yiannikouris, Univ of Kentucky, Lexington, KY

Obesity and lipodystrophy models, two opposite ends of adipose tissues dysfunction, can lead to hypertension and constitute relevant tools to decipher mechanisms involved in blood pressure control. Since sex related difference exists in blood pressure control, we aimed to determine whether the mechanism leading to hypertension in adipocyte prorenin receptor (PRR) KO mice was similar in male and female mice.
Adipocyte PRR KO and control littermate (CTL) male (n=8 to 9 mice/group) and females mice (n=6 to 7 mice/group) were fed a high fat diet. The systolic blood pressure (SBP) was evaluated by radiotelemetry. Adipose-PRR KO significantly increased SBP in both male and female mice (Day SBP, CTL male=122.2±1.9 mmHg, KO male=128.7±2.4 mmHg, P=0.046; CTL female=120.4±0.9 mmHg, KO female=125.5±1.9 mmHg, P=0.03). Acute losartan injection, an AT_1R inhibitor, blunted elevated SBP in both CTL female and male mice. Interestingly, losartan decreased SBP to a greater extent in adipose PRR KO female mice compared with control female mice (Delta SPB, CTL male=-7.9±1.5 mmHg; KO male=-6.4±3.1 mmHg, P=0.68; CTL female=-5.3±1.9 mmHg; KO female=-14.2±0.9 mmHg, P=0.015). To assess the contribution of the autonomic nervous system, male and female mice were injected with propranolol, atropine and chlorisondamine. The tachychardic response to atropin was significantly greater in adipose-PRR KO male mice compared with control male mice (Delta Heart Rate, CTL male=+114.2±12.1 bpm; KO male=+156.0±11.1 bpm, P=0.023) but did not change in adipose-PRR KO female mice compared with control female mice (Delta Heart Rate, CTL female=+84.8±10.1 bpm; KO female=+89.8±21.3 bpm, P=0.85).
In conclusion, our data strongly suggest that the elevation of SBP in adipose PRR KO mice is mediated by an AngII-dependent mechanism in female mice and by the para-sympathetic nervous system in male mice. These results support the importance of sex-specific approach for the development of personalized drugs in hypertension treatment.

Disclosures: E. Gatineau: None. **M. Gong:** None. **F. Yiannikouris:** None.

Funding: Yes

Funding Component: Great Rivers Affiliate (Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)

P177

The Roles of Estrogen-Related Receptor Alpha in Cardiometabolic Control

Authors: Kenji SAITO, Yue Deng, Kevin C Davis, Jing Wu, Masashi Mukoda, Curt D Sigmund, Huxing Cui, The Univ of Iowa, Iowa City, IA

The estrogen-related receptor alpha (Esrra) is an orphan nuclear receptor with strong homology to estrogen receptor alpha (ERα), whereas it exhibits estrogen-independent constitutive transcriptional activity to regulate a number of genes involving in cellular energy metabolism. Esrra is broadly expressed throughout the body including those metabolically active tissues such as skeletal muscles, heart, brown adipose tissues, kidney, and brain. While previous reports showed that Esrra knockout (KO) mice were hypotensive and resistant to high fat diet-induced obesity (DIO), systemic understanding of the roles of Esrra in cardiometabolic control is limited. We therefore performed a variety of metabolic and cardiovascular measures to evaluate the cardiometabolic consequences of mice lacking Esrra globally. Our results revealed that Esrra KO mice were hypoactive and were resistant to DIO mainly due to decreased food intake. Despite of lower body weight and plasma leptin level, female Esrra KO mice tend to have elevated blood glucose level (p=0.08)

without notable changes of insulin and glucagon levels. Non-invasive blood pressure measurement by tail-cuff sphygmomanometer showed that male Esrra KO mice had significantly lower blood pressure when the measurement was performed during light period (WT vs. KO: 99.8±1.4 vs. 93.9±1.8 mmHg, $p<0.05$). On the other hand, the blood pressure measured during dark period was significantly lower in female, but not male, Esrra KO mice compared to their WT littermates (106.1±1.8 vs. 98.8±1.5 mmHg, $p<0.05$). Pulse wave velocity test showed that vascular stiffness was comparable between genotypes in both genders. Echocardiographic measurements revealed that the ejection fraction of male, but not female, KO mice was significantly higher than that of WT littermates (WT vs. KO: 75.2±0.02% vs. 85.4±0.01%, $P<0.01$), while female KO mice did not show any significant changes.
These results indicate a multifaceted role of Esrra in the regulation of metabolic and cardiovascular functions likely in a gender- and circadian cycle-dependent manner. Future studies with conditional deletion approach are necessary to tease apart complex roles of Esrra in distinct cardiometabolic processes.

Disclosures: K. Saito: None. **Y. Deng:** None. **K.C. Davis:** None. **J. Wu:** None. **M. Mukoda:** None. **C.D. Sigmund:** None. **H. Cui:** None.

Funding: No

Funding Component:

P179

Placental Ischemia-Induced Hypertension is Attenuated in Melanocortin-4 Receptor (MC4R)-Deficient Pregnant Rats

Authors: Frank T Spradley, Joey P Granger, U of Mississippi Medical Ctr, Jackson, MS

The incidence of preeclampsia (PE), a pregnancy-specific disorder of new-onset hypertension that is linked to placental ischemia, has increased over the last several decades. While obesity is a major risk factor for PE, the mechanisms whereby obesity impacts placental ischemia-induced hypertension are not fully understood. We recently reported that placental ischemia-induced hypertension is partially dependent upon an intact sympathetic nervous system (SNS). In various hypertensive models including obesity, blockade of MC4R signaling leads to increased food intake and body weight but protects against the development of hypertension via suppression of the SNS. Although blockade of MC4R may lead to increased body weight during pregnancy, we tested the hypothesis that placental ischemia-induced hypertension is attenuated in obese MC4R-deficient pregnant rats. Wild-type (WT) and MC4R heterozygous-deficient rats were mated with genotype-matched males at ~12 wks old. All rats were maintained on NIH31 chow diet. On gestational day 14, rats were subjected to chronic placental ischemia via the reduced uterine perfusion pressure (RUPP) procedure or Sham surgery. By day 19, conscious mean arterial blood pressure (MAP, carotid catheter) was elevated in RUPP WT (N=21) over Sham WT rats (N=14) (117±2 vs. 101±1 mmHg, $P<0.05$) with reduced average fetus (1.71±0.04 vs. 1.89±0.05 g, $P<0.05$) but no change in average placenta weights (0.46±0.02 vs. 0.47±0.01 g), respectively. In Sham MC4R-def vs. Sham WT, respectively, there was increased ($P<0.05$) body weight (366±8 vs. 337±6 g), total body fat mass (72±4 vs. 44±2 g), and circulating leptin adipokine levels (6.9±0.7 vs. 3.3±0.3 ng/mL) but with similar average fetus (1.92±0.02 vs. 1.89±0.05 g) and placenta weights (0.49±0.01 vs. 0.47±0.01 g). Even though blood pressure was elevated in Sham MC4R-def (109±2 mmHg, $P<0.05$) over Sham WT, the hypertensive response to RUPP was attenuated in MC4R-def rats (114±2 mmHg). RUPP did not alter leptin levels in MC4R-def rats (6.4±1 ng/mL). In conclusion, these data suggest that MC4R heterozygous-def pregnant rats are obese and have higher blood pressure during pregnancy. However, placental ischemia may require a full complement of MC4R expression to elicit a hypertensive response.

Disclosures: F.T. Spradley: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 4R00HL130577-02. **J.P. Granger:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; P20GM104357.

Funding: No

Funding Component:

P180

Cardiac Small Vessel Imaging by Light Sheet Microscopy and Micro CT - Discovering the Missing Link Between Preeclampsia and Higher Risk for Further Cardiovascular Disease

Authors: Kristin Kraeker, Nadine Haase, Experimental and Clinical Res Ctr; Max-Delbrück-Ctr for Molecular Med; Berlin Inst of Health; German Ctr for Cardiovascular Res, Berlin, Germany; Florian Herse, Experimental and Clinical Res Ctr; Max-Delbrück-Ctr for Molecular Med; Charité – Univsmedizin Berlin; Berlin Inst of Health, Berlin, Germany; Stefan Verlohren, Charité – Univsmedizin, Berlin, Germany; Arnd Heuser, Anje Sporbert, Matthias Richter, Max-Delbrueck-Ctr for Molecular Med, Berlin, Germany; Jamie O'Driscoll, Basky Thilaganathan, St George's Healthcare NHS Trust, London, United Kingdom; Dominik N. Mueller, Experimental and Clinical Res Ctr; Max-Delbrück-Ctr for Molecular Med; Charité - Univsmedizin; Berlin Inst of Health; German Ctr for Cardiovascular Res, Berlin, Germany; Ralf Dechend, Experimental and Clinical Res Ctr; Charité – Univsmedizin Berlin; Berlin Inst of Health; Helios Klinikum, Berlin, Germany

Introduction: In preeclampsia, symptoms like high blood pressure and albuminuria are caused by a state of anti-angiogenic and immune imbalance resulting in endothelial dysfunction. The evaluation of smaller vessels is a challenge, but clinically of increasing importance. **Objective:** We want to examine whether the increased risk for postpartum maternal cardiovascular disease after preeclamptic pregnancy is resultant from microvascular changes in connection with the structural remodelling processes. **Methods:** We compared echo data from a human cohort with data from our transgenic animal model (hAGTxhRen) after preeclamptic pregnancy. In addition, we investigated cardiac changes in gene (qPCR) and protein expression levels (ELISA, IHC staining) in maternal rats, as well as alterations in microvascular 3D remodeling using LSFM and Micro CT. **Results:** We were able to show that the echo changes in our transgenic rat model are comparable to human data. Basic parameters like ejection fraction (human 0,91; animal 0,86), end-systolic volume (human 1,26; animal 1,22) or heart rate (human 1,13; animal 1,16) are altered at the end of pregnancy in same direction. Also markers of hypertrophy like relative wall thickness (human 1,19; animal 1,21) are changed. With the help of speckle trackle analysis, a more sensitive method to detect subclinical changes in hearts functionality, both groups show a reduction of global longitudinal strain (human 0,75; animal 0,6) and strain rate (human 0,81; animal 0,73). The microvasculature and entire vascular network has been visualized so far only in cleared mouse brains and partially in adult mouse hearts. Here, we present the 3D network of lectin-labeled blood vessels in cleared adult rat hearts with the analysis of cardiac small vessels with regard to branching points, vessel length and up to a diameter of 6µm. **Discussion:** Preeclampsia leads to a weakened functionality in postpartum hearts, in a human cohort as well as in our transgenic animal model. Studies are underway to quantify coronary microvascular pathology as a possible missing link between preeclampsia and higher risk for further cardiovascular disease. (values show postpartum change compared to non-pregnant)

Disclosures: K. Kraeker: None. **N. Haase:** None. **F. Herse:** None. **S. Verlohren:** None. **A. Heuser:** None. **A. Sporbert:** None. **M. Richter:** None. **J. O'Driscoll:** None. **B. Thilaganathan:** None. **D. Mueller:** None. **R. Dechend:** None.

Funding: No

Funding Component:

P181

Hypertension Treatment Disruption During Pregnancy Among Women With Chronic Hypertension

Authors: Lu Chen, Susan M Shortreed, Kaiser Permanente Washington Health Res Inst, Seattle, WA; Thomas Easterling, Univ of Washington, Seattle, WA; T. Craig Cheetham, Kaiser Permanente Southern California, Dept of Res & Evaluation, Pasadena, CA; Victoria Holt, Univ of Washington, Seattle, WA; Lyndsay A Avalos, Kaiser Permanente Northern California, Div of Res, Oakland, CA; Rod Walker, Aruna Kamineni, Kaiser Permanente Washington Health Res Inst, Seattle, WA; Kristi Reynolds, Kaiser Permanente Southern California, Dept of Res & Evaluation, Pasadena, CA; Sascha Dublin, Kaiser Permanente Washington Health Res Inst, Seattle, WA

Objective: In the U.S., 1.3 million women of reproductive age take antihypertensive medications for chronic hypertension. However, some of these medications are considered unsafe during pregnancy. We evaluated the burden of switching and stopping medications during pregnancy among women receiving treatment for chronic hypertension.
Study design: We identified a population-based cohort of women with chronic hypertension who received antihypertensive medications within 120 days before pregnancy and gave birth to a singleton between 2005 and 2014 within three Kaiser Permanente regions. We characterized women's antihypertensive medication use from 120 days before pregnancy through delivery. We ascertained the highest systolic and diastolic blood pressures in the 120 days before the start of pregnancy and supplemented these values with measures up through 8 weeks' gestation when pre-pregnancy values were missing.
Results: 5,782 pregnant women were included. Prior to pregnancy, the most commonly used medication classes were thiazide diuretics (2,370/5,782, 41%), beta-blockers (1,569/5,782, 27%) and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEIs/ARBs) (1,391/5,782, 24%). In contrast, the predominant medications during pregnancy were labetalol (2,165/5,782, 37%) and methyldopa (1,593/5,782, 28%). During pregnancy, 18% of the cohort (1,037/5,782) had no antihypertensive medication fills. Women taking ACEI/ARBs or thiazides before pregnancy were the most likely to have no medication fills in pregnancy (23% and 20%, respectively). Many women (n=881) had at least one severe high BP (SBP ≥160 or DBP ≥110) before pregnancy, and in this group, 15% (132/881) filled no medications throughout pregnancy.
Conclusion: Women with chronic hypertension frequently stop antihypertensive medications during pregnancy, even women with a severely high BP documented before pregnancy. Research is needed to understand impact of these treatment interruptions on pregnancy outcomes and women's long-term health.

Disclosures: L. Chen: None. **S.M. Shortreed:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; S.M.S. has worked on grants awarded to Kaiser Permanente Washington Health Research Institute (KPWHRI) by Pfizer and also serves as a co-Investigator on grants awarded to KPWHRI from Syneos Health.. **T. Easterling:** None. **T. Cheetham:** None. **V. Holt:** None. **L.A. Avalos:** None. **R. Walker:** None. **A. Kamineni:** None. **K. Reynolds:** None. **S. Dublin:** None.

Funding: No

Funding Component:

P182

Daily Subcutaneous Administration of a Biopolymer-Stabilized VEGF Chimera Optimizes Therapeutic Efficacy in Treating a Rodent Model of Placental Ischemia

Authors: Omar C Logue, Stephen P Burke, Eric M George, Gene L Bidwell III, Univ Mississippi Medical Ctr, Jackson, MS

Preeclampsia (PE) complicates 3-5% of pregnancies with gestational hypertension, and programs later life outcomes in both mother and offspring. The unknown etiology arises from abnormal remodeling of the maternal spiral arteries,

creating an ischemic placenta that releases pathogenic factors that drive the syndrome. One of these factors, soluble fms-like tyrosine kinase-1 (sFlt-1), contributes to the hypertensive response by sequestering vascular endothelial growth factor (VEGF). Currently, no effective pharmacological therapies exist for PE. With the goal of developing PE treatments that are safe for both the mother and fetus, novel therapies that target sFlt-1 are being tested in the reduced uterine perfusion pressure (RUPP) rodent model. Previously, we demonstrated that intraperitoneal administration of human VEGF-A121 fused to the bioengineered protein polymer drug carrier elastin-like polypeptide (ELP-VEGF), at a dose of 5 mg/kg/d, blocked hypertension by sequestering sFlt-1, enhanced renal nitric oxide signaling, and simultaneously prevented fetal exposure to VEGF. However, chronic intraperitoneal infusion was associated with adverse ascites production, and at the 10 mg/kg/day dose, fetal loss. In order to minimize dose-dependent side effects while retaining the therapeutic efficacy of ELP-VEGF, chronic biodistribution studies were conducted in non-pregnant female hairless Sprague-Dawley rats. ELP-VEGF was fluorescently labeled, and rats (*n*=4) received a single 50 mg/kg bolus subcutaneously (SC) between the scapulae or intravenously (IV) by daily bolus injection (5 mg/kg/day) via jugular catheter. Blood draws and *in vivo* whole-animal imaging were conducted at specific timepoints to assess plasma levels and mean tissue polypeptide levels. Pharmacokinetic fits demonstrated a plasma half-life of 2.18 +/- 1.1 hours following IV administration, and the plasma clearance rate was consistent after each daily injection. Tissue levels accumulated over the five-day IV dosing time course. When administered SC, plasma levels peaked 3 hours after administration and cleared slowly over a 24 - 48 h period. Based on these data, the therapeutic efficacy of daily SC administration of ELP-VEGF (50 mg/kg/d) is being assessed in the RUPP model.

Disclosures: O.C. Logue: None. **S.P. Burke:** None. **E.M. George:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; Patent. **G.L. Bidwell:** 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; Leflore Technologies.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P183

Maternal Obesity is Associated With Altered Cardiovascular Remodeling During Pregnancy

Authors: Katherine Shreyder, Maira Carrillo, James Maher, Natalia Schlabritz-Loutsevitch, Texas Tech Health Sciences Ctr at Permian Basin, Odessa, TX

Introduction: Maternal obesity (MO) is the strongest risk factor for hypertensive disorders of pregnancy. Both, pregnancy and obesity are associated with the activation of inflammatory pathways, however, the information regarding pregnancy-related cardiac changes in MO as well as the role of leptin in the process is conflicting.
The aim of this study was to evaluate echocardiographic and serum inflammatory changes in normal/overweight (NPW) (BMI 18.0-29.9) and obese pregnant women (OPW) (BMI >30) in the first and second trimesters of pregnancy.
Material and methods: Nine obese (BMI 33.6±2.9) and seven non-obese (BMI 25.5±2.3) nulliparious women, who met inclusion criteria, were enrolled into IRB approved protocol in the first trimester of pregnancy. Blood pressure (systolic: SBP and diastolic (DBP) and heart rate (HR) were measured, transthoracic echocardiography was performed in the first and second pregnancy trimesters. Data was compared using Mann-Whitney test.
Results: SBP, DBP and HR were within normal range, but higher in OPW, compared to NPW in both trimesters (Table 1). The left ventricular mass (LVM) was higher, and ratio of the early (E) to late (A) ventricular filling velocities (E/A ratio) was lower in OPW, compared to NPW in the first trimester. There was increase in stroke volume (SV), left atrium (LA) size, LVM and decrease in E/A in second, compared to first trimesters in both groups. SV enhanced less in OPW. Leptin

concentration was higher in OPW and inversely correlated with stroke volume (SV) in the second trimester.
Conclusion: Maternal obesity alters pregnancy-driven changes in systolic and diastolic heart function.

	First trimester			Second trimester		
	Obese (n=7)	Non-obese (n=9)	p-value	Obese (n=7)	Non-obese (n=9)	p-value
SBP, mmHg	125± 12.6	109 ±9.3	<0.05	121.3±10.8	104±8.1	<0.05
DBP, mmHg	79.7± 12.7	68.8 ±6.73	<0.05	72.2±12.0	67±8.0	<0.05
HR, bpm	74.8 ± 3.5	69.9 ± 3.2	<0.05	76.2±4.0	72.9±3.8	<0.05
SV, ml	60.4 ±4.9	57.4 ±6.3	>0.05	67.8 ±3.3	77.0 ±4.5	<0.05
SV/BSA ml/m2	34.5 ± 6.1	32.4±5.6	>0.05	34.8±2.6	47.6±1.8	<0.05
LVM (g)	122.6 ±29.5	97.4 ±21.6	<0.05	164.0± 33.8	135.3± 16.8	>0.05
LVM/BSA g/m2	60.1±5.3	56.9±9.0	>0.05	83.0±18.1	80.3±6.7	>0.05
E/A	1.5 ±0.2	1.83 ±0.19	<0.05	1.3±0.3	1.5±0.3	>0.05
LA, cm	3.2±0.4	3.0±0.2	>0.05	3.6±0.3	3.3±0.1	<0.05
Leptin, ng/ml	73.1±14.7	48.9±14.0	<0.05			
TNFα, pg/ml	37.0±14.5	21.6±9.2	>0.05			

Disclosures: K. Shreyder: None. **M. Carrillo:** None. **J. Maher:** None. **N. Schlabritz-Loutsevitch:** None.

Funding: No

Funding Component:

P184

Coordinated Control of Cardiac Contractility By Multiple RGS Proteins

Authors: Tyler F Bernadyn, Jackson G Marshall, Shelby A Dahlen, Matthew M Meleka, Elizabeth A Owens, **Patrick Osei Owusu**, Drexel Univ Coll of Med, Philadelphia, PA

Signaling via heterotrimeric G proteins is critical to maintaining cardiovascular homeostasis. Abnormal G protein signaling due to the loss of G protein regulation by proteins such as regulators of G protein signaling (RGS) proteins is implicated in several cardiovascular disorders such as hypertension, cardiac hypertrophy, and heart failure. RGS act as GTPase activating proteins (GAPs) to control the kinetics and amplitude of G protein signaling. Multiple RGS proteins are prominently expressed in the cardiovascular system; however, it is unknown whether their activities/functions are coordinated to control G protein signaling and cardiovascular function. This study used mice concurrently lacking RGS2 and 5 (*Rgs2/5* dbKO) to determine how the dual loss of potent GAPs for Gq/11 and Gi/o class G proteins affects cardiovascular function. Blood pressure and heart rate in conscious, freely moving mice were monitored via radiotelemetry. Surgical implantation of the radiotelemetry device induced marked systolic blood pressure increase in *Rgs2/5* dbKO mice (WT: 140 ± 6 vs. dbKO: 170 ± 2 mmHg; $p<0.01$) at baseline, which gradually declined but remained elevated above wild type (WT) control level several days later. Whereas all WT mice survived the surgery, ~70-80% of male *Rgs2/5* dbKO mice died 72-96 hr post-surgery. When subjected to cardiac stress test using acute dobutamine infusion and echocardiography, male *Rgs2/5* dbko mice showed hypocontractile response relative to WT mice. Freshly isolated ventricular cardiomyocytes from male *Rgs2/5* dbKO mice showed decreased fractional shortening (WT: 16.1 ± 4.3 vs. dbKO: 7.4 ± 1.1 %; $p<0.01$) but high calcium transients (WT: 117 ± 20 vs. dbKO: 198 ± 50 au; $p=0.07$) at baseline, and application of electrical field stimulation or the non-selective β-adrenergic receptor agonist, isoproterenol (ISO), triggered premature calcium transients, tachyarrhythmia and death of cells from *Rgs2/5* dbKO mice. Interestingly, cells from mice harboring just one copy of *Rgs2* (*Rgs2+/-,Rgs5-/-*) but not *Rgs5* (*Rgs2-/-,Rgs5+/-*) were resistant to low-dose ISO-induced arrhythmia. These results together suggest that RGS2 and 5 coordinate their activity to control cardiomyocyte excitation-contraction coupling and normal cardiac rhythm.

Disclosures: T.F. Bernadyn: None. **J.G. Marshall:** None. **S.A. Dahlen:** None. **M.M. Meleka:** None. **E.A. Owens:** None. **P. Osei Owusu:** None.

Funding: Yes

Funding Component: National Center

P185

Periostin Increases Inducible Nitric Oxide Synthase Expression in Right Ventricular Fibroblasts From Pulmonary Arterial Hypertension Rats

Authors: Keisuke Imoto, Muneyoshi Okada, Hideyuki Yamawaki, Sch of Veterinary Med, Kitasato Univ, Towada, Aomori, Japan

[Background] Pulmonary arterial hypertension (PAH) leads to a lethal right heart failure (RHF), effective treatment for which has not been established. Inducible nitric oxide synthase (iNOS) is thought to be associated with the pathogenesis of heart failure. Inflammatory cytokines, which are increased in RHF, are known to induce iNOS expression in cardiac fibroblasts. We previously demonstrated that the mRNA expression of periostin, a matricellular protein, was upregulated in right ventricles (RVs) of monocrotaline (MCT)-induced PAH model rats. In the present study, we investigated the effects of periostin on iNOS expression in right ventricular fibroblasts isolated from PAH model rats.
[Methods] MCT (60 mg/kg) was injected intraperitoneally to 4-week-old male Wistar rats. Two weeks after the MCT-injection, cardiac fibroblasts were enzymatically isolated from RVs using a modified Langendorff apparatus. A soluble form of recombinant rat periostin protein was produced by *Escherichia coli*. The right ventricular fibroblasts (RVFs) were stimulated with a recombinant rat periostin (10-1000 ng/ml, 30 min-24 h). The expression of iNOS and activation of extracellular signal-regulated kinase (ERK) 1/2 and c-jun N-terminal kinase (JNK) were investigated by Western blotting.
[Results] Periostin (1000 ng/ml, 24 h) significantly increased iNOS (3.9 ± 0.6-fold, $P<0.01$, n=4) but not eNOS in RVFs. Periostin (1000 ng/ml, 30 min) increased phosphorylation of ERK1/2 (4.0 ± 2.3-fold, n=3) and JNK (13.2 ± 2.7-fold, $P<0.05$, n=3) in RVFs. An ERK pathway inhibitor, PD98059 (10 μM) or JNK inhibitor, SP600125 (30 μM) almost completely suppressed the periostin-induced iNOS expression (PD98059, $P<0.01$; SP600125, $P<0.05$, n=3).
[Conclusion] We for the first time demonstrated that periostin increases iNOS expression via the activation of ERK and JNK in RVFs isolated from PAH model rats. It is suggested that inflammatory reaction mediated by periostin might be a novel target for treatment of PAH-induced RHF.

Disclosures: K. Imoto: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; JSPS KAKENHI Grant Number 18J11540. **M. Okada:** None. **H. Yamawaki:** None.

Funding: No

Funding Component:

P186

Reversion of Severe Cardiac and Renal Dysfunction With Surgical Correction of Atypical Aortic Coarctation in Adult Patient: Case Report

Authors: Alexandre L Bortolotto, Faculty of Med , São Paulo Univ, São Paulo, Brazil; Rogerio B Filho, Everton L Moreira, Faculty of Med, Sao Paulo Univ, São Paulo, Brazil; Luis A Dallan, Luiz Bortolotto, Heart Inst (InCor), São Paulo, Brazil

Background: Heart and renal dysfunction are the most frequent complications of arterial hypertension (AH). Secondary causes of AH can lead to severe organ damage, potentially reversible after treatment. **Objective:** To report a rare cause of AH in adult, atypical aortic coarctation, where surgical correction promoted reversion of organ damage. **Report:** Woman, 55 years-old, history of AH for 10 years, worsening of blood pressure (BP) control for the last 10 months, and

progressive dyspnea on exertion, reaching dyspnea at rest. Admission: BP=260x110mmHg and acute pulmonary edema. Improvement of clinical condition with intensive measures, and after, the patient showed differences in BP between left (120x100mmHg) and right upper arm (180x110mmHg), systolic murmur on mitral region, absence of pulse in lower limbs. Exams: Serum creatinine (Cr) = 1,7mg/dL; EKG: left chamber overload; Echocardiogram: table; Magnetic resonance angiography of the aorta: severe stenosis of the aortic arch (13.6mm in its largest diameter). Patient was submitted to a bypass (Dacron #18 tube) from the ascending to the descending segment of the aorta. In the post-operatory period the patient improved renal function (Cr 1.0mg/dl) and controlled BP under use of 1 drug. One month after surgery, patient maintained BP adequately controlled significantly improved echocardiographic parameters (table). In long-term follow-up, patient remained asymptomatic with BP=124x72mmHg, and normal renal and cardiac function, under use of 3 antihypertensive drugs. **Conclusion:** Atypical aortic coarctation at adult age can lead to severe cardiac and renal impairment, which is reversible after surgical correction.

	Septum Wall Thickness (mm)	Left ventricle diastolic diameter (mm)	Left atrium (mm)	Ejection fraction (%)	Left ventricle Mass Index (g/m²)	Serum Creatinine (mg/dL)
Admission exam	13	64	60	23	230	1.7
1 month post-op	13	62	60	55	230	1
6 years post-op	9	47	43	69	72	-
8 years post-op	11	46	43	61	72	0.67

Disclosures: A.L. Bortolotto: None. **R.B.R. Filho:** None. **E.L.S. Moreira:** None. **L.A. Dallan:** None. **L. Bortolotto:** None.

Funding: No

Funding Component:

P187

Elevated Resting Heart Rate is Associated With Dyslipidemia in Chinese Rural Population: The Henan Rural Cohort Study

Authors: Xia Zhang, Chongjian Wang, Coll of Public Health, Zhengzhou Univ, Zhengzhou, China; Yuqian Li, Sch of Pharmaceutical Science, Zhengzhou Univ, Zhengzhou, China; Songcheng Yu, Xiaotian Liu, Runqi Tu, Haiqing Zhang, Kaili Yang, Dou Qiao, Xue Liu, Gongyuan Zhang, **Zhenxing Mao**, Coll of Public Health, Zhengzhou Univ, Zhengzhou, China

Aim: The aim of this study was to explore the association between resting heart rate (RHR) and dyslipidemia in Chinese rural population. Simultaneously, we examined whether this association was mediated by some degree of fasting plasma glucose (FPG). **Methods:** A total of 38727 participants aged 18-79 years were derived from the Henan Rural Cohort Study during 2015-2017 in China. Restricted cubic splines and logistic regression were used to estimate the *OR*s and 95% *CI*s. Mediation analysis using bootstrap was performed to examine the contribution of FPG to RHR-related dyslipidemia. **Results:** The results showed that RHR were associated with the risk of dyslipidemia, and the corresponding adjusted *OR*s (95%*CI*s) for each quartiles were 1.00, 1.12(1.05-1.20), 1.22(1.15-1.30), 1.45(1.36-1.54), respectively. In continuous analysis, each 1SD increment in RHR was significantly associated with a 15% increased risk of dyslipidemia.

The restricted cubic splines showed that the risk of dyslipidemia increased gradually with continuous RHR. In mediation analysis, the percentage of excess relative risk mediated for FPG was 24.63%. **Conclusion:** Elevated RHR was significantly associated with the risk of dyslipidemia in Chinese rural population. In addition, RHR -related dyslipidemia was mediated by some degree of FPG. However, the potential clinical application remains to be determined.

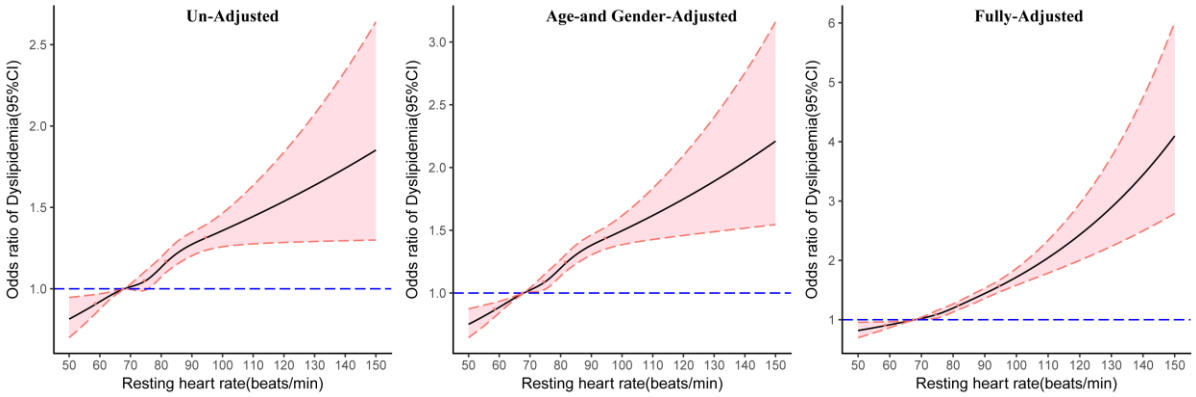


Figure *OR* (solid lines) and 95% *CI* (dashed lines) for the risk of dyslipidemia along with the changes of resting heart rate from restricted cubic splines.
Adjusted for age, gender, education level, marital status, smoking, drinking, physical activity, family history of dyslipidemia and body mass index.

Disclosures: X. Zhang: None. **C. Wang:** None. **Y. Li:** None. **S. Yu:** None. **X. Liu:** None. **R. Tu:** None. **H. Zhang:** None. **K. Yang:** None. **D. Qiao:** None. **X. Liu:** None. **G. Zhang:** None. **Z. Mao:** None.

Funding: No

Funding Component:

P188

Adipose-Specific Deletion of Angiopoietin-like 4 Protects Against High Fat Diet Induced Dyslipidemia

Authors: Kathryn M Spitler, Emily M Cushing, Shwetha Shetty, Brandon S Davies, Univ of Iowa, Iowa City, IA

Hypertriglyceridemia is a risk factor for the development of hypertension and diabetes. Plasma triglycerides (TGs) are hydrolyzed by lipoprotein lipase (LPL) to release free fatty acids for uptake into tissues. A circulating inhibitor of LPL is angiopoietin-like 4 (A4). A4 is expressed in liver and adipose and is present in circulation. In both humans and mice, A4 deficiency reduces plasma TGs. We previously reported that A4 deficient mice clear more TGs from plasma to adipose tissue. However, it is not clear if this effect is mediated by circulating ANGPTL4 or from local, adipose-derived A4. In this study we sought to characterize the effects of adipose- or liver-specific deletion of A4 on lipid homeostasis. Liver-specific or adipose-specific A4 knockout mice (A4-LivKO/AdipoKO) were generated by crossing A4 floxed mice (A4-fl/fl) with albumin-cre mice or adiponectin-cre mice, respectively. At 8 weeks of age, mice were randomized to either a normal chow diet (NCD) or a high fat diet (60% fat/kCal) for 12 weeks. TG levels (mg/dL) were significantly decreased in A4-AdipoKO mice at baseline (90±4) vs. A4-fl/fl (132±5). No differences were seen in A4-LivKO vs. A4-fl/fl mice (132±3 vs 130±4). After 12 weeks on diets, the A4-AdipoKO mice on NCD (92±6) and HFD (73±6) maintained lower plasma TG levels than A4-fl/fl mice (145±12) on NCD. No differences were seen in TG levels between A4-LivKO mice on HFD or NCD compared to A4-fl/fl mice. TG uptake was increased into adipose tissue of HFD fed A4-AdipoKO [(%-injected dose/mg tissue) (epididymal (eWAT) 0.82± 0.2 and brown adipose tissue (BAT) 1.74± 0.6)] compared to floxed controls on HFD (eWAT 0.44± 0.3 and BAT 0.93± 0.9). Liver-specific loss of ANGPTL4 had no differences in TG uptake from floxed controls

on HFD. Overall, we anticipate that identifying tissue-specific contributions will be valuable in informing therapeutic approaches for treating hypertriglyceridemia.

Disclosures: K.M. Spitler: None. **E.M. Cushing:** None. **S. Shetty:** None. **B.S. Davies:** None.

Funding: No

Funding Component:

P189

The Prorenin Receptor and its Soluble From Stimulate Hepatic Triglyceride and Cholesterol Metabolism in Male Mice

Authors: Eva Gatineau, Frédérique Yiannikouris, Univ of Kentucky, Lexington, KY

Non-alcoholic fatty liver disease is strongly associated with obesity and metabolic syndrome. Our laboratory previously demonstrated that mice lacking adipose prorenin receptor (PRR) have elevated hepatic triglycerides and cholesterol contents. In addition, lack of adipose PRR induced a counter regulatory increase of hepatic PRR and plasma soluble PRR (sPRR). Therefore, our study aimed to determine whether PRR and sPRR stimulated endogenous hepatic triglycerides and cholesterol synthesis.
PRR floxed male mice fed a standard diet were injected with a single dose of an adeno-associated virus thyroxine-binding globulin Cre (KO, n=5) or saline (CTL, n=6). Body weight were measured weekly for 3 weeks. The deletion of PRR in liver did not change body weight curves. Hepatic levels of TG were significantly decreased in liver PRR KO mice compared with control mice (CTL, 615 ± 196 mg/g prot; KO, 114 ± 18 mg/g prot; P=0.047). PRR KO decreased significantly PPARγ gene expression suggesting that PRR positively regulates TG contents through PPARγ pathway. Surprisingly, similar to adipose PRR KO, liver PRR KO increased significantly plasma sPRR (CTL, 5942 ± 199 pg/ml; KO, 8903 ± 559 pg/ml; P=0.001) and hepatic total cholesterol levels (CTL, 40 ± 3 mg/g prot; KO, 102 ± 6 mg/g prot; P<0.0001). This elevation was associated with an increase in SREBP-2 and HMGCoA-reductase gene expression. To determine whether sPRR stimulated cholesterol synthesis, HepG2 cells were treated with sPRR and sPRR was infused in C57BL/6J mice (n=7 to 8 mice/group). Results showed that sPRR significantly up-regulated hepatic SREPB2 mRNA expression in HepG2 cells (Veh, 1.00 ± 0.04; sPRR, 1.36 ± 0.08; P=0.009) and in the liver of mice (Veh, 1.01 ± 0.07; sPRR, 1.23 ± 0.07; P=0.04).
In conclusion, our results demonstrated that hepatic PRR positively regulated TG contents through a PPARγ-dependent mechanism and that sPRR stimulated cholesterol synthesis via SREBP-2 pathway. Futures studies will investigate the mechanism by which PRR and sPRR modulate lipid metabolism.

Disclosures: E. Gatineau: None. **F. Yiannikouris:** None.

Funding: Yes

Funding Component: Great Rivers Affiliate (Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)

P190

The Angiotensin AT_{1A} Receptor Couples to G-alpha-i inAgouti-Related Peptide-ExpressingNeurons to Control Resting Metabolic Rate

Authors: Guorui Deng, Sarah A Sapouckey, Charles A Warwick, Yuriy M Usachev, Julien A Sebag, Huxing Cui, Justin L Grobe, Univ of Iowa, Iowa City, IA

We recently demonstrated that leptin stimulates resting metabolic rate (RMR) through a mechanism that requires angiotensin (ANG) type 1A receptors (AT_{1A}) localized to neurons of the arcuate nucleus (ARC) which express Agouti-related peptide (AgRP). Genetic disruption of AT_{1A} in AgRP neurons results in the loss of RMR responses to leptin, high fat diet, and various other stimuli which correlates with the disinhibition of AgRP, neuropeptide Y (NPY), and production enzymes and transporters (GAD1, GAD2, VGAT) for γ-aminobutyric acid (GABA) within the ARC. We hypothesize that AT_{1A} activation in AgRP neurons causes disinhibition of AgRP, NPY, GAD1, GAD2 and VGAT expression and thus increased inhibitory neurotransmission to pre-autonomic nuclei, and we seek to understand the second-messenger network activated by AT_{1A} which mediates transcriptional control of these genes. Intracellular calcium ($[Ca^{2+}]_i$, determined by FURA-2 fluorescence), cyclic AMP (cAMP, by LANCE assay), receptor surface localization (by HiBiT-tagging), and gene expression responses (by qPCR) to ANG were examined in immortalized mouse hypothalamic cells (N43/5 and N39 cells) that express typical markers of AgRP neurons. ANG (0.1 and 1 μM, n = 3) had no effect to modulate $[Ca^{2+}]_i$, but caused a dose-dependent reduction in forskolin-stimulated cAMP (EC_{50} = 43 nM) which could be blocked by losartan (1 μM). ANG increased GTP-bound Gαi (PBS vehicle = 0.57±0.08, ANG 1 μM = 0.94±0.07 ratio vs IgG, $p < 0.05$, n = 3), reduced cell-surface localization of HiBiT-tagged AT_{1A} (PBS vehicle = 0.22±0.02, ANG 0.1 μM = 0.15±0.01, $p < 0.05$, n = 4), and significantly ($p < 0.05$, n = 6) reduced AgRP and GAD1 expression by 43% and 27%, respectively. Lastly, preliminary studies suggest that pretreatment with pertussis toxin (PTX, 100 ng/mL), an inhibitor of Gαi, abrogated AgRP and GAD1 suppression by ANG (0.01 μM). Collectively these findings support the novel concept that within immortalized cells that express markers of AgRP neurons, AT_{1A} couples to Gαi to reduce cAMP, which suppresses AgRP, NPY and GABA. This should disinhibit pre-motor, pre-autonomic circuits within the hypothalamus, which results in increased thermogenic sympathetic nerve activity, ultimately increasing RMR and energy expenditure.

Disclosures: G. Deng: None. **S.A. Sapouckey:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA. **C.A. Warwick:** None. **Y.M. Usachev:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH. **J.A. Sebag:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH. **H. Cui:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH. **J.L. Grobe:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH.

Funding: Yes

Funding Component: National Center

P191

Sexually Dimorphic Metabolic or Lethal Consequences of Disrupting Angiotensinogen in Agouti Related Peptide-Expressing Cells

Authors: Sarah A Sapouckey, Guorui Deng, Nicole A Pearson, Justin L Grobe, Univ of Iowa, Iowa City, IA

Genetic disruption of angiotensin (ANG) type 1A receptors (AT1A) in cells which express Agouti-related peptide (AgRP) abolishes thermogenic sympathetic nerve activity (SNA) and resting metabolic rate (RMR) responses to leptin and high fat diet (HFD). Although it is established that glia secrete angiotensinogen (AGT), *in silico* reanalysis of published single-cell RNAseq datasets describing transcriptomes of cells from mouse hypothalamus (GSE74672) and RNAscope fluorescent *in situ* hybridization independently confirm that AgRP neurons of the hypothalamic arcuate nucleus (ARC) in adult wildtype mice express AGT mRNA. We therefore sought to explore the functional significance of this expression pattern. Why, if AgRP neurons are bathed in AGT in the interstitial space, would they synthesize AGT? Mice were generated which lack AGT specifically in AgRP cells ($AGT^{AgRP\text{-}KO}$ mice) using the Cre-Lox approach (sire: AgRP-Cre^{+}, $AGT^{F/wt}$ x dam: AgRP-Cre^{-}, $AGT^{F/F}$). Of the first 116 offspring, this resulted in expected overall distributions across sex (53% female), Cre (47% Cre^{+}) and AGT (46% $AGT^{F/F}$) genotypes, but an underrepresentation of targeted $AGT^{AgRP\text{-}KO}$ mice at

weaning (n=5 females + 1 male; X^2=52.0, df=3, p<0.001), indicating pre-weaning or *in utero* lethality of the genotype. Before and at 8 weeks of age, female $AGT^{AgRP\text{-}KO}$ mice exhibited normal body mass (n=5, 17.3±0.4 vs n=12 littermates 17.3±0.3 g); 5 weeks of 45% HFD caused significant weight gain in all mice (p<0.05), but $AGT^{AgRP\text{-}KO}$ mice gained less (body mass: 18.3±0.4 vs 19.7±0.3 g, p<0.05, and fat mass by NMR: 1.6±0.4 vs 2.3±0.2 g, p<0.05), which was not due to suppression of food intake (p=NS) or digestive efficiency as assessed by bomb calorimetry (p=NS). We conclude that (i) AGT is expressed by AgRP cells of the ARC, (ii) disruption of AGT in AgRP cells causes a developmental lethality that is more penetrant in males, and (iii) in surviving females, the disruption of AGT in AgRP cells causes resistance to weight gain through increased energy expenditure.

Disclosures: S.A. Sapouckey: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA. **G. Deng:** None. **N.A. Pearson:** None. **J.L. Grobe:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH.

Funding: Yes

Funding Component: National Center

P192

Trpc6 Deficiency Causes Obesity, Impaired Glucose Tolerance and Leptin Resistance

Authors: Zhen Wang, Jussara M. do Carmo, Alexandre A. da Silva, Sydney P. Moak, Kandice C. Bailey, John E. Hall, Univ of Mississippi Medical Ctr, Jackson, MS

Transient receptor potential cation channel subfamily C member 6 (TPRC6) is a receptor-operated cation channel that contributes to changes in cytosolic free Ca^{2+} concentration. TRPCs have been suggested to play a role in the regulation of neurite outgrowth, synapse formation, and neuronal survival. However, the role of TRPC6 in controlling metabolic and cardiovascular functions is still unclear. We therefore investigated the impact of TRPC6 on regulation of energy balance, metabolic and cardiovascular functions and anorexic responses to leptin in male and female TRPC6 knockout mice and control B6/129 mice, including assessment of body weight (BW), food intake (FI), body fat/lean composition, responses to acute leptin injection, glucose tolerance tests (GTTs), and blood pressure (BP) and heart rate (HR). TRPC6 null mice of both sexes were heavier than control mice from 6 to 16 weeks of age when fed a standard diet (39.5 ± 1.5 vs 31.1 ± 1.2 g in males and 31.1 ± 1.2 vs 22.9 ± 1.0 in females at 16 weeks of age, n=7, p<0.05). EchoMRI scans showed that the higher body weight in TRPC6 null mice was mainly due to increased body fat compared to controls (30.0 % vs 22.6 % of fat mass/BW in males and 32.7 % vs 21.0 % in females at 16 weeks of age), and was associated with increased daily FI (3.4 ± 0.1 vs 2.7 ± 0.2 g in males and 2.7 ± 0.1 vs 2.0 ± 0.1 in females at 16 weeks of age, n=7, p<0.05). Acute leptin injections (5 mg/kg, i.p. at 18 weeks of age, n=7) significantly reduced 24-hr FI by 41 % and 32 % in male and female control mice, while only an 8 % and 18 % reduction in FI were observed in male and female TRPC6 null mice. At 20 weeks of age, male and female TRPC6 null mice also exhibited impaired glucose tolerance during GTT compared to controls (AUC: 16,138 ± 1,877 vs 8,016 ± 1,476 mg/dL x 120 min in males, and 6,152 ± 1,173 vs 1,975 ± 512 mg/dL x 120 min in females, n=7, p<0.05). BP and HR measured by telemetry for 5 consecutive days at 22 weeks of age were similar between groups. Our results indicate that TRPC6 plays an important role in normal control of food intake, body weight and glucose homeostasis as well as for leptin's anorexic action. Although TRPC6 deficiency caused obesity and metabolic abnormalities, BP and HR did not increase.

Disclosures: Z. Wang: None. **J.M. do Carmo:** None. **A.A. da Silva:** None. **S.P. Moak:** None. **K.C. Bailey:** None. **J.E. Hall:** None.

Funding: No

Funding Component:

P193

Angiotensin-(1-7) Acutely Induces Browning of White Adipose Tissue in Mice

Authors: Amanda J Miller, Melissa White, Sarah S Bingaman, Amy C Arnold, Pennsylvania State Univ Colle, Hershey, PA

Angiotensin (Ang)-(1-7) is a beneficial hormone of the renin-angiotensin system that is emerging as a promising target for obesity. We previously showed that chronic Ang-(1-7) treatment attenuates high fat diet (HFD)-induced weight gain in mice by increasing markers of thermogenesis in subcutaneous white adipose tissue ("browning") to increase energy expenditure. In this study, we tested the hypothesis that Ang-(1-7) could acutely increase adipose thermogenesis in mice. To test this, adult male C57BL/6J mice were placed on a chow diet or 60% HFD for 12 weeks and then received a single subcutaneous injection of Ang-(1-7) (2mg/kg) or saline. This study included 4 groups of mice: chow+saline (n=9), chow+Ang-(1-7) (n=15), HFD+saline (n=8), HFD+Ang-(1-7) (n=10). Core temperature was measured at baseline and at 6 hours after injection. Subcutaneous white and brown adipose tissues were collected 6 hours post-injection. Gene expression of the thermogenic marker uncoupling protein 1 (UCP1) was measured in tissues by quantitative real-time PCR and quantified with 2-ΔΔCT methods. Acutely, Ang-(1-7) did not alter body mass in chow or HFD mice (P=0.992). There were 5 chow fed mice (33%) and 2 HFD mice (20%) that did not respond to Ang-(1-7) injection. In the chow diet responders, Ang-(1-7) increased core temperature (36.7±0.2 vs 35.9±0.2°C saline; P=0.012) and UCP1 gene expression in subcutaneous white adipose tissue (14.3±5.8 vs 1.9±0.6 saline; P=0.050), with no effect in brown adipose tissue (1.7±0.6 vs 1.0±0.2 saline, P=0.284). In the HFD responders, core temperature was not altered by Ang-(1-7) (37.8±0.1 vs 37.8±0.1 saline, P=0.689). There was a trend towards decreased UCP1 expression in white adipose of HFD-saline mice compared to chow-saline mice (P=0.196). Ang-(1-7) significantly increased UCP1 expression in subcutaneous white adipose of HFD responders (6.2±3.1 vs 0.9±0.2 saline, P=0.036), with no effect in brown adipose tissue (1.2±0.1 vs saline: 1.7±0.4 saline, P=0.402). These data provide further evidence that targeting Ang-(1-7) may be a promising strategy to increase white adipose thermogenesis, an effect that could serve to enhance energy expenditure and restore energy balance in obesity.

Disclosures: A.J. Miller: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; AHA 18POST33960087. **M. White:** None. **S.S. Bingaman:** None. **A.C. Arnold:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; HL122507.

Funding: Yes

Funding Component: National Center

P194

Body Silhouette Trajectories Across the Lifespan and Vascular Aging: The Paris Prospective Study 3

Authors: Thomas van Sloten, Pierre Boutouyrie, Quentin Lisan, Muriel Tafflet, Inserm U970, Paris, France; Frédérique Thomas, Preventive and Clinical Investigation Ctr, Paris, France; Catherine Guibout, Rachel Climie, Bruno Pannier, Inserm U970, Paris, France; James Sharman, Menzies Inst for Medical Res, Hobort, Australia; Stéphane Laurent, Xavier Jouven, Jean-Philippe Empana, Inserm U970, Paris, France

Background
Vascular aging, i.e. accumulation of functional and structural changes of vessels throughout life, is a major contributor to cardiovascular disease. It can be quantified by higher carotid stiffness, intima-media thickness and diameter, and hypertension. Weight gain across the lifetime may be an important, modifiable determinant of vascular aging.
Aim

To assess lifetime body silhouette trajectories (a marker of weight change across the lifespan) in relation to vascular aging in late adulthood.

Methods

We used cross-sectional data from a community-based study (n=8,083; age 59.4; 38.6% women). A linear mixed model was used to assess trajectories of recalled body silhouettes from age 8 to 45. We assessed carotid stiffness, IMT and diameter (ultrasonography), resting hypertension (blood pressure ≥140/90 mmHg or use of antihypertensives), and exaggerated exercise blood pressure, a marker of masked hypertension (systolic blood pressure ≥150 mmHg during submaximal exercise).

Results

We identified 5 trajectories: lean-stable (32%), lean-increase (11%), moderate-stable (33%), lean-marked increase (16%) and heavy-stable (8%). Compared to those in the lean-stable trajectory, individuals in the moderate-stable, lean-marked increase and heavy-stable trajectories had more evidence of vascular aging (Figure).

Conclusions

Vascular aging was most prominent among individuals who were lean in early life but gained weight during young adulthood, and among those who were heavy in early life and maintained weight. This suggests that prevention of weight gain across the life course, especially during young adulthood, is important to promote healthy vascular aging.

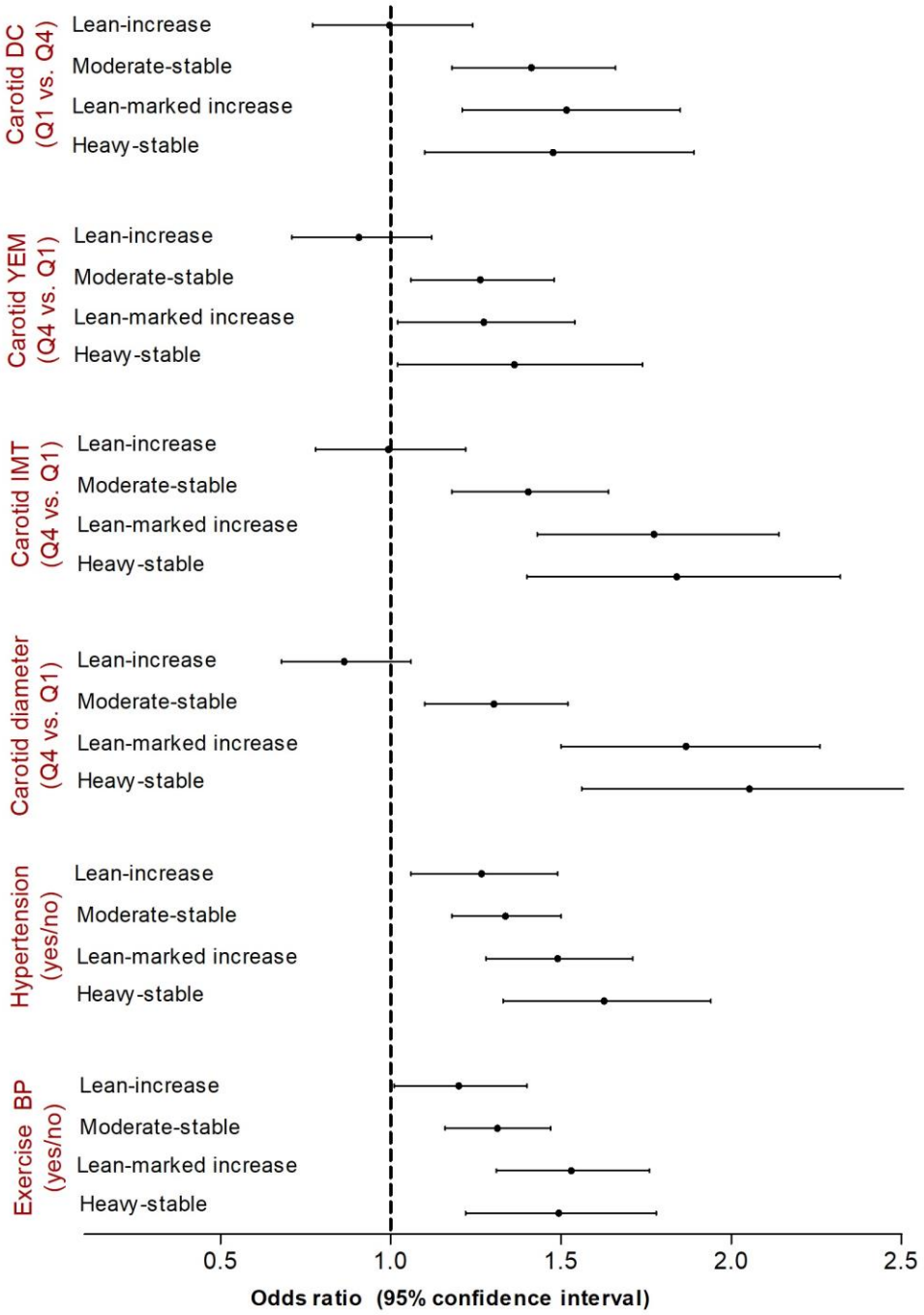


Figure. Associations between body silhouette trajectories and manifestations of vascular aging. Results adjusted for age, sex, education, and lifestyle and cardiovascular risk factors. DC=distensibility coefficient, YEM=Young's elastic modulus, IMT=intima-media thickness, BP=blood pressure

Disclosures: T. van Sloten: None. **P. Boutouyrie:** None. **Q. Lisan:** None. **M. Tafflet:** None. **F. Thomas:** None. **C. Guibout:** None. **R. Climie:** None. **B. Pannier:** None. **J. Sharman:** None. **S. Laurent:** None. **X. Jouven:** None. **J. Empana:** None.

Funding: No

Funding Component:

P195

Blood Presure is Related to Glucose Fluctuation in Longstanding Type 1 Diabetes

Authors: Anna Shalimova, Beata Graff, Anna Szyndler, Jacek Wolf, Magdalena Blaszkowska, Elzbieta Orlowska-Kunikowska, Bogumil Wolnik, Krzysztof Narkiewicz, Medical Univ of Gdansk, Gdansk, Poland

Mechanisms underlying relationship between hypertension and metabolic abnormalities are well established in type 2 diabetes. Much less is known about this link in type 1 diabetes. None of the previous studies has assessed relationship between glucose variability and diurnal blood pressure profile in patients with longstanding type 1 diabetes (DM1).
The aim: to investigate the possible association of glucose fluctuation with BP levels in longstanding DM1.
Design and methods: We examined 36 patients with longstanding (>20 years) history of DM1 (without overt cardiovascular disease, including hypertension) and episodes of hyperglycemia >160 mg/dL during 24-hour continuous glucose monitoring (CGM). In all patients simultaneous 24-hour CGM and ambulatory blood pressure monitoring (ABPM) were performed. Intima-media thickness (IMT) of the common carotid artery was also assessed. Patients were divided into two groups: with and without severe hypoglycemia <50 mg/dL (n=18 in each group).
Results: Compared to patients with hypoglycemia, patients without hypoglycemia had a significantly lower day-time SBP variability expressed as standard deviation (12.6 ± 2.5 and 10.9 ± 3.0 mmHg, respectively, $p<0.05$). In patients without hypoglycemia, mean amplitude of glycemic excursion both up and down was associated with increase in DBP ($r=0.49$, $p<0.05$ and $r=0.59$, $p<0.05$, respectively), whereas in patients with hypoglycemia it was associated with increase in SBP ($r=0.53$, $p<0.05$). In patients without hypoglycemia, time of hyperglycemia was associated with increase in DBP ($r=0.57$, $p<0.05$) and in patient with hypoglycemia – with increase in SBP ($r=0.67$, $p<0.05$). Furthermore, in patients with short hypoglycemic episodes, time of hypoglycemia was associated with increase in SBP ($r=0.57$, $p<0.05$). In patients with hypoglycemia, SBP level had also positive correlation with IMT ($r=0.50$, $p<0.05$).
Conclusions: In patients with longstanding DM1, BP is related to glucose fluctuation. While the change in glucose levels towards hypoglycemia is associated with an increase in SBP, hyperglycemia is linked to an increase in DBP. The mechanisms underlying these associations remain to be determined.

Disclosures: A. Shalimova: None. **B. Graff:** None. **A. Szyndler:** None. **J. Wolf:** None. **M. Blaszkowska:** None. **E. Orlowska-Kunikowska:** None. **B. Wolnik:** None. **K. Narkiewicz:** None.

Funding: No

Funding Component:

P196

Fenofibrate Improves Vascular Contractility and Endothelial Function in Diabetic Mice

Authors: Nan Xu, Qin Wang, Zhejiang Univ, HangZhou, China; Qi J Wang, Affiliated Women's Hosp of Zhejiang Univ, HangZhou, China; Shan Jiang, Su H Zhou, Liang Zhao, En Y Lai, Zhejiang Univ, HangZhou, China

Aim: To exam the hypothesis that fenofibrate improves vascular endothelial dysfunction via balancing endothelium-dependent relaxation and constriction of aorta in diabetic mice. **Methods:** The streptozotocin-induced diabetic mice were treated with fenofibrate (100 mg/Kg/d, 8 weeks). The responses were assessed from serum and aortic parameters, vascular reactivity by wire myograph and aortic protein expression of endothelial nitric oxide synthase (eNOS), peroxisome proliferator-activated receptor α (PPARα), AMP-activated protein kinase-α (AMPKα) and liver kinase B1 (LKB1) and cyclooxygenase-2 (COX-2) in control and diabetic mice given placebo or fenofibrate. **Results:** Comparing with the control mice (12.2±2.0 μmol/L), serum creatinine increased by 114.8% in diabetic mice (26.2±2.0 μmol/L) and by 66.2% in fenofibrate-treated diabetic mice (21.6±2.8 μmol/L, $p<0.01$). NO level in aorta was 49.5±8.2 (μmol/g) in diabetic mice and 71.7±5.2 (μmol/g) in fenofibrate-treated diabetic mice ($p<0.01$). Compared with placebo-treated

diabetic group, phosphorylated-eNOS protein in aorta was 3.6±1.0 fold higher ($p<0.01$) and endothelium-dependent relaxation was increased in fenofibrate-treated diabetic mice ($p<0.01$). The aorta vasodilation by fenofibrate treatment was reversed with PPARα inhibitor or AMPKα inhibitor. Fenofibrate treatment elevated PPARα expression, subsequently induced LKB1 translocation from nucleus to cytoplasm to activate AMPKα, thus activated eNOS in diabetic aorta ($p<0.01$). Fenofibrate treatment reduced vascular contractility and expression of COX-2 in aorta from diabetic mice($p<0.01$). Prostaglandin E2 and thromboxane A2 were 174.9±16.4 and 128.9±4.4 (pg/ml) in diabetic mice, and reduced to 142.7±14.1 and 105.9±5.7 (pg/ml) in fenofibrate-treated mice ($p<0.01$). **Conclusion:** Fenofibrate treatment in diabetic mice normalizes endothelial function by balancing vascular reactivity via increasing NO production and suppressing vasoconstrictor prostaglandin. Our results suggest that implication of fenofibrate as a putative mediator of diabetic vascular complications.

Disclosures: N. Xu: None. **Q. Wang:** None. **Q.J. Wang:** None. **S. Jiang:** None. **S.H. Zhou:** None. **L. Zhao:** None. **E.Y. Lai:** None.

Funding: No

Funding Component:

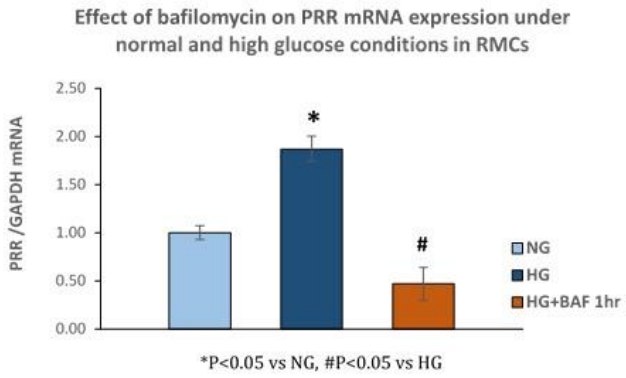
P197

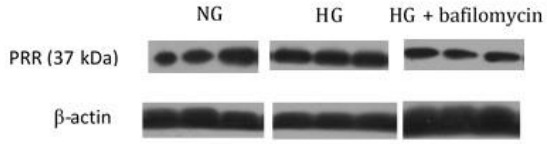
Vacuolar-type H^+-ATPase Enhances Renal (Pro)renin Expression and Inflammation in Response to High Glucose

Authors: Safia Akhtar, Rebecca Mc Devitt, Silas A Culver, Caixia Li, Helmy M Siragy, Dept of Endocrinology, Sch of Med, Univ of Virginia, Charlottesville, VA

(Pro)renin receptor (PRR) is an accessory subunit for vacuolar-type H-ATPase (V-ATPase). Recently, we demonstrated that PRR expression is upregulated in diabetes and promotes renal inflammation. We hypothesized that V-ATPase enhances renal PRR expression and inflammation in response to high glucose. Rat renal mesangial cells (RMCs) were cultured for 3 days in medium containing 25 mM D-glucose (high glucose) for experiments groups and 5 mM D-glucose plus 20 mM L-glucose (normal glucose) for control group. Cells were serum starved for 6 hrs at the end of the 3 day glucose exposure, thereafter 100nM bafilomycin, a V-ATPase inhibitor, treatment was given for 1 hour. At the end of experiments, cells were harvested for total RNA and protein for the determination of PRR and interleukin-6 (IL-6). High glucose significantly ($P<0.05$) increased mRNA and protein expression of PRR (Fig 1) by 87 (1 vs 1.87) and 52% (1 vs 1.52) respectively, and IL-6 level (Fig 2) by 44% (1 vs 1.44, $P<0.05$), when compared to their respective normal glucose cells. Bafilomycin treatment attenuated the increase in mRNA and protein expression of PRR by 74 (1.87 vs 0.47, $P<0.05$) and 45% (1.52 vs 0.83, $P<0.05$). Similarly, IL-6 mRNA level was also lower by 62% (1.44 vs 0.54, $P<0.05$) in bafilomycin treated high glucose cells compared to its non-treated counterpart. The present study conclude that V-ATPase contributes to increased renal PRR expression and inflammation in presence of hyperglycemia.

FIG 1





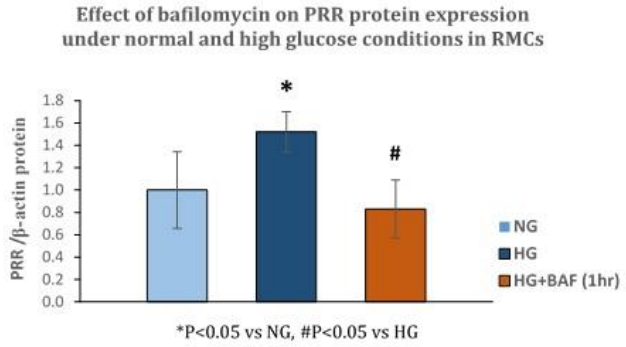
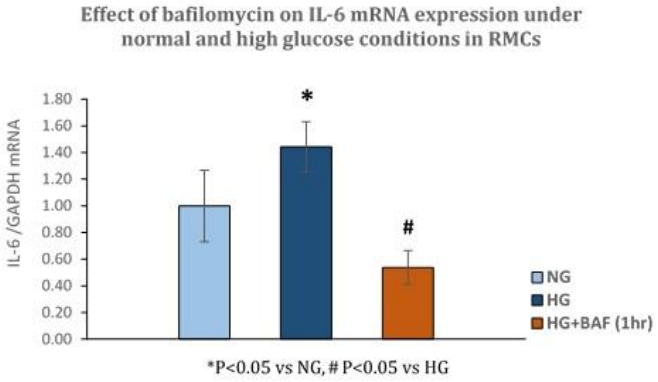


FIG 2



Disclosures: **S. Akhtar:** None. **R.M. Devitt:** None. **S.A. Culver:** None. **C. Li:** None. **H.M. Siragy:** None.

Funding: No

Funding Component:

P198

WNK-SPAK-NKCC1 Cascade Activation Contributes to Worsened Brain Damage in Mice With Hypertension Co-Morbidity after Ischemic Stroke

Authors: Mohammad Iqbal H Bhuiyan, Huachen Huang, Univ of Pittsburgh, Pittsburgh, PA; Ting Zhang, Xiamen Univ, Xiamen, Fujian, China; Bradley J Molyneaux, Samuel M Poloyac, Univ of Pittsburgh, Pittsburgh, PA; Jinwei Zhang, Univ of Exeter Medical Sch, Exeter, United Kingdom; Xianming Deng, Xiamen Univ, Xiamen, Fujian, China; Dandan Sun, Univ of Pittsburgh, Pittsburgh, PA; Dandan Sun, Veterans Affairs Pittsburgh Health Care System, Pittsburgh, PA

Objectives: The WNK-SPAK/OSR1 kinase complex plays an important role in renal salt handling and pathogenesis of hypertension by regulating ion transporters and channels. Hypertension is the most common risk factor for stroke and stroke patients with hypertension comorbidity have worsened outcome with an increased risk of dependency or death. However, the mechanisms underlying the worsened ischemic stroke pathophysiology with hypertension comorbidity remain poorly defined. In this study, we investigated roles of the WNK-SPAK-NKCC1 signaling pathway in ischemic brain damage in mice with hypertension comorbidity. **Methods:** Hypertension was induced in C57BL/6j male mouse (12-15 weeks) by subcutaneous infusion of 1000 ng/kg/min angiotensin II (AngII, mini-osmotic pump) for two weeks. Permanent ischemic stroke was induced by permanent occlusion of the distal branches of the left middle cerebral artery (pd-MCAO). Brain tissues were harvested for immunoblot assessment of expression levels of NKCC1, SPAK/OSR1 or WNK1-4. Infarct volume and hemisphere swelling were determined by TTC staining, and behavioral deficits were analyzed by foot fault test, cylinder test and adhesive tape removal test. **Results:** pd-MCAO stimulated expression of WNK proteins (isoforms 1, 2, 4), total and phosphorylated SPAK/OSR1 and NKCC1 proteins in ischemic brains of the AngII-infused hypertensive mice compared to normotensive saline controls. In parallel with the increased activation of WNK-SPAK-NKCC1 signaling, hypertensive mice displayed significantly larger infarct volume and hemispheric swelling at 24 h after pd-MCAO compared to normotensive controls. Moreover, hypertensive mice exhibited a slow recovery of neurological function after ischemic stroke compared to normotensive counterparts as assessed by sensory-motor sensitive tests. **Conclusions:** These results suggest that activation of the WNK-SPAK-NKCC1 complex in hypertensive ischemic brains associates, at least in part, with the worsened brain damage and neurological deficits. Pharmacological inhibition of WNK-SPAK complex has therapeutic potentials for stroke therapy with hypertension comorbidity.

Disclosures: M.H. Bhuiyan: None. **H. Huang:** None. **T. Zhang:** None. **B.J. Molyneaux:** None. **S.M. Poloyac:** None. **J. Zhang:** None. **X. Deng:** None. **D. Sun:** None. **D. Sun:** None.

Funding: No

Funding Component:

P199

The Associations Between Blood Pressure Parameters and Stroke in Chinese Rural Population: The Henan Rural Cohort Study

Authors: Xia Zhang, Xiaotian Liu, Coll of Public Health, Zhengzhou Univ, Zhengzhou, China; Yuqian Li, Sch of Pharmaceutical Science, Zhengzhou Univ, Zhengzhou, China; Xinyu Zhao, The First Affiliated Hosp, Zhengzhou Univ, Zhengzhou, China; Min Liu, Henan Provincial People's Hosp, Zhengzhou, China; Runqi Tu, Xinling Qian, Haiqing Zhang, Wen Zhou, Zhongyan Tian, Dou Qiao, Linlin Li, Zhenxing Mao, Gongyuan Zhang, **Wang Chongjian**, Coll of Public Health, Zhengzhou Univ, Zhengzhou, China

Aim: This study aimed to verify the associations of blood pressure (BP) parameters with the risk of stroke in Chinese rural population. Simultaneously, we quantified how much of the effects of BP on stroke were mediated through metabolic factors. **Methods:** A total of 38880 subjects aged 18 to 79 were derived from the Henan Rural Cohort study. Restricted cubic splines and logistic regression were used to estimate the *OR*s and 95% *CI*s. We used factor analysis to create continuous variables of metabolic dysfunctions to be examined as potential mediator of the association between

BP and stroke. Mediation analysis using bootstrap was performed to examine the contribution of metabolic factors to BP parameters related stroke. **Results:** After adjusting for potential confounders, the *OR*s (95% *CI*s) of SBP, DBP, PP and MAP in the highest quartile with the risk of stroke were 1.96 (1.70-2.27), 1.80 1.58-2.05), 1.66 (1.42-1.94), 1.95 (1.70-2.24). The restricted cubic splines showed that the risk of stroke increased gradually with continuous BP levels. We further reported that 11.45%-20.98% of excess relative risk of stroke was mediated through metabolic factors for BP parameters. **Conclusion:** Elevated BP were significantly associated with the risk of stroke especially in SBP and MAP. In addition, metabolic factors explained part of the associations.

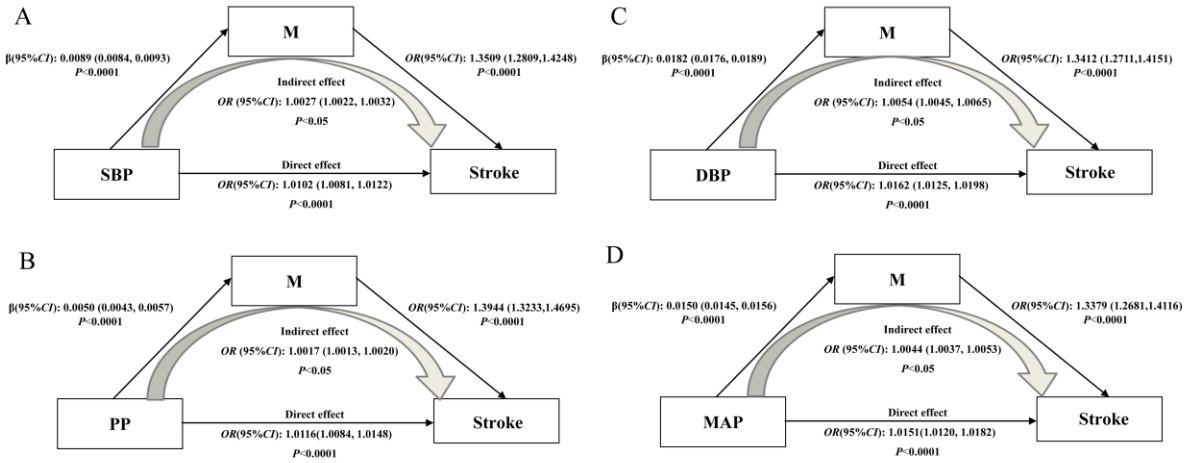


Figure 3 Mediation analysis to determine the relationship between SBP (A), DBP (B), PP (C), MAP (D) and stroke through metabolic factors.
M, metabolic factors.OR(95% CI)of total effect for SBP, DBP, PP and MAP on total stroke was 1.0122 (1.0102-1.0142), 1.0205 (1.0169-1.0240), 1.0131 (1.0099-1.0163) and 1.0185 (1.0155-1.0214), respectively.

Disclosures: X. Zhang: None. **X. Liu:** None. **Y. Li:** None. **X. Zhao:** None. **M. Liu:** None. **R. Tu:** None. **X. Qian:** None. **H. Zhang:** None. **W. Zhou:** None. **Z. Tian:** None. **D. Qiao:** None. **L. Li:** None. **Z. Mao:** None. **G. Zhang:** None. **W. Chongjian:** None.

Funding: No

Funding Component:

P200

Night Sleep Duration and Hypertension in Chinese Rural Population: The Henan Rural Cohort Study

Authors: Haiqing Zhang, Coll of Public Health, Zhengzhou Univ, Zhengzhou, China; Yuqian Li, Sch of Pharmaceutical Science, Zhengzhou Univ, Zhengzhou, China; Zhenxing Mao, Coll of Public Health, Zhengzhou Univ, Zhengzhou, China; Min Liu, Henan Provincial People's Hosp, Zhengzhou, China; Wenqian Huo, Ruihua Liu, Xiaotian Liu, Runqi Tu, Kaili Yang, Xinling Qian, Jingjing Jiang, Xia Zhang, Zhongyan Tian, Ronghai Bie, **Chongjian Wang**, Coll of Public Health, Zhengzhou Univ, Zhengzhou, China

Aim: The purpose of the study was to evaluate the association between night sleep duration and hypertension, and to examine whether the mediation effect of night sleep duration and hypertension exists in rural population based on the factors of blood lipids.
Methods: Night sleep duration was classified into <5h, 5-h, 6-h, 7-h, 8-h, 9-h and ≥10h. Logistic regression and restricted cubic spline were performed to evaluate the association of night sleep duration with hypertension. Mediation analysis was used to explore the mediation effect of night sleep duration and hypertension.

Results: Among the 37, 317 participants, a total of 12, 333 suffered from hypertension. Compared with reference, fully-adjusted *OR* of hypertension, undiagnosed and diagnosed hypertension were 1.52(1.25-1.84), 1.68(1.32-2.14) and 1.32(1.03-1.69) respectively among male sleeping for 10h at night. Additionally, the dose-response relationship of night sleep duration with prevalent hypertension risk was also found in male (Figure 1, *P*<0.05). Mediation analyses showed that the *OR* for hypertension among male (total effect, 1.089; *95% CI*, 1.040, 1.139). Low density lipoprotein cholesterol (LDL-C) showing the *OR*, 1.003(95% *CI*, 1.001, 1.005) for indirect effect and 1.085(1.038, 1.137) for direct effect partly mediated their association.

Conclusion: A dose-response association of night sleep duration and hypertension was found in male but not in female. Additionally, LDL-C mediated the association between night sleep duration and hypertension among male.

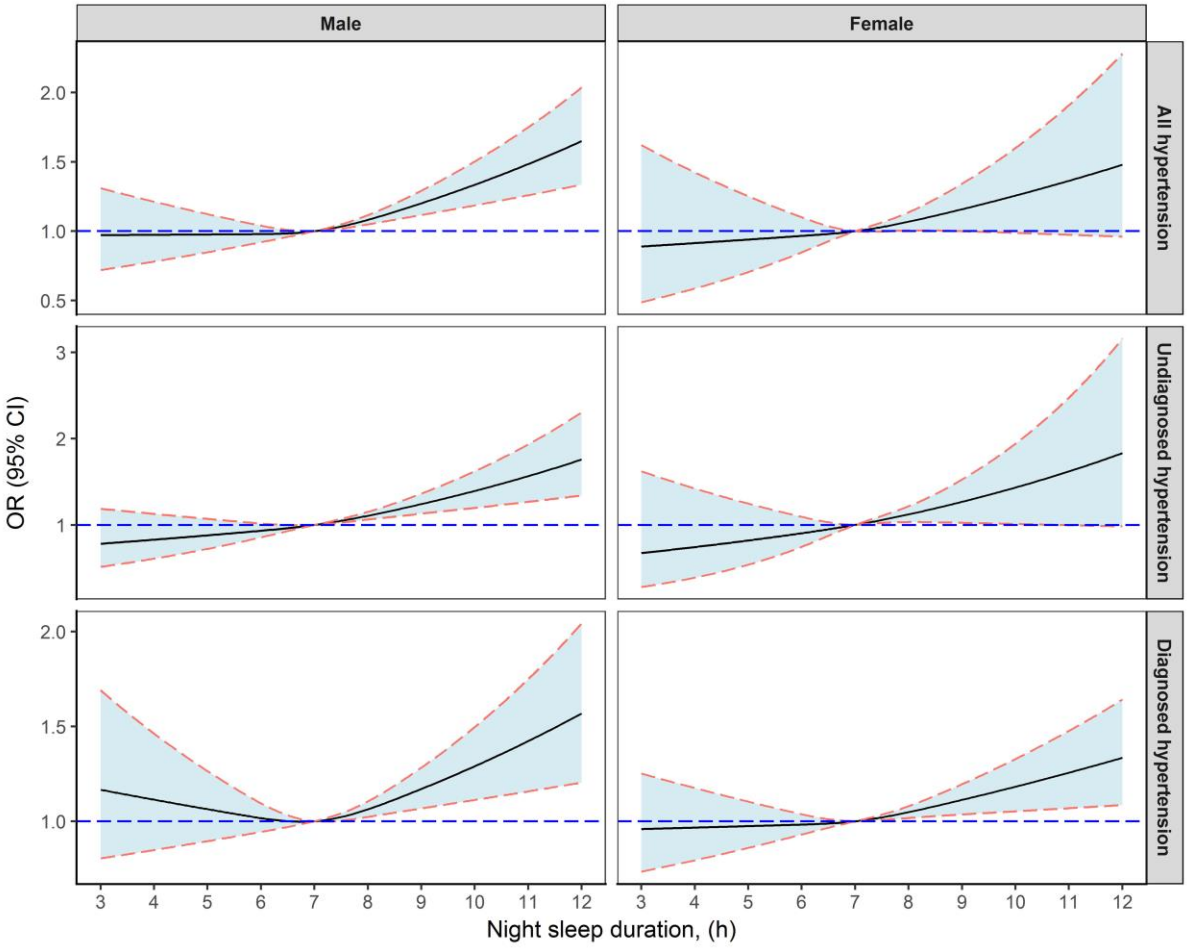


Figure 1. Relation of night sleep duration for hypertension from restricted cubic splines.

Disclosures: **H. Zhang:** None. **Y. Li:** None. **Z. Mao:** None. **M. Liu:** None. **W. Huo:** None. **R. Liu:** None. **X. Liu:** None. **R. Tu:** None. **K. Yang:** None. **X. Qian:** None. **J. Jiang:** None. **X. Zhang:** None. **Z. Tian:** None. **R. Bie:** None. **C. Wang:** None.

Funding: No

Funding Component:

P201

Exposure to Stress and Low Salt Diet Increases Blood Pressure in Mice Lacking the Brain-Specific Renin Isoform Renin-b

Authors: Pablo Nakagawa, Javier A Gomez, Fernando De Azevedo Cruz Seara, Curt D Sigmund, Univ of Iowa, Iowa City, IA

Previous experience to various hypertensive conditions including psychosocial stress and high or low salt diet enhances the response to subsequent hypertensive challenges. Importantly, the activation of the brain renin angiotensin system (RAS) is required for the sensitization to these hypertensive challenges. We previously reported that the brain-specific renin isoform (Ren-b) tonically inhibits the activation of the brain RAS and the ablation of Ren-b results in brain RAS disinhibition leading to hypertension. Interestingly, we observed a high degree of variability in the blood pressure (BP) between Ren-b KO cohorts that can be attributed to different levels of environmental stress. Thus, BP and heart rate (HR) from five separate experimental cohorts which were subjected to different levels of stress were re-analyzed and compared. In cohorts that were not subjected to any surgical procedures, Ren-b KO mice housed in a stressful environment exhibited higher systolic BP (cohort 1, KO: 130 ± 1 vs WT: 122 ± 1 mmHg, $p<0.05$) in comparison with animals housed in a quiet and more regulated environment (cohort 2; KO: 116 ± 1 mmHg vs WT: 116 ± 1). BP was selectively higher in Ren-b KO mice subjected to a surgical procedure required to study a BP mechanism (Cohort 3, KO: 137 ± 2 vs WT: 117 ± 3, $p<0.05$; Cohort 4, KO: 139 ± 2 vs WT: 112 ± 6, $p<0.05$; Cohort 5, KO: 137 ± 3 vs WT: 113 ± 3 mmHg, $p<0.05$). Similarly, elevation of BP was associated with increased HR. Altogether this reanalysis of pre-existing data suggests that Ren-b KO mice might be sensitive to environmental or surgical stressors. Next, we hypothesized that Ren-b KO mice are sensitized to high salt diet, thus BP was acquired in WT or Ren-b KO fed low or high salt diet (0.3% and 4.0% NaCl, respectively) for a week in a quiet environment. On high salt diet, Ren-b KO did not manifest an increase in mean BP (WT: 113±2 vs KO: 109±2 mmHg, $p=0.18$), indicating that Ren-b KO are not salt-sensitive. In contrast, on low salt diet Ren-b KO exhibited modest increase in mean BP compared to WT (WT: 108±2 vs KO: 113±1 mmHg, $p<0.05$) indicating that Ren-b KO might be sensitive to conditions that exacerbate activation of peripheral RAS. We conclude that disinhibition of brain RAS by the ablation of Ren-b leads to exaggerated response to hypertensive challenges.

Disclosures: P. Nakagawa: None. **J.A. Gomez:** None. **F. De Azevedo Cruz Seara:** None. **C.D. Sigmund:** None.

Funding: Yes

Funding Component: National Center

P202

Targeting the Cardiac Sympathetic Afferent Reflex in Hypertrophic Cardiomyopathy

Authors: Robert A Larson, Yongjun Lu, Christopher J Benson, Mark W Chapleau, Univ of Iowa, Iowa City, IA

Sarcomere gene mutations cause hypertrophic cardiomyopathy (HCM). Hallmark features include cardiac hypertrophy, fibrosis, excessive cardiac sympathetic tone, and increased risk of arrhythmias and sudden death. The mechanism(s) of increased sympathetic tone is poorly understood. We hypothesized that cardiac spinal (sympathetic) afferents are sensitized by metabolites (e.g., H+) produced during myocyte energy depletion in HCM, resulting in an enhanced cardiac sympathetic afferent reflex (CSAR). We studied mice with cardiac-specific expression of mutated alpha-tropomyosin (Glu180Gly), an established model of HCM. The CSAR was assessed by measuring mean arterial pressure (MAP) and heart rate (HR) responses to epicardial application of the TRPV1 agonist capsaicin (2.5mM, 2μL) to the left ventricle in anesthetized, ventilated HCM (n=5) and wild-type (WT) littermate control (n=5) mice. Pressor and tachycardic responses to capsaicin were markedly enhanced in HCM mice (Table). Blocking the tonic influence of cardiac afferent activity by epicardial application of the local anesthetic lidocaine (2%) caused small, significant decreases in MAP in both groups of

mice; but decreased HR markedly in HCM mice only (n=5) (Table). mRNA expression (qPCR) of acid-sensing ion channel 3 was increased by 2-fold in dorsal root ganglia (T1-T9) of HCM vs. WT mice (n=6 each, P<0.05), suggesting a mechanism for sensitization of cardiac sympathetic afferents in HCM. We conclude that the CSAR is enhanced in HCM mice and speculate that increased activity of cardiac 'sympathetic' afferent nerves contributes to increased sympathetic tone, arrhythmias and disease progression in HCM.

	∆MAP (mmHg)		∆HR (bpm)	
* P<0.05 vs. WT	WT	HCM	WT	HCM
Capsaicin	+9 ± 3	+19 ± 2*	+8 ± 2	+33 ± 10*
Lidocaine	–5 ± 0	–5 ± 1	+2 ± 1	–42 ± 5*

Disclosures: **R.A. Larson:** None. **Y. Lu:** None. **C.J. Benson:** None. **M.W. Chapleau:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH HL14388.

Funding: No

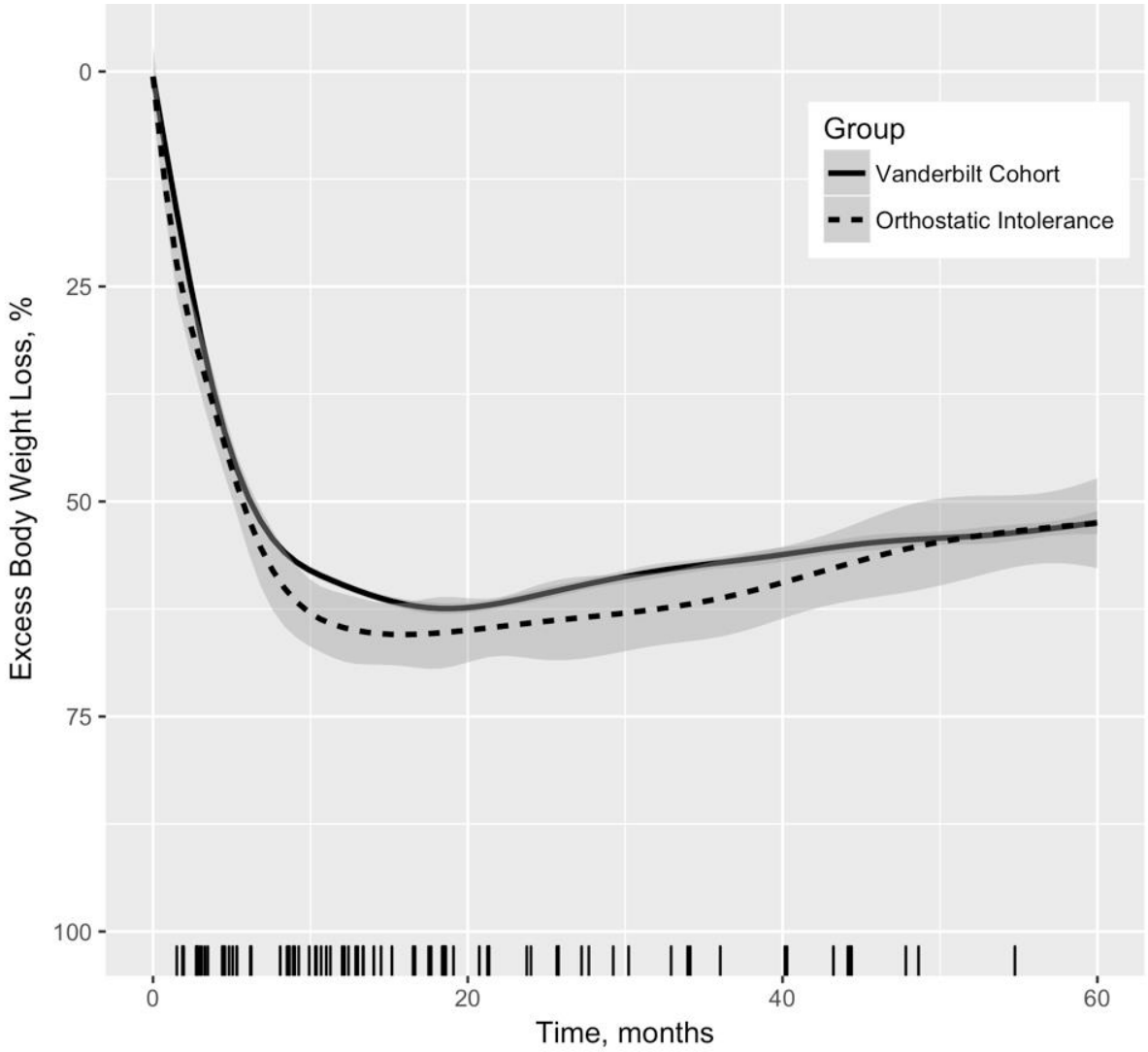
Funding Component:

P203

Cumulative Incidence of Orthostatic Intolerance After Bariatric Surgery

Authors: James B Zhang, Robyn A Tamboli, Vance L Albaugh, Donna M Kilkelly, Carlos G Grijalva, David B Williams, Cyndya A Shibao, Vanderbilt Univ Medical Ctr, Nashville, TN

Several retrospective studies describe an orthostatic intolerance (OI) syndrome after bariatric surgery. However, its incidence remains unknown. Using a de-identified registry of 4547 bariatric surgery patients, we identified cases of OI syndrome via structured chart reviews for all subjects who reported new-onset post-operative symptoms of OI. Cases of OI were confirmed using an operational case definition developed by the Vanderbilt Autonomic Dysfunction Center, and available autonomic function test results were examined. The cumulative incidence of post-bariatric surgery OI syndrome was estimated using a life table. Of 741 patients reporting new OI symptoms after surgery, we confirmed the presence of post-bariatric surgery OI syndrome in 85 patients (1.9% of 4547), 14 with severe OI requiring pressor agents. At 5 years post-surgery, follow-up was reduced to 15% (682 of 4547), and the estimated cumulative incidence of OI syndrome adjusted for losses to follow-up was 4.2%. Most OI cases developed during relatively weight-stable months, and weight change was not significantly different between patients with and without OI syndrome (Figure 1). Of OI cases with available autonomic function test data, 52% (11 of 21) showed evidence of impaired sympathetic vasoconstrictor activity. In conclusion, new onset orthostatic intolerance is relatively frequent in the bariatric surgery population. Some patients with OI exhibit evidence of impaired autonomic function.
Figure 1. Excess Weight Loss for All Bariatric Operations (solid) vs. Patients with OI Syndrome (dotted). Shaded Areas Represent 95% Confidence Interval. Rug Plot on X-axis Indicates Time of OI Onset per Patient.



Disclosures: J.B. Zhang: None. **R.A. Tamboli:** None. **V.L. Albaugh:** None. **D.M. Kilkelly:** None. **C.G. Grijalva:** None. **D.B. Williams:** None. **C.A. Shibao:** None.

Funding: No

Funding Component:

P204

Induction of Endoplasmic Reticulum Stress Upregulates Renin Expression in Immortalized Mouse Hypothalamic N43/5 Cells

Authors: Javier A. Gomez, Pablo Nakagawa, Curt D. Sigmund, Univ of Iowa, Iowa City, IA

Dysregulation of the brain renin-angiotensin system (RAS) has been implicated in many forms of hypertension, including resistant hypertension. However, the mechanism by which the brain RAS is activated is unknown. We have recently published on the activation of brain RAS following disinhibition of renin-a expression after deletion of the brain-specific renin-b isoform. Endoplasmic reticulum (ER) stress, which is an accumulation of unfolded proteins in the ER, has been linked to hypertension as central angiotensin II administration induced ER stress markers. Here, we hypothesize that ER stress induces brain RAS activation by upregulating renin expression in the brain. We first tested if ER stress could induce renin expression *in vitro* in a mouse neuroblastoma hypothalamic cell line, N43/5 cells. After treating cells with ER stressor thapsigargin, a SERCA pump inhibitor, (100nM) for 8 hours we observed transcriptional upregulation of ER

stress marker Bip (28.9±5.0 fold increase vs vehicle, p<0.05, n=6). This same treatment also induced an increase in renin expression (13.8±5.8 fold increase vs vehicle, p<0.05, n=6). We have previously shown that deletion of renin-b in the brain causes an increase in renin-a expression specifically in the rostral ventrolateral medulla (RVLM). This data suggests that ER stress may induce RAS activation through a similar renin-a disinhibitory mechanism. Under basal conditions, the RVLM of renin-b knockout mice showed no difference in gene expression of ER stress marker Bip (0.7±0.5 fold increase vs control, p=0.67, n=3-4) or Chop (0.7±0.35 fold increase vs control, p=0.64, n=3-4). This suggests that ER stress may be upstream of renin induction. To test this, we treated C57BL/6 mice with tunicamycin (40mg/mL, 2uL) via intracerebellar ventricle cannula. We detected a positive endogenous renin transcript signal via *in* situ hybridization bilaterally in the medulla in the vicinity of the RVLM (possibly the nucleus ambiguous) from vehicle and tunicamycin treated mice. We are currently testing if ER stress quantitatively increases renin expression using qPCR. These data extend previous studies showing that RAS activation in the brain causes ER stress by suggesting that ER stress may also induce the brain RAS.

Disclosures: J.A. Gomez: None. **P. Nakagawa:** None. **C.D. Sigmund:** None.

Funding: Yes

Funding Component: National Center

P205

Age-dependent Alteration in Expression of Brain-derived Neurotrophic Factor and its Receptors in Central Nervous System of Spontaneously Hypertensive Rats.

Authors: Kosuke Otani, Muneyoshi Okada, Hideyuki Yamawaki, Sch of Veterinary Med, Kitasato Univ, Towada, Aomori, Japan

Background & aim: Essential hypertension is a cause unknown disease leading to heart disease, stroke and renal failure. Spontaneously hypertensive rats (SHR) are animal model of human essential hypertension and develop hypertension at 7-15-week-old. Several studies reported expression pattern of brain-derived neurotrophic factor (BDNF) changes in central nervous system (CNS) in SHR, suggesting that BDNF and its receptors might be related to increased blood pressure. However, age-dependent change in BDNF and receptors (TrkB and p75NTR) remains to be elucidated. This study aimed to clarify it.
Method: Isolated brain tissues from SHR and control Wistar Kyoto Rats (WKY) at 4-, 7- and 15-week-old were dissected into five regions (cerebral cortex: CC; cerebellum: CB; brainstem: BS; hypothalamus: HT; hippocampus: HC). Protein and mRNA expression of BDNF, TrkB isoforms (FL, T1 and T2) and p75NTR as well as an inflammatory marker NOX-2 was examined by Western blotting and real-time PCR.
Results: The following data showed significant difference in SHR compared with WKY (n = 4): pro-BDNF protein was lower by 49.9% (4-week, $p < 0.05$) and 38.2% (7-week, $p < 0.05$) in HT, while mature BDNF protein was lower by 21.9% in HC (15-week, $p < 0.05$). TrkB FL mRNA was higher by 32.5% in CB (7-week, $p < 0.05$) and 43.1% in BS (7-week, $p < 0.05$), while TrkB FL protein was higher by 51.4% in BS (15-week, $p < 0.05$). TrkB T1 mRNA was higher by 22.8% in BS (7-week, $p < 0.05$) and 17.2% in HT (15-week, $p < 0.05$), while TrkB T1 protein was higher by 56.1% in BS (4-week, $p < 0.01$). p75NTR protein was higher by 46.9% in CB (7-week, $p < 0.05$). NOX-2 mRNA was higher by 29.1% (4-week, $p < 0.01$), 104.2% (7-week, $p < 0.01$) and 75.8% (15-week, $p < 0.05$) in CC, 27.0% in CB (15-week, $p < 0.05$), 34.6% in BS (4-week, $p < 0.05$), 326.1% in HT (15-week, $p < 0.01$) and 29.8% in HC (7-week, $p < 0.05$).
Conclusion: We for the first time revealed age-dependent changes in expression of BDNF and its receptors in CNS of SHR. Specifically, our results suggest that alteration of BDNF and TrkB expression is already occurred at juvenile before a stable blood pressure increase. The upregulation of NOX-2 was also identified. Further detailed study might identify whether and how BDNF-receptor-oxidative stress axis is related to pathogenesis of essential hypertension.

Disclosures: **K. Otani:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; JSPS KAKENHI Grant Number 17J02328. **M. Okada:** None. **H. Yamawaki:** None.

Funding: No

Funding Component:

P206

Interarm Difference in Blood Pressure: Prevalence, Risk Factors, and Relevance for Diagnosis of Disease of the Aorta Among Patients Referred to Specialized Regional Hypertension Center

Authors: Januvi Jegatheswaran, Swapnil Hiremath, Cedric Edwards, Marcel Ruzicka, Ottawa Hosp, Ottawa, ON, Canada

Background: Initial evaluation of hypertension (HTN) should include assessment of blood pressure (BP) in both arms. The prevalence of high interarm differ ranges from 3% in the general adult population to about 10% in the patients with HTN. However, we lack proper estimates of its prevalence, and existing practice of follow up for these patients in a referred population. **Methods:** We performed a retrospective chart review of all prevalent patients followed at the Hypertension Center at the Ottawa Hospital. BP data from the first visit were used for assessment for interarm BP difference. We considered interarm difference in either systolic or diastolic BP in excess of 10 mmHg for casual BP by mercury sphygmomanometry to be clinically significant. **Results:** 493 patients of 580 patients were included in this study based on available data. The prevalence of clinically significant interarm difference in systolic or diastolic BP was 16.2% and was similar among men and women. These patients were more likely to be smokers (current or previous; 53.5% vs 36.8%) with peripheral arterial disease (PAD, 15% vs 8%). None of these patients had undergone further investigations of ascending aorta/aortic arch. **Conclusions:** A significant proportion of referred patients have a high interarm difference in systolic or diastolic BP. No clinical investigations were ordered to evaluate for ascending aorta/aortic arch disease reflecting the physicians' lack of understanding of its clinical relevance. The association with smoking and PAD suggests underlying aortic/large vessel disease as a potential mechanism in some patients.

Disclosures: **J. Jegatheswaran:** None. **S. Hiremath:** None. **C. Edwards:** None. **M. Ruzicka:** None.

Funding: No

Funding Component:

P207

Unattended Automated Office Blood Pressure Compared to Loaned Self-Blood Pressure Measurement in Hypertensive Patients

Authors: Jose Rafael Vega Ramirez, César Gonzálo Calvo Vargas, Hosp Civil de Guadalajara, Guadalajara, Mexico

PURPOSES The Unattended Automated Office Blood Pressure (UAOBP) has been proposed as a model to reduce the white-coat effect. In the present research this technique is compared to the Loaned Self-Blood Pressure Measurement at home (LSEM), which has a good correlation to Automated Office Blood Pressure (AOBP). **METHODOLOGY** A Cross-Sectional study was applied in 67 hypertensive patients who were under antihypertensive treatment for at least 12 weeks, and, in the office, they showed numbers in the Hypertension (HT) rank (≥140 /90 mmHg, measurement according to the conventional AHA technique). In another visit, UAOBP was performed with an oscillometric device (BP-

Tru100), which made the measurements with the patient alone in the room, after five minutes of rest, five automatic readings were taken in intervals of two minutes, and an average was obtained. The LSEM model was applied in another visit, this method consists in lending a number of sphygmomanometers, owned by the clinic, to the patients for a period of three days (Omron HEM 7320), doing 27 readings in this period of time and obtaining their average. Finally, a daytime AOBP was made by using the equipment (Space lab 90207); the measurements were made from 6:00 to 22:00 hours. **RESULTS** The population was of 67 patients having an average age of 62 $\pm$ 9 years, from which 64% were women (43/67). The Bland-Altman analysis revealed a bias (ie, mean of the differences) in the numbers of the systolic pressure for the daytime AOBP of -5.3 mmHg (-28.39 to 18.12*), versus UAOBP a bias of 1.75 mmHg (-25.66 to 29.16*), versus LSEM of -7.94 mmHg (-37.61-21.72*) versus the Conventional Office BP. (*95% Confidence Interval). **CONCLUSIONS** The findings obtained show that the measurement of BP with LSEM is closer to the daytime AOBP; thus, the technique is more reliable than UAOBP in making clinical decisions in hypertensive subjects.

Disclosures: J.R. Vega Ramirez: None. **C.G. Calvo Vargas:** None.

Funding: No

Funding Component:

P208

Association of Baseline Medication Burden With Blood Pressure Control, Patient-Reported Outcomes, and Serious Adverse Events: Findings from the SPRINT Trial

Authors: Catherine G Derington, Univ of Colorado Skaggs Sch of Pharmacy and Pharmaceutical Sciences, Dept of Clinical Pharmacy, Aurora, CO; Tyler H Gums, Univ of Texas at Austin, Div of Health Outcomes and Pharmacy Practice, Austin, TX; Adam P Bress, Univ of Utah, Dept of Population Health Sciences, Sch of Med, Salt Lake City, UT; Jennifer S Herrick, Univ of Utah, Salt Lake City, UT; Tom H Greene, Univ of Utah, Div of Biostatistics, Salt Lake City, UT; Andrew E Moran, Ian M Kronish, The Columbia Hypertension Ctr, Columbia Univ Medical Ctr, New York, NY; Donald E Morisky, Univ of California Los Angeles, Fielding Sch of Public Health, Los Angeles, CA; Katy E Trinkley, Joseph J Saseen, Univ of Colorado Skaggs Sch of Pharmacy and Pharmaceutical Sciences, Dept of Clinical Pharmacy, Aurora, CO; Kristi Reynolds, Kaiser Permanente Southern California, Res and Evaluation, Pasadena, CA; Jordan B King, Kaiser Permanente Colorado, Dept of Pharmacy, Aurora, CO

Background: Achieving intensive systolic blood pressure (SBP) control (<120 mmHg) requires more antihypertensive medications than standard control (<140 mmHg). It is unclear how total medication burden impacts hypertension outcomes. **Objective:** To evaluate the association of total medication burden at baseline and SBP control, serious adverse events (SAEs), medication adherence, and treatment satisfaction in the Systolic Blood Pressure Intervention Trial (SPRINT). **Methods:** Baseline medication data were obtained by pill bottle review at randomization and categorized as high (≥ 5 prescription medications) vs low (< 5 medications) medication burden. SBP control per randomization goal, eight item Morisky Medication Adherence Scale (MMAS 8), and Treatment Satisfaction Questionnaire were evaluated at 1-year. SAEs were collected over the trial. We calculated adjusted risk ratios (RR) and hazard ratios (HR) for each outcome associated with medication burden using multivariable regression models stratified by randomized group. **Results:** Among 8454 participants with available data at 1-year follow up, 4797 (57%) and 3657 (43%) had high and low medication burden, respectively (mean medications ± SD; 8 ± 3 vs 3 ± 1). High medication burden was associated with reduced SBP control in the intensive arm (RR, 95%CI; intensive 0.92, 0.87-0.98; standard 0.98, 0.94-1.03; interaction p 0.01) and increased SAEs in both arms (HR, 95%CI; intensive 1.57, 1.40-1.77; standard 1.59, 1.41-1.78; interaction p 0.54). High medication burden was associated with worse medication adherence in the intensive arm and improved adherence in the standard arm (RR for MMAS 8 ≥6, 95%CI; intensive 0.97, 0.94-1.00; standard 1.05, 1.02-1.09; interaction p <0.01). High medication burden was not associated with hypertension treatment satisfaction (RR for satisfied/very satisfied, 95%CI; intensive 0.99, 0.98-1.00; standard 0.98, 0.97-1.00; interaction p 0.50). **Conclusion:** In

SPRINT, high medication burden at baseline was associated with higher risk of SAEs. The association of high medication burden on the likelihood of achieving SBP goal and medication adherence at 1 year was different by treatment arm; medication burden was not associated with hypertension treatment satisfaction.

Disclosures: C.G. Derington: None. **T.H. Gums:** None. **A.P. Bress:** None. **J.S. Herrick:** None. **T.H. Greene:** None. **A.E. Moran:** None. **I.M. Kronish:** None. **D.E. Morisky:** None. **K.E. Trinkley:** None. **J.J. Saseen:** None. **K. Reynolds:** None. **J.B. King:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Consultant on investigator-initiated Novartis grant..

Funding: No

Funding Component:

P209

Antihypertensive Medication Non-Adherence is Common in Patients With Suspected Refractory Hypertension

Authors: Mohammed Siddiqui, Eric K Judd, Tanja Dudenbostel, Univ of Alabama at Birmingham, Birmingham, AL; Pankaj Gupta, Univ of Leicester, Leicester, United Kingdom; Maciej Tomaszewski, Univ of Manchester, Manchester, United Kingdom; Prashanth Patel, Univ of Leicester, Leicester, United Kingdom; Suzanne Oparil, David A Calhoun, Univ of Alabama at Birmingham, Birmingham, AL

Introduction: Refractory hypertension is a phenotype of antihypertensive treatment failure defined as uncontrolled BP (> 135/85 mmHg) despite the use of five or more different antihypertensive agents, including long-acting thiazide like diuretic (chlorthalidone) and mineralocorticoid receptor antagonist (Spironolactone or Eplerenone). Recently white coat effect has been shown to be uncommon in patients with refractory hypertension. However, the degree of medication non-adherence, an important cause of apparent refractory hypertension, is unknown.
Methods: In this prospective evaluation, 37 refractory hypertensive patients were recruited from the University of Alabama at Birmingham Hypertension Clinic after having uncontrolled BP at three or more clinic visits. All patients were evaluated by automated office BP (AOBP) with the BpTRU device, ambulatory BP monitoring (ABPM), and 24-hr urine collection to detect antihypertensive medication adherence by high-performance liquid chromatography-tandem mass spectrometry. Out of 34 patients who underwent ABPM monitoring, 30 (88%) patients were refractory hypertensive by AOBP and by ABPM. Of these, 27 patients had 24-hr urine collection for detection of prescribed antihypertensive medications or their metabolites.
Results: Out of 27 patients, 11 (40.7%) patients had complete adherence with five or more antihypertensive medications, indicating true refractory hypertension. Ten (37.0%) patients were partially adherent, and 6 (22.2%) patients had total non-adherence to their antihypertensive medications.
Conclusion: Of patients identified as having apparent refractory hypertension, 40% were adherent with at least 5 of their prescribed antihypertensive agents, confirming true refractory hypertension. The remaining 60% were partially or fully non-adherent. These results indicate that non-adherence is common in patients suspected of having refractory hypertension.

Disclosures: M. Siddiqui: None. **E.K. Judd:** None. **T. Dudenbostel:** None. **P. Gupta:** None. **M. Tomaszewski:** None. **P. Patel:** None. **S. Oparil:** None. **D.A. Calhoun:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P210

Blood Pressure Medication Adherence Among Medicare Beneficiaries, Causes and Consequences

Authors: William Aitken, Lara Bakhos, Loyola Univ Chicago, Maywood, IL

Introduction: Medication non-adherence is a major healthcare barrier, especially among diseases which are largely asymptomatic such as hypertension. The impact of poor medication adherence ranges from patient specific adverse health outcomes to broader strains on health care system resources. Accordingly, there is rich literature examining the causes and consequences of suboptimal medication adherence.
Objective: To document the relationship between blood pressure medication adherence, socioeconomic status, health care costs, and outcomes among Medicare beneficiaries with heart disease in the United States.
Methods: The CDC Wonder database was used to retrieve Centers for Medicare and Medicaid Services' data pertaining to blood pressure medication adherence, socio-economic variables, per-capita healthcare costs, and cardiovascular outcomes among beneficiaries by county across the United States. Spearman correlation was used to analyze the relationship between various factors.
Results: Among Medicare beneficiaries, blood pressure medication non-adherence rates ranged from 15.9% to 56.2% with a mean of 26.5% ± 5.3% among the 3,196 counties reporting data. Factors that strongly correlated with non-adherence include poor educational attainment (0.678, $p<0.01$) and poverty status (0.613, $p<0.01$). Although non-adherence had a weakly positive relationship with inpatient costs (0.183, $p<0.01$) and total overall costs (0.324, $p<0.01$), it demonstrated a solid correlation with cardiovascular deaths (0.42, $p<0.01$), stroke death (0.420, $p<0.01$), stroke hospitalization (0.415, $p<0.01$), and hypertension related hospitalization (0.561, $p<0.01$).
Conclusions: Our analysis of Medicare beneficiary data across the United States demonstrates a strong correlation between socioeconomic determinants of health and poor medication adherence. Our data further demonstrates that poor medication adherence translates into both worse cardiovascular outcomes and higher health care costs. In an era where health care spending has become overwhelmingly problematic, these findings provide compelling evidence for increased efforts focusing on educational and incentive based programs to improve medication adherence.

Disclosures: W. Aitken: None. **L. Bakhos:** None.

Funding: No

Funding Component:

P211

Increased Salt Intake Decreases Postprandial Energy Expenditure in Healthy Volunteers - A Randomized Clinical Study

Authors: Anja Mähler, Nicola Wilck, Charité Univsmedizin Berlin, Berlin, Germany; Samuel Klamer, DZHK (German Ctr for Cardiovascular Res), partner site Berlin, Berlin, Germany; Andras Balogh, Lars Klug, Michael Boschmann, Charité Univsmedizin Berlin, Berlin, Germany; Dominik N Müller, Max Delbruck Ctr for Molecular Med, Berlin, Germany

High salt intake is a potential risk factor for obesity independent of energy intake, though underlying mechanisms remain unclear. Diet-induced thermogenesis (DIT) accounts for about 10% of total energy expenditure. We hypothesized that high salt intake decreases DIT in healthy volunteers. We enrolled 40 healthy subjects (sex ratio 1:1) in a randomized, double-blind, placebo-controlled, parallel-group study (NCT03024567). They received either 6 g salt or placebo daily in capsules over 14 days on top of their habitual diet. Before and after the intervention, resting and postprandial energy expenditure, ambulatory blood pressure, bioelectrical impedance analysis (BIA), and food intake from 3-day food records were obtained. Energy expenditure was measured by indirect calorimetry (canopy hood) after a 12h overnight fast and a standardized 440 kcal, high-protein meal. In both groups, 19 subjects completed the study (placebo: nine men, 29 ± 6 years, BMI 23.1 ± 0.5 kg/m^2; salt: ten men, 32 ± 7 years, BMI 23.3 ± 0.7 kg/m^2). Salt intake from foods was 6 g/d in both groups, both before and after the intervention. Resting energy expenditure did not change in either group.

DIT was significantly decreased after salt ($P = 0.049$) but not after placebo (*NS*). Decreased DIT was accompanied by a decreased fat and therefore increased carbohydrate oxidation after salt ($P = 0.03$). However, this was also the case after placebo ($P < 0.0001$). Surprisingly, systolic blood pressure was increased in four and 11 subjects after salt and placebo, respectively (both *NS*). Diastolic blood pressure was higher in seven subjects, both after salt and placebo (both *NS*). Body composition and hydration did not change due to increased salt intake or placebo. In 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.

Disclosures: A. Mähler: None. **N. Wilck:** None. **S. Klamer:** None. **A. Balogh:** None. **L. Klug:** None. **M. Boschmann:** None. **D.N. Müller:** None.

Funding: No

Funding Component:

P212

Splanchnic and Hepatic Portal Hemodynamics in the Hypotensive Actions of 5-HT

Authors: Bridget Mahon Seitz, Gregory D Fink, Stephanie W Watts, MIchigan State Univ, East Lansing, MI

Circulating 5-hydroxytryptamine (5-HT) regulates mean arterial pressure (MAP) under some conditions. Infusion of low doses of 5-HT into rats leads to a sustained decrease in MAP which is prevented by the selective 5-HT7 receptor (5-HT7R) antagonist SB269970 or by inhibition of nitric oxide (NO) production. Time-dependent changes in the splanchnic circulation play a critical role in the sustained depressor response to 5-HT. We hypothesized that activation of 5-HT7Rs reduces MAP in part by causing decreased splanchnic vascular resistance and/or increased hepatic portal vascular resistance. Anesthetized male Sprague Dawley rats were instrumented with arterial and venous lines for pressure measurements and 5-HT/SB269970 infusion, and a Transonic probe on the portal vein to measure portal flow. Within 20 minutes of starting an infusion of 5-HT (25 ug/kg/min), MAP was significantly reduced (63±2 vs baseline 82±4 mmHg), whereas splanchnic vascular resistance (4.1±0.6 vs 2.4±0.1 ml/min/mmHg) and portal vascular resistance (0.18±0.08 vs 0.11±0.3) were increased. In a separate group of rats subjected to 24 hours of 5-HT infusion, MAP was reduced compared to control values (70±3 vs 84±2 mmHg), but portal vascular resistance (0.23±0.07 vs 0.17±0.04) and splanchnic vascular resistance (2.6±0.3 vs 2.4±0.1) were near control values. These animals then received an infusion of SB269970 to determine the direct contribution of the 5HT7R to hemodynamics after 24 hours of 5-HT exposure. After 20 minutes of SB269970 infusion, MAP (83±3 mmHg) was completely restored to baseline values while splanchnic vascular resistance (2.1 ±0.1 vs 2.6±0.1) and portal vascular resistance (0.12±0.3 vs 0.23±0.7) were decreased. These data suggest that the ability of 5-HT7R activation to increase splanchnic vascular resistance is effectively opposed over time by an endogenous vasodilator, which we suspect is nitric oxide. The data also indicate that changes in splanchnic vascular resistance are unlikely to participate in the chronic hypotensive effect of 5-HT, whereas increased resistance to flow through the hepatic portal system could play a role.

Disclosures: B.M. Seitz: None. **G.D. Fink:** None. **S.W. Watts:** None.

Funding: No

Funding Component:

P213

Angiotensin Converting Enzyme Inhibitors (ACEi) Increase Antifibrotic Biomarkers in African American Patients With Hypertension and Left Ventricular Hypertrophy

Authors: Cesar A Romero, Henry Ford Health System, Detroit, MI; Brian Reed, Shobi Mathew, Aaron Brodi, Rachel Dawood, MIchael Twiner, Dept of Emergency Med and Integrative Biosciences Ctr, Wayne State Univ, Detroit, MI; Candance McNaughton, Dept of Emergency Med, Vanderbilt Univ, Nashville, TN; John M Flack, Dept of Internal Med, Southern Illinois Univ, Springfield, IL; Oscar A Carretero, Henry Ford Health System, Detroit, MI; Phillip D Levy, Dept of Emergency Med and Integrative Biosciences Ctr, Wayne State Univ, Detroit, MI

ACEi are first line treatment in hypertension; however, there is controversy regarding the benefit over other antihypertensive drugs. ACEi have pressure independent effects that may make them preferable for certain patients. We aimed to evaluate the impact of ACEi on antifibrotic biomarkers in hypertensive patients with left ventricular hypertrophy (LVH). We conducted a post hoc analysis of a randomized controlled trial where hypertensive African American patients with LVH and vitamin D (VTD) deficiency were randomized to receive standard antihypertensive therapy plus VTD supplementation or placebo. We selected patients who had detectable lisinopril in plasma at one year follow-up and compared them to subjects who did not. The profibrotic marker propeptide of procollagen type I (CPIP) and the antifibrotic markers: matrix metalloproteinase-1 (MMP-1), tissue inhibitor of MMP1 (TIMP-1), the MMP-1/TIMP-1 ratio, telopeptide of collagen type I (CITP) and Ac-SDKP peptide were measured. Sixty six patients were included. Table 1 shows patients characteristics. Patients with lisinopril had lower blood pressure at one-year but no differences were observed in left ventricular mass index (LVMI). The antifibrotic markers Ac-SDKP (3.9±2.6 vs 6.3±2.8; p<0.001), MMP1 and MMP1/TIMP-1 ratio were higher in patients with detectable ACEi (all p<0.05). In a model adjusted for systolic blood pressure, low LVMI (p=0.01), MMP1/TIMP-1 (p=0.02) and Ac-SDKP (p<0.001) levels were associated with lisinopril. We conclude that ACEi increase antifibrotic biomarkers in African Americans with hypertension and LVH, suggesting that they may be offer added benefit over other agents in such patients.

Table 1. General and Clinical characteristic of those patients treated or not with ACE inhibitors

	Control group (No ACEi)	Lisinopril	*p*
n	30	36	
Age (years)	43.7±7.9	48.7±7.5	**0.01**
Female (%)	60	44	0.21
BMI (Kg/m2)	35.9±10.8	35.1±7.1	0.98
Number of prescribed anti-hypertensive drugs	2.3±0.7	2.7±0.8	0.07
ACEi(%)	13	94.4	**<0.001**
ARB(%)	56.7	2.8	**<0.001**
Amlodipine(%)	53.3	66.7	0.27
Diuretics(%)_	86.7	77.8	0.35
Supplemental Vitamin D(%)	56.7	41.7	0.22
SBP at one year (mmHg)	139.5±20.7	129.6±13.8	**0.02**
DBP at one year (mmHg)	94.3±12.8	86.3±9	**<0.01**
Left ventricular mass LVMI (g/m2) at one year	84±13.6	80.8±12.7	0.33
Changes LVMI g/m2) (1 year-Basal)	-12.9±15.5	-17.9±11.2	0.1
Pulse wave velocity (m/sec)	6.6±3.4	6.6±3.2	0.94
CIP_ICTP ratio	41.1±32.7	46.6±44.2	0.59
CICP	84.7±31.9	85.2±28.4	0.83
ICTP	3.6±2.8	3.3±2.9	0.63
MMP1_TIMP1 ratio	0.02±0.02	0.04±0.03	**0.04**
MMP1	3.4±2.6	5.5±4	**0.04***
TIMP1	166.8±36.6	155±34.6	0.25
Ac-SDKP (nM)	3.9±2.6	6.3±2.8	**<0.001**

Disclosures: C.A. Romero: None. **B. Reed:** None. **S. Mathew:** None. **A. Brodi:** None. **R. Dawood:** None. **M. Twiner:** None. **C. McNaughton:** None. **J.M. Flack:** None. **O.A. Carretero:** None. **P.D. Levy:** None.

Funding: No

Funding Component:

P214

Serum Glucocorticoid Kinase 1 and Sympathetic Nerve Activity in Salt-Sensitive Hypertension.

Authors: Justin P Van Beusecum, Natalia R Barbaro, Liang Xiao, Arvind K Pandey, David G Harrison, Annet Kirabo, Vanderbilt Univ Medical Ctr, Nashville, TN

Salt-sensitive hypertension affects nearly 50% of the population and reducing salt intake decreases blood pressure and cardiovascular events in the general population. The precise mechanism of how dietary salt contributes to blood pressure (BP) elevation, renal injury, and cardiovascular disease remains unclear. Substantial evidence supports a role of increased sympathetic output in salt-dependent hypertension and this promotes renal inflammation and dysfunction. It has been shown that salt accumulates in the interstitium of hypertensive humans and animals and drives immune cells toward a proinflammatory phenotype through the salt sensing kinase serum/glucocorticoid kinase 1 (SGK1). We have also shown an important role of monocytes and monocyte-derived dendritic cells in hypertension. In this study, we tested the hypothesis that SGK1 in myeloid CD11c$^+$ cells promote salt-sensitive hypertension by increasing sympathetic outflow. To test this hypothesis, we created mice lacking SGK1 in CD11c$^+$ cells (SGK1^{CreCD11c} mice) and used SGK1$^{fl/fl}$ mice as controls. To induce salt-sensitivity, mice received 0.5 mg/ml of N-Nitro-L-arginine methyl ester hydrochloride (L-NAME) in the drinking water for 2 weeks. This was followed by a 2-week washout period and then a 4% high salt diet for 3 weeks. BP was monitored using telemetry. We found that BP elevation during high salt feeding was significantly attenuated in SGK1^{CreCD11c} mice compared to SGK1$^{fl/fl}$ mice (125 ± 1 vs. 141 ± 1 mmHg; $p < 0.01$). SGK1^{CreCD11c} mice had a significant reduction in CD45$^+$, CD3$^+$, CD4$^+$ CD8$^+$ and CD19$^+$ cells in the kidney compared to SGK1$^{fl/fl}$ mice as assessed by flow cytometry ($p < 0.05$). Interestingly, on high salt, SGK1^{CreCD11c} mice had a significant reduction in heart rate (HR) compared to SGK1$^{fl/fl}$ mice (528 ± 2 vs. 592 ± 7 bpm; $p = 0.006$). Microglial cells of the brain express CD11c, and we found that the reduction in HR was associated with a marked reduction in HR variability in SGK1^{CreCD11c} mice compared to SGK1$^{fl/fl}$ mice (1.65 ± 0.38 vs. 3.00 ± 0.57; $p = 0.0018$), indicating a reduction in sympathetic outflow. Our data indicate that SGK1 in CD11c$^+$ myeloid cells and likely those of the central nervous system, modulate BP and sympathetic outflow promoting the pathogenesis of salt-sensitive hypertension.

Disclosures: J.P. Van Beusecum: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 5T32HL069765-13, F32HL143927- Pending. **N.R. Barbaro:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; POST290900. **L. Xiao:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; 17SDG33670829. **A.K. Pandey:** None. **D.G. Harrison:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; SFRN204200, R01HL125865, R01HL039006. **A. Kirabo:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; K01HL130497.

Funding: Yes

Funding Component: National Center

P215

Role of Nox4 - H_2O_2 Production in Cctivation of mTORC1 in Salt-Induced Hypertension and Kidney Injury in Dahl S Rats

Authors: Vikash Kumar, Theresa Kurth, Allen Cowley, Medical Coll Wisconsin, Milwaukee, WI

Nox4 is the most abundant NADPH-oxidase isoform in the kidney. It predominantly releases H_2O_2 and has been shown to contribute importantly to hypertension and renal injury in Dahl salt-sensitive (SS) rats fed a high salt diet. Recently, we reported that systemic inhibition of mTORC1 with chronic rapamycin treatment reduces salt-induced hypertension and

kidney injury in SS rats. In the present study, we hypothesized that elevations of H_2O_2 would activate mTORC1 and that reduction of Nox4 related H_2O_2 production would reduce the mTORC1 contribution to salt-induced hypertension in SS rats. We first examined the effect of H_2O_2 on mTORC1 activity *in vivo* by comparing the activity of mTORC1 in SS rats which have high levels of H_2O_2 to SS-Nox4 knockout ($SS^{Nox4-/-}$) rats which have low levels of renal H_2O_2. As analyzed by Western blot, pS6 $^{S235/236}$/S6 (index of mTORC1 activity) was found to be significantly lower ($P<0.05$) in the renal cortex of $SS^{Nox4-/-}$ rats indicating that Nox4 generated H_2O_2 was upstream in the mTORC1 pathway. We then examined whether systemic inhibition of mTORC1 with chronic rapamycin treatment would fail to reduce salt-sensitivity and kidney injury in nine week old male $SS^{Nox4-/-}$ rats. It was found that mean arterial pressure (MAP) of $SS^{Nox4-/-}$ rats treated with rapamycin (I.P.,1.5 mg/kg/day) fed a high salt diet (4.0%NaCl) for 21 days was not significantly different to vehicle treated $SS^{Nox4-/-}$ rats on the final day 21 (150 ± 7; n=8; vs 145 ± 6 mmHg; n=5; $P>0.05$). Neither did rapamycin treatment have a significant effect on kidney injury in $SS^{Nox4-/-}$ rats compared to vehicle treated rats determined at day 21 as reflected by albumin excretion rate (8 ± 2 vs 20 ± 13 mg/day, respectively; $P>0.05$). Rapamycin treated $SS^{Nox4-/-}$ rats exhibited a slow rate of body weight gain (302 ± 14 g at control and 313 ± 12 g at 21 days; $P>0.05$) compared to vehicle treated rats (316 ± 9 g at control and 366 ± 15 g at 21 days; $P<0.05$), although average food intake did not differ significantly ($P>0.05$) between the two groups. We conclude that Nox4 - H_2O_2 production contributes importantly to mTORC1 activation leading to blood pressure salt-sensitivity, and renal injury in SS rats.

Disclosures: V. Kumar: None. **T. Kurth:** None. **A. Cowley:** None.

Funding: No

Funding Component:

P216

The Circadian Clock Protein BMAL1 in the Kidney Contributes to Blood Pressure Control in Male Mice

Authors: Gene Ryan Crislip, Lauren G. Douma, Sarah Masten, Kit-Yan Cheng, I. Jeanette Lynch, Meaghan Holzworth, Dominique Barral, Amber Miller, Karyn A. Esser, Charles S. Wingo, Michelle L. Gumz, Univ of Florida, Gainesville, FL

Blood pressure (BP) regulation is influenced by the circadian clock as seen by its daily rhythm, with a peak occurring in the active phase and a dip during the rest phase. BMAL1 is a core circadian transcription factor which regulates the circadian clock feedback loop. BMAL1 also controls the expression of thousands of genes important for physiological functions. Others have shown that global BMAL1 knockout male mice have lower mean arterial pressure (MAP) and a loss of circadian rhythm compared to controls. Because the kidney is a critical regulator of BP, the goal of this study was to determine the BP phenotype of renal distal nephron-specific BMAL1 knockout mice (KO) vs. littermate controls (CNTL). KO were generated using Ksp-cadherin Cre. Histological analysis validated the BMAL1 KO model. MAP was measured by radiotelemetry. KO had 7 mmHg lower MAP compared to CNTL (103±2 vs. 110±0.2; P=0.03; N=4-5), however, the circadian rhythm of BP was apparent in both groups. There was no difference in activity or heart rate (P=0.7, P=0.1). Kidneys were collected at noon (midpoint of rest phase) for gene expression analysis, Table 1. HKa2 expression was increased in untreated KO vs. CNTL (1.7±0.2 vs. 1±0.1; P=0.01). We have previously linked HKa2 to renal Na handling and the mineralocorticoid response. Therefore, we assessed the BP response to a high salt diet plus mineralocorticoid treatment (HS/DOCP). MAP increased in both groups in response to HS/DOCP (P=0.03) where KO rose 6 mmHg (109±1) and WT only 2 mmHg (112±2). These results suggest that BMAL1 in the kidney plays a critical role in the regulation of BP but does not appear to contribute to the circadian rhythm of BP under the conditions tested.

Table 1. Effect of Kidney-Specific Bmal1 KO on Candidate Gene Expression in Male Mice		
Gene	Kidney Region	Expression in KO vs. WT
αENaC	Cortex	1.0 vs. 1.0
ATP4a	Cortex	1.0 vs. 1.0
	Medulla	0.9 vs. 1.0
ATP12a	Cortex	1.1 vs. 1.0
	Medulla	*1.7 vs. 1.0*
Edn1	Cortex	1.2 vs. 1.0
	Medulla	*0.8 vs. 1.0*
NHE3	Cortex	1.1 vs. 1.0
NKCC2	Medulla	1.1 vs. 1.0
TGFβ*	Cortex	*0.7 vs. 1.0*
Wnk1	Cortex	1.2 vs. 1.0
Wnk4	Cortex	1.1 vs. 1.0
*P<0.05; N=6 per group.		

Disclosures: G.R. Crislip: None. **L.G. Douma:** None. **S. Masten:** None. **K. Cheng:** None. **I.J. Lynch:** None. **M. Holzworth:** None. **D. Barral:** None. **A. Miller:** None. **K.A. Esser:** None. **C.S. Wingo:** None. **M.L. Gumz:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P217

Evidence for Impaired Diurnal Cardio-Vagal Tone Due to p67phox in Dahl Salt-Sensitive Rats

Authors: Bryan K Becker, Daian Chen, Jackson C Colson, Chunhua Jin, Univ of Alabama at Birmingham, Birmingham, AL; Allen W Cowley Jr., Aron Geurts, Medical Coll of Wisconsin, Milwaukee, WI; Martin E Young, Jennifer S Pollock, David M Pollock, Univ of Alabama at Birmingham, Birmingham, AL

Salt-sensitive hypertension is associated with increased reactive oxygen species (ROS) generation and sympathetic tone, and diurnal alterations in the rhythm of blood pressure control further increase cardiovascular disease risk. However,

the interaction between ROS and diurnal autonomic rhythms is unclear. We hypothesized that ROS generation impairs diurnal autonomic tone in the Dahl salt-sensitive model of hypertension. Dahl salt-sensitive (Dahl SS; n=14), Dahl SS p67phox null (SSp67; n=8), and Dahl SS with an introgressed chromosome 13 from Brown-Norway (SS.BN13; n=7) rats were instrumented with telemetry transmitters and fed a normal (0.4% NaCl), low (0.04% NaCl), or high salt (4.0% NaCl) diet. Data were analyzed on days 7 - 10 of each diet. SSp67 and SS.BN13 have lower blood pressure than Dahl SS on high salt diet. Surprisingly, we saw no difference between groups in 24-hr heart rate or systolic blood pressure low frequency to high frequency (LF/HF) on each diet suggesting a similar 24-hr autonomic tone across genotypes. However, SSp67 had a reduced LF/HF amplitude (day-night) compared to Dahl SS during high salt (0.36 ± 0.05 vs. 0.67 ± 0.11 LF/HF; respectively p=0.01). SSp67 also had higher 24-hr root mean squared of successive differences (RMSSD), a marker of cardio-vagal tone, within each salt diet (table). Also, RMSSD amplitude was higher in SSp67 (table) than other groups within each salt diet. These results support the hypothesis that reductions in cardio-vagal tone and diurnal autonomic function in the Dahl SS rat is due to NADPH oxidase activity, most likely via production of ROS. Inhibition of ROS may be an effective therapy to improve diurnal cardio-vagal tone.

	Dahl SS	SSp67	SS.BN13
Normal Salt 24-hr RMSSD (ms)	2.24 ± 0.06	3.41 ± 0.28*	2.00 ± 0.23†
Low Salt 24-hr RMSSD (ms)	2.64 ± 0.39	3.77 ± 0.53*	2.21 ± 0.24†
High Salt 24-hr RMSSD (ms)	2.56 ± 0.15	4.18 ± 0.47*	2.32 ± 0.28†
Normal Salt RMSSD Amp (ms)	0.23 ± 0.07	0.86 ± 0.26*	0.19 ± 0.09†
Low Salt RMSSD Amp (ms)	0.17 ± 0.03	0.98 ± 0.31*	0.31 ± 0.08†
High Salt RMSSD Amp (ms)	0.37 ± 0.09	0.90 ± 0.18*	0.52 ± 0.14

RMSSD = Root Mean Squared of Successive Differences, Amp = Amplitude; *, $p<0.05$ vs. Dahl SS; †, $p<0.05$ vs. SSp67; 2-way ANOVA, Tukey's multiple comparisons

Disclosures: B.K. Becker: None. **D. Chen:** None. **J.C. Colson:** None. **C. Jin:** None. **A.W. Cowley:** None. **A. Geurts:** None. **M.E. Young:** None. **J.S. Pollock:** None. **D.M. Pollock:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P218

Downregulation of Renal Tumor Necrosis Factor-Alpha Production in Response to Hypotonic Stress Facilitates Adaptive Increases in Renal Cortical Angiotensinogen and Nkcc2b

Authors: Shoujin Hao, Joseph Salzo, Mary Hao, Nicholas R. Ferreri, New York Medical Coll, Valhalla, NY

We previously showed that diverse stimuli activate the thick ascending limb of Henle's loop (TAL) to synthesize tumor necrosis factor-alpha (TNF), which subsequently acts as an endogenous inhibitor of the Na(+)-K(+)-2Cl(-) cotransporter (NKCC2). Moreover, renal-specific silencing of TNF unmasks salt-dependent increases in blood pressure via a mechanism involving NKCC2A. The objective of the present study was to determine the effects of TNF on NKCC2 isoform and angiotensinogen (AGT) expression in the renal cortex under conditions of hypotonic stress. Mice given a low salt (0.02% NaCl) diet (LS) for 7 days exhibited a 62±7.4% decrease ($p<0.5$, n=4) in TNF mRNA expression in the renal cortex, compared with mice ingesting a normal salt diet (NSD), which was associated with a concomitant 4-fold increase in renal cortical AGT mRNA accumulation ($p<0.05$, n=4). Mice ingesting LS also exhibited about a 63% increase ($p<0.05$, n=4) in cortical TAL phospho-NKCC2 (pNKCC2) expression and a 3-fold increase in NKCC2B mRNA abundance without a concurrent change in NKCC2A mRNA accumulation. The increases in AGT and NKCC2B mRNA abundance were increased

by 6-fold ($p<0.05$, n=4) and 5-fold ($p<0.05$, n=4), respectively, in mice ingesting LS that also received an intrarenal injection of a lentivirus construct designed to specifically silence TNF in the kidney (U6-TNF-ex4) compared with mice injected with control lentivirus (U6). The effects of a single intrarenal injection of murine recombinant TNF (5ng/g body weight) or saline control for 24 hr on renal AGT and NKCC2 mRNA levels were then determined in mice that ingested LS for 7 days. Administration of TNF inhibited the increase of AGT and NKCC2B mRNA abundance by approximately 42±5.9% and 49±6.5% respectively, $p<0.05$, n=4) in mice exposed to hypotonic stress. Similarly, intrarenal injection of TNF inhibited the increase in pNKCC2 by approximately 54% ($p<0.05$, n=4) in renal cortex from mice given LS for 7 days. Collectively, these findings suggest that downregulation of renal TNF production in response to hypotonic conditions contributes to the regulation of sodium chloride reabsorption via adaptive increases in AGT as well as a selective effect on NKCC2B, which is exclusively expressed by macula densa cells in the renal cortex.

Disclosures: S. Hao: None. **J. Salzo:** None. **M. Hao:** None. **N.R. Ferreri:** None.

Funding: Yes

Funding Component: National Center

P219

Site-1 Protease-Derived Soluble (Pro)Renin Receptor Contributes to Salt-Sensitive Hypertension via Activation of the Intrarenal RAS

Authors: Fei Wang, Kexin Peng, Shiying Xie, Kevin Yang, Renfei Luo, Tianxin Yang, Univ of Utah, Salt Lake Cty, UT

Dahl salt-sensitive (SS) hypertensive rats exhibit increased expression of renal (pro)renin receptor (PRR) and aberrant activation of the intrarenal renin-angiotensin system (RAS) with unclear functional implication. Recent evidence demonstrates that soluble PRR (sPRR) serves as a potential regulator of tubular transport via activating Wnt/ β-catenin pathway in the distal nephron and is derived from the cleavage by site-1 protease (S1P). The present study attempted to define the role of sPRR during salt-sensitive hypertension in SS rats. The effect of S1P inhibitor PF-429242 (PF at 20 mg/kg/d via mini-pump) on blood pressure, proteinuria, and urinary renin were tested in SS rats during high salt loading. In a separate group, a recombinant sPRR (sPRR-His at 20 μg/kg/d) was infused via catheterization of jugular vein to SS rats during PF treatment. In High sodium (HS)-loaded SS rats, administration of PF attenuated the hypertension development (MAP on day 10, HS+PF group: 124.5 ± 4.6 mmHg vs. HS group: 144.1 ± 8.2 mmHg) as assessed by radiotelemetry and proteinuria (HS+PF group: 50.9 ± 5.5 μg/24h vs. HS group: 105.3 ± 7.4 μg/24h). PF treatment reduced HS-stimulated urinary sPRR excretion by 65% in SS rats. Supplement of sPRR restored HS-induced hypertension during PF treatment (MAP on day 10, HS+PF+sPRR-His group: 138.7 ± 6.1 vs. HS+PF group: 124.5 ± 4.6 mmHg) and proteinuria (HS+PF+sPRR-His group: 79.0 ± 5.8 μg/24h vs. HS+PF group: 50.9 ± 5.5 μg/24h) in SS rats. Concurrently, PF treatment reduced HS-stimulated urinary renin activity (by 80%), aldosterone excretion (by 36%) and renal medullary prorenin and renin (by 60%), which were all restored by sPRR-His infusion (1.5- to 3-fold). Indices of Wnt/β-catenin pathway including protein levels of renal cytosolic Axin-2 and nuclear β-catenin as assessed by immunoblotting were elevated by HS (3.4-fold, 2.2-fold respectively) in SS but not SR rats, which were all suppressed by S1P inhibition (by 80% and 50% respectively) and restored by sPRR-His infusion. Given β-catenin signaling as a known regulator of intrarenal RAS, our results suggest that S1P-derived sPRR plays a pathogenic role in salt-sensitive hypertension in SS rats via β-catenin/intrarenal RAS pathway.

Disclosures: F. Wang: None. **K. Peng:** None. **S. Xie:** None. **K. Yang:** None. **R. Luo:** None. **T. Yang:** None.

Funding: No

Funding Component:

P220

High Salt Intake Induces Catabolism Accompanied by Hepatic Urea Osmolyte Production and Decreases Renal Sympathetic Nerve Activity

Authors: Norihiko Morisawa, Div of Nephrology & Hypertension, Dept of Internal Med, Jikei Univ Sch of Med, Tokyo, Japan; Kento Kitada, Cardiovascular and Metabolic Disorders, Duke-NUS Medical Sch, Singapore, Singapore; Daisuke Yamazaki, Daisuke Nakano, Dept of Pharmacology, Faculty of Med, Kagawa Univ, Kagawa, Japan; Yoshihide Fujisawa, Life Science Res Ctr, Kagawa Univ, Kagawa, Japan; Jens Titze, Cardiovascular and Metabolic Disorders, Duke-NUS Medical Sch, Singapore, Singapore; Akira Nishiyama, Dept of Pharmacology, Faculty of Med, Kagawa Univ, Kagawa, Japan

Introduction: We have recently reported that high salt intake induces the energy-intensive nature of hepatic urea osmolyte production that accompanied the decrease in heart rate (HR). These findings suggest that high salt intake decreases sympathetic nerve activity to reduce cardiovascular energy expenditure for the hepatic urea production. Therefore, we examined the effects of high salt intake on renal sympathetic nerve activity (RSNA) by using a radiotelemetry system in rats. Methods: We inserted the radiotelemetry system into male Sprague Dawley (SD) rats for recording RSNA and HR. After 2 weeks recovery, we fed the rats 0.3% NaCl diet (NS) with tap water to drink (NS + tap), 4% NaCl diet with tap water (HS + tap), and 4% NaCl diet with saline (HS + saline) for 4 days in this order, and kept monitoring their HR and RSNA. In the separate experiment, we also fed male SD rats NS + tap or HS + saline for 6 consecutive weeks to confirm a catabolic state in HS + saline rats. Results: Rats exhibited marked reductions in RSNA and HR during HS + saline phase compared with other phases. HS + saline rats significantly decreased body weight (369±35 g) compared with NS + tap rats (405±48 g) despite of the similar daily food intake (18.5±1.3 vs. 19.2±1.6 g/day). And HS + saline rats increased liver arginase, a urea producing enzyme, activity (1561±121 units/L/mg) compared with NS + tap rats (1337±131 units/L/mg) at 6 weeks after the feeding. Conclusion: High salt intake decreased RSNA accompanied by the catabolic urea production in rats. These findings suggest that the sympathetic nervous system is one of the key regulators to reduce cardiovascular energy expenditure, which could support the energy-intensive nature of hepatic urea production in high salt-fed rats.

Disclosures: N. Morisawa: None. **K. Kitada:** None. **D. Yamazaki:** None. **D. Nakano:** None. **Y. Fujisawa:** None. **J. Titze:** None. **A. Nishiyama:** None.

Funding: No

Funding Component:

P221

The Mechanism of Salt-Sensitive Hypertension in Intermittent Hypoxia

Authors: Alimila Yeerbolati, Sayoko Ogura, Univ of Tokyo, Faculty of Med, Tokyo, Japan; Tatsuo Shimosawa, Intl Univ of Health and Welfare, Sch of Med Mita Hosp, IUHW Dept. of Clinical Lab, Tokyo, Japan; Hideyuki Maeda, The Medical Univ of Tokyo, Tokyo, Japan

The mechanism of Salt-sensitive Hypertension in Intermittent Hypoxia (IH) **[Object]** The morbidity of sleep apnea syndrome (SAS) is about 4% of the general population and it has been recognized as an independent risk factor for hypertension as well as other cardiovascular diseases. We established IH condition for rats and mice to investigate the development of salt-sensitive hypertension under IH. **METHODS:** FiO2 was adjusted to cyclically drop to 4-5% every 90 seconds for 8 hours. Male Sprague-Dawley rats at age of 11weeks old and C57B6j mice at age of 8 weeks were kept in IH for 2 weeks with normal salt (0.5%) and high salt (8% NaCl) diet. Blood pressure (BP) and heart rate (HR) were monitored by telemetry. To explore the mechanism of salt sensitivity, Na excretion was measured. At the end of study, we evaluated the activity of sodium-chloride cotransporter (NCC) and epithelial sodium channel (ENaC) by treatment with

either hydrochlorothiazide (HCTZ) and Amiloride, respectively. mRNA of WNK4, ACE, ACE-2, Angiotensinogen, 11bHSD-2 were examined. In addition, we analyzed T cell population both by histologically and by FACS analyzer. **Results:** Only during IH, high salt diet-induced hypertension (+20-30mmHg) and BP decreased after changed to a low-salt diet. In normoxic controls, BP did not change by high salt diet. Natriuresis by HCTZ but not by amiloride was significantly enhanced IH group than normoxic control. There was no significant difference in mRNA level of WNK4, ACE, angiotensinogen, 11bHSD-2. CD3+ T cell was found around the renal tubule cells. **Conclusion**: The current study revealed that IH induces salt-sensitive hypertension and the enhanced Na+ re-absorption through the NCC may be involved. T-cell inflammation might be one of the reasons for activation of NCC.

Disclosures: A. Yeerbolati: None. **S. Ogura:** None. **T. Shimosawa:** None. **H. Maeda:** None.

Funding: No

Funding Component:

P222

Oxidative Stress Causes Salt Sensitive Hypertension via Activation of Na^+-Cl^- Cotransporter (ncc)

Authors: Conghui Wang, Tatsuo Shimosawa, Reheman Latapati, SuangSuang Koid, Intl Univ of Health and Welfare, Narita, Japan; Yeerbolati Alimila, BeiBei Liu, The Univ of Tokyo, Tokyo, Japan; Sayoko Ogura, Nihon Univ, Tokyo, Japan; Fumiko Mori, Mitsui Memorial Hosp, Tokyo, Japan; Toshiro Fujita, The Univ of Tokyo, Tokyo, Japan

Background:
We have reported that blockade of nitric oxide synthesis by L-NAME causes salt-sensitive hypertension via activating NCC. which was confirmed by the finding that systemic administration of L-NAME failed to cause salt-sensitive hypertension in NCC deficient mice. In vitro study showed when mDCT cells are treated with L-NAME, NCC phosphorylation is increased and NO donner reduced its phosphorylation. However, the precise direct effect of NO on NCC phosphorylation is not clear. In this study we clarified the role of oxidative stress but not angiotensin signaling in NCC phosphorylation
Method:
The passages 3 - 15 of mDCT cells were used in this study. The cells were starved 1 hour before the experiment. L-NAME (10-40 μM), SOD mimetic 4-Hydroxy-TEMPO (10 μM), NO donor sodium nitroprusside, (SNP 100 μM - 2 mM), pSPAK inhibitor STOCK2S-26016, and Losartan (10 μM) were used to treat the mDCT cells for 10 min and 30 min. Oxidative stress was measured by ROS production using the lucigenin method. To confirm the oxidative stress function, we performed vivo experiment with SOD mimetics--4-hydroxy-TEMPO. The C57BL/6J Kwl mice received implantation of subcutaneous osmotic mini-pump containing TEMPO (20 mg/kg/day) with 8 % high salt diet and L-NAME (3.7 μM/day in drinking water) for 4 weeks.
Result:
L-NAME increased oxidative stress by ROS production (2.0 ± 0.15 times higher than control) and p-SPAK signaling, which was normalized by TEMPO, an SOD mimetic and p-SPAK inhibitor. In contrast, AT1R inhibitor Losartan did not affect L-NAME-induced activation of NCC in mDCT cells. Moreover, the function of TEMPO was also confirmed in vivo study, which attenuated the L-NAME- induced increases in superoxide, mean BP (HS 108.7 ± 3.7 mmHg; HSL 130.5 ± 2.9 mmHg; HSL + TEMPO 116.0 ± 1.7 mmHg) and p-NCC expression in the C57BL/6J mice.
Conclusion:
Oxidative stress - pSPAK pathway plays a pivotal role in the NO-induced inhibition of NCC.

Disclosures: C. Wang: None. **T. Shimosawa:** None. **R. Latapati:** None. **S. Koid:** None. **Y. Alimila:** None. **B. Liu:** None. **S. Ogura:** None. **F. Mori:** None. **T. Fujita:** None.

Funding: No

Funding Component:

P223

Identification of Chromosome 2 Differentially Expressed Genes Linked to Vascular Inflammation Using Congenic Rats Fed a Normal and High-Salt Diet

Authors: Olga Berillo, Sofiane Ouerd, Ku-Geng Huo, Asia Rehman, Lady Davis Inst for Medical Res, Montreal, QC, Canada; Chantal Richer, Daniel Sinnett, Sainte-Justine Univ Hosp, Montreal, QC, Canada; Anne E Kwitek, Univ of Iowa, Iowa, IA; Pierre Paradis, Ernesto L Schiffrin, Lady Davis Inst for Medical Res, Montreal, QC, Canada

Background: The immune system plays an important role in hypertension. Chromosome 2 (Chr2) introgression from normotensive Brown Norway (BN) rats into hypertensive Dahl salt sensitive (SS) background (consomic SB2) reduced blood pressure (BP), and vascular inflammation under a normal-salt diet (NSD). We hypothesized that BN Chr2 contains anti-inflammatory genes that could prevent BP elevation and vascular inflammation in rats fed NSD and high-salt diet (HSD). These genes will be identified using microRNA (miRNA) and total RNA sequencing in aorta of congenic rats containing different portions of BN Chr2 under NSD and HSD. **Methods and results:** Four-to-6 week-old male SS and congenic SB2a and SB2b rats were fed a NSD or HSD (4% NaCl) for 8 weeks. Telemetry systolic BP (SBP) was lower in SB2a and SB2b compared to SS (125±3, 127±6 vs 146±2 mm Hg, *P*<0.05) under NSD and tended to be higher in SB2b but not in SB2a when compared to SS (185±8, 167±7 vs 168±5 mm Hg) under HSD. RNA was extracted from aorta and used for small and total RNA sequencing using Illumina HiSeq-2500. Differentially expressed (DE) miRNAs and genes (mRNAs and non-coding RNAs [ncRNAs]) encoded in BN Chr2 introgressed portions were identified with fold change ≤ or ≥1.5 and FDR<0.05 in SB2a vs SS (miRNAs: 2 up (↑) and 2 down (↓), mRNAs: 8 ↑ and 10 ↓, ncRNA: 1 ↓) under NSD and (miRNAs: 12 ↑ and 14 ↓; mRNAs: 188↑ and 259 ↓, ncRNAs: 1 ↑ and 11 ↓) under HSD; and SB2b vs SS (miRNAs: 2 ↑ and 4 ↓, mRNAs: 26 ↑ and 85 ↓; ncRNAs: 1 ↑ and 2 ↓) under NSD and (mRNAs: 6 ↑ and 11 ↓, ncRNAs: 1 ↑ and 3 ↓) under HSD. *Ddah1* was down-regulated in SB2a vs SS rats fed NSD and HSD, while *Acad9*, *Agtr1b,* ncRNA AABR07012047.1*, Fbxw7, Ptgfrn* were down-regulated and ncRNA AABR07012585.3*, Bbs12, Kcnab1* were up-regulated in SB2b vs SS rats fed NSD and HSD. DE RNAs were confirmed by reverse transcription-quantitative PCR (RT-qPCR). Correlation between RNA-sequencing and RT-qPCR data was demonstrated for 6 of 9 tested RNAs: *Kcnab1* (r=0.86, *P*<3.3E-9), *Agtr1b* (r=0.85, *P*<6E-9), *Ddah1* (r=0.78*, P*<5.0E-7)*, Bbs12* (r=0.72, *P*<6.4E-6), *Ptgfrn* (r=0.54, *P*<0.005), and *AABR07012047.1* (r=0.48, *P*<0.05). **Conclusions:** BN Chr2 encoded DE genes were identified in aorta of congenic SB2a and SB2b rats fed NSD and HSD. Whether these genes play a role in vascular inflammation remains to be determined.

Disclosures: O. Berillo: None. **S. Ouerd:** None. **K. Huo:** None. **A. Rehman:** None. **C. Richer:** None. **D. Sinnett:** None. **A.E. Kwitek:** None. **P. Paradis:** None. **E.L. Schiffrin:** None.

Funding: No

Funding Component:

P224

Urinary Excretion of Sodium, Potassium, Calcium and Magnesium and Blood Pressure Among a Population of ≥ 20-Year-Olds: Evidence From Southwest Coastal Bangladesh

Authors: Abu Mohd Naser Titu, Dept of Environmental Health Sciences, Rollins Sch of Public Health, Emory Univ, Atlanta, GA; Mahbubur Rahman, Leanne Unicomb, Solaiman Doza, Intl Ctr for Diarrhoeal Disease Res, Bangladesh (icddr,b), Dhaka, Bangladesh; Kazi Matin Uddin Ahmed, Dept of Geology, Univ of Dhaka, Dhaka, Bangladesh, Dhaka, Bangladesh; Shahjada Selim, Dept of Endocrinology, Bangabandhu Sheikh Mujib Medical Univ, Dhaka, Bangladesh;

Shuchi Anand, Div of Nephrology, Stanford Sch of Med, Stanford Univ, Stanford, CA; Howard Chang, Dept of Biostatistics and Bioinformatics, Rollins Sch of Public Health, Emory Univ, Atlanta, GA; Stephen Luby, Woods Inst for the Environment, Stanford Univ, Stanford, California, Stanford, CA; Thomas Clasen, Matthew Gribble, Dept of Environmental Health Sciences, Rollins Sch of Public Health, Emory Univ, Atlanta, GA; KM Venkat Narayan, Emory Global Diabetes Res Ctr, Hubert Dept of Global Health, Rollins Sch of Public Health, Emory Univ, Atlanta, GA

We analyzed data from a stepped wedge randomized trial to evaluate how urinary Na, K, Ca and Mg excretion are associated with the change in blood pressure (BP) among the adult population.
We followed up a cohort of 1,191 participants (>20 years old) from 540 households in 16 communities of southwest coastal Bangladesh for five visits during December 2016 - April 2017 when they were exposed to high salinity drinking water. In all visits, we measured participants' BP (N=5,746) and 24-hour urinary Na, K, Ca and Mg. We used multilevel linear regression models to determine the association among change in urinary excretion of Na, K, Ca and Mg with differences in mean systolic blood pressure (SBP) and diastolic blood pressure (DBP). Models included participant-, household-, and community-level random intercepts and were adjusted for age, sex, BMI, physical activity, smoking status, and household wealth. We restricted analysis among participants who had complete 24-hour urine collection based on measured versus expected urinary creatinine ratio of >=0.7 (N=5,103).
The median urinary 24 hours Na excretion was 164 mmol, K was 34 mmol, Ca was 126 mg, and Mg was 83 mg in visit 1. The mean population SBP was 115.6 mmHg in visit 1 and 110.2 in visit 5, and the mean DBP was 68.9 mmHg in visit 1 and 65.9 in visit 5. Compared to visit 1, we found 85% higher urinary Ca in visit 3 and 57% higher urinary Mg in visit 5. We found 100 mmol per 24 hours increase in urinary Na was associated with 1.74 (95% CI: 0.85, 2.62) mmHg higher SBP and 0.51 (95% CI: -0.06, 1.08) mmHg higher DBP, and 50 mmol per 24 hours increase in urinary K was associated with 2.39 (95% CI: 0.84, 3.94) mmHg lower SBP and 0.79 (95% CI: -1.76, 0.17) mmHg lower DBP. We found 100 mg per 24 hours increase in urinary Ca was associated with 0.29 (95% CI: 0.02, 0.60) mmHg lower SBP and 0.32 (0.07, 0.57) mmHg lower DBP, and 100 mg per 24 hours increase in urinary Mg was associated with 1.09 (95% CI: 0.55, 1.64) mmHg lower SBP and 0.41 (0.07, 0.76) mmHg lower DBP. We found SBP lowering effect of urinary Mg increased if urinary Ca was lower (p = 0.048).
We found urinary K, Ca, and Mg are associated with lower BP in coastal Bangladesh. Our results suggest a high intake of these beneficial minerals alongside Na reduction may successfully lower mean BP of the population.

Disclosures: A. Titu: None. **M. Rahman:** None. **L. Unicomb:** None. **S. Doza:** None. **K. Ahmed:** None. **S. Selim:** None. **S. Anand:** None. **H. Chang:** None. **S. Luby:** None. **T. Clasen:** None. **M. Gribble:** None. **K. Narayan:** None.

Funding: No

Funding Component:

P225

Associations Between Sleep, Physical Activity and Blood Pressure Among Black Women: Baseline Results From the*SisterTalk At Home*Intervention for Weight Loss

Authors: Augustine W. Kang, Patricia M. Risica, Brown Univ, Providence, RI

Significance: Sleep is a key factor affecting hypertension and obesity, but limited evidence is available examining the relationship between sleep and predictors of hypertension/obesity (e.g. physical activity (PA)) among populations at risk for health disparities. This study seeks to fill gaps in research by (a) describing sleep patterns among overweight/obese Black women, (b) examining the association between sleep and PA, and (c) examining the association between PA and blood pressure (BP). **Methods**: We examine baseline data from the *SisterTalk at Home* study, an RCT assessing the efficacy of a church-based weight management program for Black women in Northeastern USA. The study sample included 361 participants (Age = 49.9 ± 13.0 years). Mean BMI was 34.8 ± 6.7 kg/$m^2$and mean systolic/diastolic BP (SBP

and DBP) were 125.9 ± 17.8 and 77.9 ± 9.2 mm/Hg. Study instruments include the Epworth Sleepiness Scale (ESS), self-reported sleep duration, and PA. Chi-square, ANCOVA and linear regression were used for data analysis. **Results**: 49% of the sample reported short sleep duration (SSD) (<7 hrs); 46% were optimal sleepers (7-9 hrs), and 5% were long sleepers (>9 hrs). For sleepiness (ESS), 38% of the sample were classified as "normal", 35% were "high normal", and 27% had "excessive daytime sleepiness". Age, unemployment, and BMI were associated with higher SBP (p <.001, .006, and .002) and thus controlled for in subsequent analysis. Participants in the "high normal" ESS category reported significantly lower vigorous and moderate PA compared to "normal' participants (p = .028 and .035). Vigorous PA was in turn inversely associated with SBP and DBP (p = .009 and .031); Moderate PA was inversely associated with SBP (p = .039). **Conclusions**: The present sample reports a higher prevalence of short sleepers and excessive daytime sleepiness compared to the general population, supporting prior findings that poor sleep quality may be comorbid with obesity. Results also indicate a relationship between poor sleep and lower physical activity, suggesting that intervention efforts to address obesity among this population should consider targeting sleep. Lastly, PA was associated with BP, extending conclusions drawn in other populations to our sample.

Disclosures: A.W. Kang: None. **P.M. Risica:** None.

Funding: No

Funding Component:

P226

Reducing Sympathetic Activity and Cardiac Pre-Load Have Differential Effects on Mean and Pulsatile Blood Pressure Components

Authors: Luca Faconti, Bushra Farukh, Phil Chowienczyk, King's Coll London, London, United Kingdom

Objective: Blood pressure (BP) can be divided into steady state (mean arterial pressure, MAP) and pulsatile (pulse pressure, PP) components that are differentially associated with target organ damage and cardiovascular events. Here, we tested the effects of non-pharmacological interventions that reduce sympathetic activity (SA) and cardiac pre-load on MAP and PP.
Material and methods: Pulse-wave analysis of the radial artery (SphygmoCor, AtCor Medical, Sydney, Australia), calibrated from 3 consecutive readings of brachial BP, was used for the evaluation of central PP and MAP. Measurements were obtained before and after supervised device-guided paced breathing (DGB), and bilateral lower limb venous occlusion (LVO, performed non-invasively using thigh cuffs), in random order. DGB reduces SA whereas LVO produces an acute reduction in pre-load. High resolution heart rate variability (HRV) as log-ratio of low-frequency/high-frequency range (LF/HF),(Schiller Medilog AR12plus, United States) was used to assess the effects of DGB on SA, while reduction of pre-load was evaluated by inferior vena cava diameter (IVCd) measured with cardiac ultrasound.
Results: Thirty six HT patients (16 female) age (mean±SD) 46±12 years, BP 144.8±17.7/88.2±10.3 mmHg were studied. DGB decreased log LF/HF from 0.306±0.27 to 0.171±0.3 and LVO decreased IVCd from 15.16±3.96 mm to 12.39±3.43mm. Reduction of brachial BP was greater during DGB compared to LVO: (mean (95% confidence interval)):-9.7(-11.6,-7.8) mmHg vs -3.8(-5.8,-1.7) mmHg for systolic BP and -4.1(-5.6,-2.5) mmHg vs 1.1(-0.1, 2.2) mmHg for diastolic BP, respectively (*P<0.05)*.
Effects of DGB and LVO were similar on PP: -3.5(-4.8,-2.3) mmHg for DBG and -3.1(-5.5,-0.7) mmHg for LVO, P>0.1. MAP reduction was significant only during DBG (-6.4 (-8.1,-4.7) mmHg) compared to LVO (-1.2 (-2.7, 0.1) mmHg, *P<0.01* between the interventions.
Conclusion: Reducing sympathetic activity via DGB decreases both steady state and pulsatile components of BP whereas pre-load reduction selectively decreases PP. Depending on individual BP components, interventions targeting sympathetic activity and/or pre-load might be used to personalized treatment.

Disclosures: L. Faconti: None. **B. Farukh:** None. **P. Chowienczyk:** None.

Funding: No

Funding Component:

P227

The Feasibility of Interventions to Increase Potassium Intake for Hypertension: A Systematic Review of the Evidence

Authors: Maryam Basim Zaree, Marcel Ruzicka, Rana Hassan, Swapnil Hiremath, Ottawa Hosp, Ottawa, ON, Canada

BackgroundIncreased potassium (K) intake has been reported to decrease blood pressure (BP) in animal studies as well as clinical trials. On this basis, major organizations including the American Heart Association recommend increasing K intake, preferably by diet, as a non pharmacological mean of reducing BP. However, it is not clear if the interventions for efficaciously increasing K intake are reproducible or feasible for translation into public health. Hence, we conducted a systematic review of the evidence to review this from randomized controlled trials (RCTs).
MethodsWe conducted a literature search using an information specialist of MEDLINE, EMBASE and Cochrane CENTRAL till November 2017. Two reviewers selected RCTs that were in adults, with an intervention aimed at increasing K intake, with blood pressure as an outcome. From RCTs which reported both a significant change in BP and K using 24 hour urine K, we evaluated the interventions for ease of reproducibility and feasibility based on prespecified criteria.
ResultsThe initial search retrieved 1199 non-duplicate citations. After applying eligibility criteria, 90 studies were selected for inclusion. In 31 studies, the change in BP or K was not significant. Of the remaining 59 studies which reported a significant change in K and BP, 47 reported a change in K based on 24 hour urinary K measurement. 32/47 studies used a K supplement, with details provided on dose and administration to make it both reproducible and feasible. 15/47 studies used a dietary intervention, of which in 4, the intervention was not described in sufficient detail to be reproducible.The remaining 11 studies were feeding trials, with intervention consisting of provision of prepared meals, or of food items on a daily basis to make them unfeasible for routine clinical practice.
ConclusionsDietary potassium interventions from trials in which there was a significant change in K based on 24 hour urine and a significant change in BP are not reproducible or feasible.

Disclosures: M.B. Zaree: None. **M. Ruzicka:** None. **R. Hassan:** None. **S. Hiremath:** None.

Funding: No

Funding Component:

P228

Text-Facilitated Home Blood Pressure Monitoring: A Qualitative Analysis of Health Behavior Change

Authors: Matthew Allen, Taya Irizarry, Julian Einhorn, Brian Suffoletto, Thomas Kamarck, Lora Burke, Bruce Rollman, Matthew F Muldoon, Univ of Pittsburgh, Pittsburgh, PA

Uncontrolled hypertension constitutes a major challenge for healthcare systems. Home blood pressure monitoring (HBPM) is widely recommended and may lower BP when combined with other supports. However, scalable and systematic HBPM interventions are lacking and the behavioral mechanism(s) through which BP is lowered remain poorly understood. Our team designed the *MyBP* program with video-based education and a fully automated, bi-directional texting to facilitate longitudinal HBPM. Exit interviews conducted after six-weeks of *MyBP* revealed that most participants made at least one healthy behavior change. The current study examines why participants made healthy behavior changes, and what specific components of the *MyBP* program facilitated those changes. Adults with

hypertension were recruited from either an urban emergency department, a primary care office, or a hypertension referral center. The 40 enrolled participants were widely representative: age range 34-70, 23 women, 24 minority, 14 completed only high school, BP range 110-250/70-130 mm Hg, and prescribed BP medications range 0-5. A thematic analysis of transcribed exit interview audio-recordings identified three themes contributing to patients' decision to initiate a behavior change: 1) improved hypertension literacy from viewing educational videos; 2) increased day-to-day salience of one's BP as a result of consistent HBPM; and 3) use of BP readings as feedback on participants' health behaviors, with high readings often triggering intrinsic motivations to make behavior changes. These themes and associated sub-themes were found to have analogous constructs in the Health Belief Model and Social Cognitive Theory. The presentation of educational materials at baseline, followed by regular BP self-monitoring, increased confidence and motivation to initiate changes in health behaviors. The receipt of bi-weekly reports then acted as feedback fueling participants' motivation to maintain or add healthy behaviors. Facilitation of HBPM with automated texting, in conjunction with educational videos and regular feedback, appears to stimulate improvements in hypertension self-management via mechanisms consistent with recognized models of behavior change.

Disclosures: M. Allen: None. **T. Irizarry:** None. **J. Einhorn:** None. **B. Suffoletto:** None. **T. Kamarck:** None. **L. Burke:** None. **B. Rollman:** None. **M.F. Muldoon:** None.

Funding: No

Funding Component:

P229

Effect of Management of Hypertensive Susceptible Population: A Case-Control Study in Shanghai, China

Authors: Liang Tongtong, Xu Dongli, Zhang Jinling, Minhang District Ctr for Disease Control and Prevention, Shanghai, China

Objective. The management of hypertensive susceptible population was carried out by community family physician in Minhang district since 2011. The management includes health education, blood pressure measurement, and so on.This study was to explore the effect of the management of hypertensive susceptible population under community family physician model.
Methods. A computerized database of hypertensive susceptible population enrolled in management of community health service centers in Minhang District,Shanghai from 2011 to 2018 was used. According to whether the management status was transferred to patients with hypertension, the subjects were divided into case group and control group. We compared the time of management, demographic characteristics, lifestyle, family history, and disease history between two groups. Univariate analysis was performed by t test and χ^2 test, and Logistic regression analysis was used for multivariate analysis.
Results. A total of 18163 subjects were enrolled, and 3623 cases were transferred to hypertension patients (case group), accounting for 19.9%(3623 of 18163). The average time of management of case group was (25.00±16.84) months, which was lower than that of control group (38.56±18.45) months ($P < 0.001$). The results of Logistic regression analysis showed that time of management (OR=0.953,95%CI:0.951-0.955) and exercise (OR=0.655,95%CI:0.554-0.775) were the protective factors for hypertension; the history of diabetes (OR=2.920,95%CI:2.472-3.450), the history of coronary heart disease (OR=1.844,95%CI:1.436-2.367), drinking (OR=1.410,95%CI:1.131-1.757), smoking (OR=1.356,95%CI:1.172-1.570), family history of hypertension (OR=1.226,95%CI:1.121-1.342), age (OR=1.043,95%CI:1.040-1.047), BMI (OR=1.023,95%CI:1.014-1.032) and pulse pressure difference (OR=1.015,95%CI:1.010-1.021) were risk factors for hypertension.
Conclusions. The management of hypertensive susceptible population under community family physician model can reduce the risk of hypertension and is important for protecting hypertensive susceptible population, after removing the influence of age, BMI, disease history, family history and lifestyle.

Disclosures: **L. Tongtong:** None. **X. Dongli:** None. **Z. Jinling:** None.

Funding: No

Funding Component:

P230

Food Insecurity is Associated With Hypertension in Children and Adolescents Independent of Obesity

Authors: Andrew Michael South, Deepak Palakshappa, Callie Brown, Wake Forest Sch of Med, Winston Salem, NC

Background: Food insecurity (FI), the limited or uncertain availability of nutritionally adequate foods, affects 40 million Americans and is an under-recognized contributor to health disparities, especially in children and adolescents. FI can lead to poor diet quality, including increased salt intake, and therefore may be a risk factor for hypertension and cardiovascular disease. While a link between FI and cardiovascular disease has been suggested in adults, this relationship in children is unknown, especially within the U.S.
Objective: To determine if a relationship exists between FI and high BP in a nationally representative cohort of children and adolescents.
Design/Methods: We performed a cross-sectional analysis of data from children aged 8-17 years in the National Health and Nutrition Examination Survey (NHANES), 2007-2014. FI was assessed using the USDA Food Security Survey Module. BP was measured three times and averaged. We defined high BP as i) SBP or DBP ≥90th %ile for age <13 years or ≥120/80 mmHg for age ≥13 years; ii) reported diagnosis of hypertension; or iii) reported current use of an antihypertensive medication. Using measured height and weight, we categorized weight status based on BMI. We used multivariate logistic regression models to determine the association between FI and high BP, controlling for age, sex, race, and BMI category. All analyses accounted for the complex survey design of NHANES including survey weights, clustering, and stratification.
Results: FI was present in 20.5% (1,460 of 7,125) of subjects and 12.4% (883 of 7,125) had high BP. On bivariate analysis high BP was more common among those with FI (14.4%, 1,026 of 7,125) than those who were food secure (11.6%, 826 of 7,125, *p*=0.001). After controlling for potentially confounding factors, FI remained associated with high BP (OR 1.25, 95% CI 1.04-1.50).
Conclusions: We found that FI was associated with an increased likelihood of high BP in a large, nationally representative cohort of U.S. children and adolescents independent of obesity. FI likely has a significant impact on health and cardiovascular disease during childhood, so efforts to address FI will also help reduce the burden of cardiovascular disease in children and in later adulthood.

Disclosures: **A.M. South:** None. **D. Palakshappa:** None. **C. Brown:** None.

Funding: No

Funding Component:

P231

Circulatory Gene Regulation in Youth With Primary Hypertension and Target Organ Damage

Authors: Fnu Mohammed Arif, Univ of Cincinnati, Cincinnati, OH; Elaine Urbina, Cincinnati Children's Hosp Medical Ctr, Cincinnati, OH; Sakthivel Sadayappan, Richard Becker, Univ of Cincinnati, Cincinnati, OH

Introduction: Primary hypertension (PH) is a multifactorial disease mainly influenced by genetic, epigenetic and environmental dynamics. Despite the occurrence of PH-associated cardiovascular (CV) events in youth, we have

acquired only a limited understanding of epigenetic and gene regulation of blood pressure (BP)-related target organ damage (TOD) in adolescents. **Aims and Objectives:** To define circulatory gene and miRNA expression levels and allied signaling pathways in hypertensive adolescents (Hyp) with TOD. **Methods and Results:** Three hundred adolescents of both genders aged 11 to 18 years participated in the AHA-funded Study of Hypertension in Pediatrics - Adult Hypertension Onset in Youth (SHIP AHOY) Project. Mean clinic SBP was 104.3 ±8.1 and 133.0 ± 8.2 mmHg in normal *vs* Hyp (*p<0.005*) and mean daytime ambulatory SBP was 115.7 ±11.4 and 128.9 ±6.4 mmHg in normal *vs* Hyp (*p<0.005*), respectively. Youth with PH also had higher BMI (22.33 ±3.67 *vs* 31.56 ±9.42; *p<0.05*), elevated serum creatinine (0.73 ± 0.18 *vs* 0.92 ±0.12; *p<0.05*), and left ventricular hypertrophy (LVM/$ht^{2.7}$; 25.4 ±1.8 *vs* 41.7 ±1.8; *p<0.05*), and trend for increased arterial stiffness (PWV; 4.8 ±0.6 *vs* 5.8 ±2.0; *p*=0.18). mRNA and microRNA seq were performed using peripheral blood cells of 10 normal (mean 15.2 ±1.4 years; 50% male), and 10 Hyp patients (mean 15.5 ±1.5 years; 70% male). Seq data analysis revealed master genes (Fig. 1) and pathways that were differentially regulated in Hyp patients with TOD. **Conclusion(s):** PH in youth is associated with TOD that relates to a distinct gene expression profile. Knowledge of allied pathways may lead to new treatments in hypertensive youth to prevent future CV diseases.

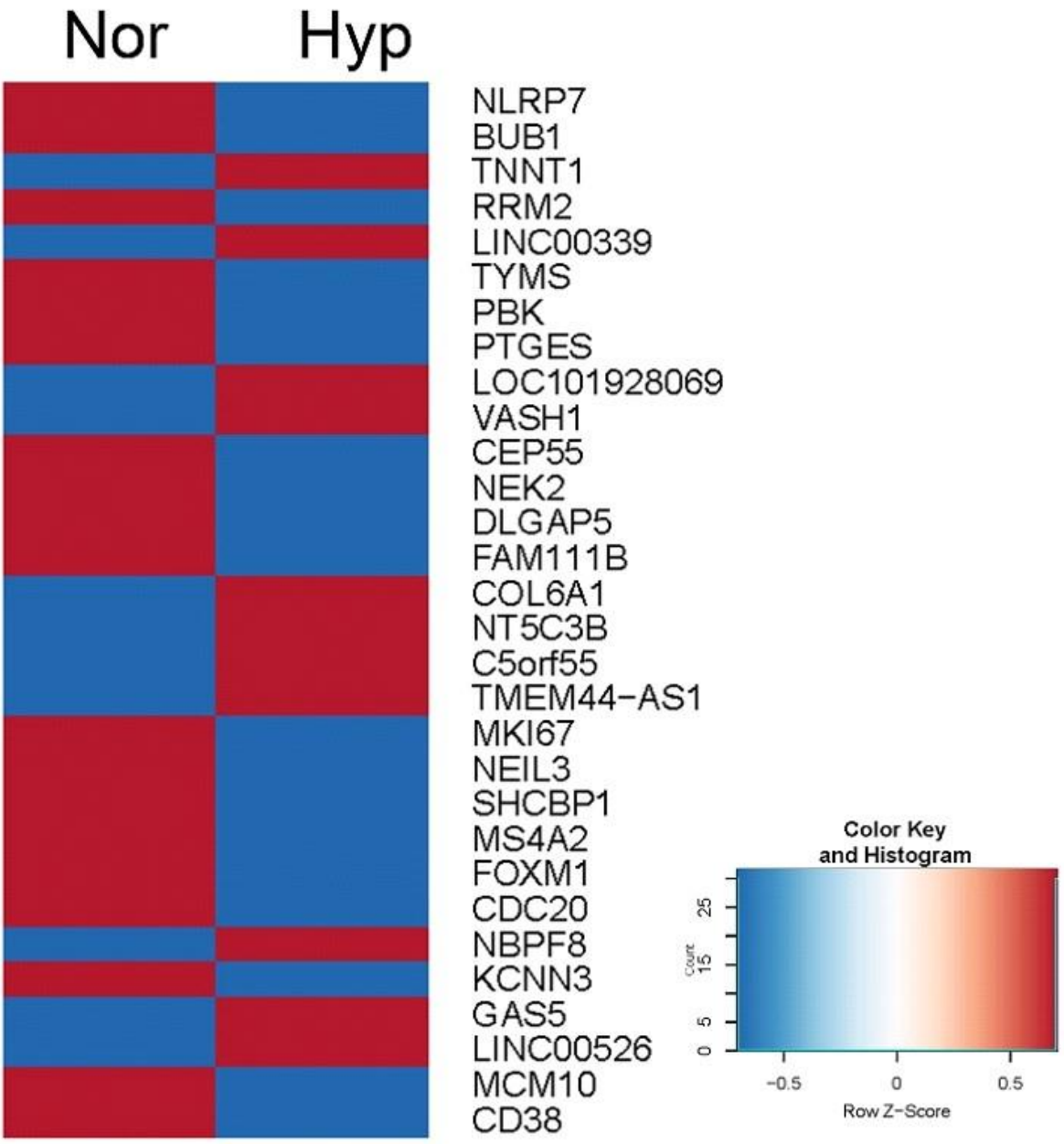


Figure | RNA-Seq data from peripheral blood cells in normotensive (Nor) and hypertensive (Hyp) patients (each group, n=10), showing differential expression of top 30 genes.

Disclosures: F. Mohammed Arif: None. **E. Urbina:** None. **S. Sadayappan:** None. **R. Becker:** None.

Funding: Yes

Funding Component: Great Rivers Affiliate (Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)

P232

Hemodynamic Phenotype and Target Organ Damage in Adolescents With Spurious Hypertension

Authors: Lukasz Obrycki, Dept of Nephrology, Kidney Transplantations and Hypertension, Children`s Memorial Health Inst, Warsaw, Poland; Janusz Feber, Div of Nephrology, Dept of Pediatrics, Children's Hosp of Eastern Ontario, Univ of

Ottawa, Ottawa, ON, Canada; Anna Niemirska, Mieczyslaw Litwin, Dept of Nephrology, Kidney Transplantations and Hypertension, Children`s Memorial Health Inst, Warsaw, Poland

Objective

Spurious hypertension (sHTN) is characterized by elevated systolic blood pressure (BP) in peripheral arteries measured by office BP and ambulatory BP monitoring (ABPM), but normal central BP. The prevalence of sHTN and its clinical significance remains unknown. The aim of the study was to assess hemodynamics and target organ damage (TOD) in adolescents with sHTN in comparison with other forms of hypertension (HTN).

Patients and methods

We analyzed 265 children (54 girls; 15.1 ±2.5 years) referred with arterial HTN, in whom secondary HTN was excluded. We assessed BP levels - office BP, ABPM, central systolic BP (cSBP), hemodynamics - cardiac output (CO), stroke volume (SV) and target organ damage (TOD) - left ventricular mass index (LVMi), carotid intima-media thickness (cIMT), pulse wave velocity (PWV). Anova with TukeyHSD post hoc test was used for analysis.

Results

115 subjects had white coat hypertension (WCH; office BP>95th perc+ABPM<95th perc), 25 had ambulatory prehypertension (preHTN; office BP<95th perc+ABPM<95th perc+ABPM load >25%), 43 had sHTN (office BP+ABPM >95th perc+cSBP<95th perc), 82 had true hypertension (tHTN; office BP+ABPM+cSBP >95th perc. There were no significant differences between groups in age, weight and BMI.

Only pts with tHTN presented features typical of hyperkinetic circulation and pts with preHTN presented intermediate hemodynamic phenotype between WCH and sHTN and those with tHTN ($p<0.001$). Median LVMi ratio to upper limit of normal (g/m$^{2.7}$/95th percentile by Khoury et. 2009) was similar in both WCH and sHTN (0.82 and 0.80, respectively), and significantly lower than in tHTN (0.89, $p=0.04$). The proportion of patients with LVH (LVMi>=95th perc) was 12% in WCH, 4% in preHTN, 9% in sHTN, 22% in tHTN ($p=0.05$).

cIMT Z-scores were also significantly lower in both WCH (0.92) and sHTN (0.90) groups compared to tHTN (1.22, $p=0.01$). Similar results were obtained for PWV Z-scores (WCH=1.8, sHTN=1.29, tHTN=2.28, $p=0.01$). Patients with preHT were not significantly different from tHTN patients in all parameters.

Conclusions

Adolescents with sHTN have similar hemodynamic phenotype and TOD as patients with WCH. However, because of lack of data on the evolution of sHTN, close monitoring and follow-up is warranted.

Disclosures: L. Obrycki: None. **J. Feber:** None. **A. Niemirska:** None. **M. Litwin:** None.

Funding: No

Funding Component:

P233

Blood Pressure Variability and Rhythmicity in Adolescents With Spurious Hypertension

Authors: Lukasz Obrycki, Dept of Nephrology, Kidney Transplantations and Hypertension, Children`s Memorial Health Inst, Warsaw, Poland; Janusz Feber, Div of Nephrology, Dept of Pediatrics, Children's Hosp of Eastern Ontario, Univ of Ottawa, Ottawa, ON, Canada; Anna Niemirska, Mieczyslaw Litwin, Dept of Nephrology, Kidney Transplantations and Hypertension, Children`s Memorial Health Inst, Warsaw, Poland

Objective

Spurious hypertension (sHTN) is characterized by elevated systolic blood pressure (BP) in peripheral arteries but normal central BP. The BP variability and characteristics of BP rhythms in sHTN is unknown. We therefore analyzed BP variability and rhythmicity in adolescents with sHTN in comparison with other forms of hypertension (HTN).

Patients and methods

We studied 265 children (54 girls; 15.1 ±2.5 years) referred with arterial HTN, in whom secondary HTN was excluded. We assessed BP levels - office BP, ABPM, central systolic BP (cSBP), systolic BP average real variability (SBPARV) and mean MAP rhythmicity (amplitudes and acrophases). Anova with TukeyHSD post hoc test was used to compare groups. One tailed t-test was used to compare MAP amplitudes and acrophases with normative values for children with Bonferroni correction for multiple tests.

Results

115 subjects had white coat hypertension (WCH; office BP>95th perc+ABPM<95th perc), 25 had ambulatory prehypertension (preHTN; office BP<95th perc+ABPM<95th perc+ABPM load >25%), 43 had sHTN (office BP+ABPM >95th perc+cSBP<95th perc), 82 had true hypertension (tHTN; office BP+ABPM+cSBP >95th perc. There were no significant differences between groups in age, weight and BMI.

The median SBPARV was 8.87 in WCH, 8.63 in preHTN, 9.25 in sHTN and 9.28 in tHTN (not significantly different between groups). The prevalence of 24h rhythms ranged from 77 to 88%, not significantly from norms or among groups. The prevalence of 12h rhythms was not significantly different compared to norms and ranged from 50 to 60% (NS among groups). There were also no significant differences in the prevalence of 8h and 6h rhythms.

All groups had significantly decreased 24h amplitudes compared to norms, but not significantly different among groups. There were no differences in 12h, 8h and 6 amplitudes between WCH, preHTN, sHTH, tHTN and norms. 24h acrophases were significantly prolonged in sHTN, 12h acrophases were significantly prolonged in preHTN, sHTN and tHTN.

Conclusions

Adolescents with spurious hypertension have significantly lower prevalence of 24h rhythms, decreased 24h amplitudes and prolonged 24h and 12h acrophases suggesting increased sympathetic drive similarly to patients with other hypertensive phenotypes.

Disclosures: L. Obrycki: None. **J. Feber:** None. **A. Niemirska:** None. **M. Litwin:** None.

Funding: No

Funding Component:

P234

Obstructive Sleep Apnea is More Common in Patients With Masked Uncontrolled Hypertension

Authors: Mohammed Siddiqui, Stephen J Thomas, Eric K Judd, Tanja Dudenbostel, Susan Harding, Suzanne Oparil, David A Calhoun, Univ of Alabama at Birmingham, Birmingham, AL

Introduction: Masked uncontrolled hypertension (MUCH) is defined as controlled automated office blood pressure (AOBP) in clinic, but uncontrolled out-of-clinic BP by 24-hr ambulatory blood pressure monitoring (ABPM). Prior studies indicate that the prevalence of masked hypertension in patients with newly diagnosed obstructive sleep apnea (OSA) is nearly 30%. In addition, patients with OSA have a higher prevalence of masked hypertension than patients without OSA.

Aim: Prospective determination of the prevalence and severity of OSA in patients with MUCH.

Methods: In this prospective evaluation, 167 treated hypertensive patients were recruited from the University of Alabama at Birmingham Hypertension Clinic after having controlled BP at three or more clinic visits. All patients were evaluated by AOBP with the BpTRU device, ABPM, and diagnostic polysomnography (PSG) to determine the presence and severity of OSA based on the apnea-hypopnea index (AHI). Out of 153 who completed ABPM, 58 patients were controlled by AOBP and by ABPM, indicating true controlled hypertension and the remaining 95 patients were controlled by AOBP, but uncontrolled by ABPM, indicative of MUCH. 49 true controlled hypertensive and 69 MUCH patients completed PSG.

Results: MUCH patients had a mean AHI of 10.3±13.3 compared to 4.5±10.2 events/hr in true controlled hypertension ($p = 0.045$). Overall, the prevalence of OSA was 48.5% in patients with MUCH compared to 26.5% in true controlled hypertension ($p = 0.01$).

Conclusion: MUCH has significant higher prevalence and severity of OSA compared to true controlled hypertension. These findings suggest that untreated OSA may contribute to the development of MUCH.

	True Controlled Hypertension (n=49)	Masked Uncontrolled Hypertension (n=69)	p-value
Apnea-Hypopnea Index (AHI) *events/hr*	4.5 ± 10.2	10.3 ± 13.3	0.045
None / Minimal Obstructive Sleep Apnea (AHI < 5)	36 (73.5%)	35 (51.5%)	0.013
Obstructive Sleep Apnea (AHI ≥ 5)	13 (26.5%)	33 (48.5%)	0.013
Mild Obstructive Sleep Apnea (AHI ≥ 5 AND < 15)	6 (12.2%)	15 (22.1%)	0.131
Moderate Obstructive Sleep Apnea (AHI ≥ 15 AND < 30)	5 (10.2%)	12 (17.6%)	0.196
Severe Obstructive Sleep Apnea (AHI ≥ 30)	2 (4.1%)	6 (8.8%)	0.269

Disclosures: M. Siddiqui: None. **S.J. Thomas:** None. **E.K. Judd:** None. **T. Dudenbostel:** None. **S. Harding:** None. **S. Oparil:** None. **D.A. Calhoun:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P235

Altered Brain-Gut-Lung Axis in Hypoxia-Induced Pulmonary Hypertension

Authors: Aline C Oliveira, Ravindra K. Sharma, Tao Yang, Victor Aquino, Gilberto O. Lobaton, Andrew J. Bryant, Elaine M. Richards, Mohan K. Raizada, Univ of Florida, Gainesville, FL

Background: Recent evidence indicate that gut microbiota plays important role in pathogenesis and progression of chronic lung diseases such as asthma, respiratory infections and COPD. However, implications of this gut-lung communication and involvement of peripheral and neural immune system in pulmonary hypertension (PH) remains unknown. Objective: Investigate the hypothesis that increased activation of microglial cells, autonomic nervous system, altered gut pathology and gut microbiota are associated with PH suggesting a dysfunctional brain-gut-lung axis in PH pathology. Methods: C57BL/6 (WT) male mice were exposed to chronic hypoxia (10%O2) or Normoxia for 4 weeks in a ventilated chamber (n=5-8/group). Millar pressure catheter was used to measure pulmonary hemodynamics, Iba1 specific antibody for immunohistochemical analysis of microglial cells and fecal 16S rDNA for gut microbiota analysis. Results: Hypoxia induced a 77% increase in right ventricular (RV) systolic pressure (RVSP: N:19.5 ± 2 mmHg Vs H:33.7 ± 2, p<0.001) and RV hypertrophy (RVH: N: 0.139 ± 0.09 Vs H: 0.192± 0.008). This was associated with substantial enhancement in sympathetic activity (LF/HF: N:0.23 ± 0.8 mmHg Vs H: 1.19 ± 0.08, p<0.001) and increase in number of microglial cells in autonomic brain regions, predominantly the paraventricular nucleus of hypothalamus. Characteristics of gut pathology such as ~50% increase in fibrotic area and muscularis layer thickness and significantly decreased villi length and number of goblet cells in small intestine were also observed. Also, principal coordinate analysis of the bacterial composition profile showed a clear separation and clustering of bacterial genera of hypoxic and normoxic mice (ANOSIM p<0.001). Conclusions: (I) Hypoxia-induced pulmonary and cardiac pathophysiology is associated with increased microglia in cardiorespiratory-relevant areas and enhanced sympathetic activity. (II) Fibrotic area and

muscularis layer thickness increased while villi length and number of goblet cells decreased in gut (III) gut microbiota are altered. Together, these observations indicated an altered brain-gut-lung axis in PH.

Disclosures: A. Oliveira: None. **R.K. Sharma:** None. **T. Yang:** None. **V. Aquino:** None. **G.O. Lobaton:** None. **A.J. Bryant:** None. **E.M. Richards:** None. **M.K. Raizada:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; HL102033.

Funding: No

Funding Component:

P236

Sodium Accumulates in the Skin of Patients and Mice With Psoriasis

Authors: Lajos Markó, Experimental Clinical Rsrch Ctr&Dzhk (German Ctr For Cardiovascular Res)&Berlin Inst Of Health (Bih)&Charité, Berlin, Germany; Johannes Wild, Ctr for Cardiology-Cardiology I, Univ Medical Ctr Mainz, Mainz, Germany; Natalia Rakova, Experimental Clinical Rsrch Ctr, Berlin, Germany; András Balogh, Experimental Clinical Rsrch Ctr&Dzhk (German Ctr For Cardiovascular Res)&Berlin Inst Of Health (Bih)&Charité, Berlin, Germany; Elisa Opitz, Experimental Clinical Rsrch Ctr, Berlin, Germany; Peter Linz, Inst of Radiology, Friedrich-Alexander-Univ Erlangen-Nürnberg, Erlangen, Germany; Anna Birukov, Experimental Clinical Rsrch Ctr, Berlin, Germany; Nicola Wilck, Ralf Dechend, Experimental Clinical Rsrch Ctr&Dzhk (German Ctr For Cardiovascular Res)&Berlin Inst Of Health (Bih)&Charité, Berlin, Germany; Jens Titze, Duke-Natl Univ of Singapore, Singapore; Dept of Nephrology and Hypertension&Friedrich-Alexander-Univ Erlangen-Nürnberg, Singapore, Singapore; Markus Kleinewietfeld, VIB Lab of Translational Immunomodulation, VIB Ctr for Inflammation Res (IRC), Hasselt Univ, Diepenbeek, Belgium; Andreas Krause, Andreas Krause, Immanuel Krankenhaus Berlin, Medical Centre for, Germany; Georgios Kokolakis, Sandra Philipp, Dept of Dermatology, Venerology and Allergology, Charité Medical Faculty, Berlin, Germany; Friedrich C Luft, Experimental Clinical Rsrch Ctr&Max Delbruck Ctr For Molecular Med, Berlin, Germany; Michael Boschmann, Experimental Clinical Rsrch Ctr&Berlin Inst Of Health (Bih), Berlin, Germany; Marcus Kelm, Titus Kühne, Inst for Cardiovascular Computer-assisted Med&German Heart Ctr Berlin, Berlin, Germany; Susanne Karbach, Ctr for Cardiology-Cardiology I, Univ Medical Ctr Mainz, Mainz, Germany; Dominik N Müller, Experimental Clinical Rsrch Ctr&Dzhk (German Ctr For Cardiovascular Res)&Berlin Inst Of Health (Bih)&Max Delbruck Ctr For Molecular Med, Berlin, Germany

Sodium can be buffered in the skin, which mechanism is altered during aging and in certain diseases such as hypertension. High salt environment can promote autoimmunity by expanding pathogenic IL-17 producing T helper (Th17) cells. Psoriasis is a relapsing and remitting inflammatory autoimmune disease affecting the skin and joints and involves proinflammatory Th17 cells. Here we tested the hypothesis if psoriatic skin has a higher sodium content in mice and humans.
We used two psoriasis mouse models; the K14-IL-17A$^{ind/+}$ mice overexpressing IL-17A in K14-positive keratinocytes and the imiquimod (IMQ) mouse model by applying 62.5 mg IMQ cream (5%) on the shaved back and ears of FVB/N mice for 5 days daily. End of the study skins of mice were collected, weighted, dried and ashed to measure water and sodium content. Additionally, skin sodium and water content were measured in psoriasis patients and aged matched healthy controls by non-invasive ^{23}Na-MRI on non-affected flexor site of the lower leg and by ^{23}Na-spectroscopy to compare affected and non-affected sites of the leg.
K14-IL-17A$^{ind/+}$ mice had significantly higher sodium content compared to control IL-17A$^{ind/+}$ mice (0.191±0.021 vs. 0.137±0.023 mg/g dry weight) together with an elevated water content. IMQ-treated back skin had significantly higher sodium content compared to untreated ventral skin of the same mice (0.175±0.023 vs. 0.143±0.014 mg/g dry weight), whereas sham mice had a significantly lower content in both regions (0.116±0.010 vs. 0.107±0.005 mg/g dry weight). IMQ treatment led to significant expansion of IL-17 producing γδT cells in the skin, regional lymph nodes and in the spleen with typical skin lesions. Patients with psoriasis area and severity index (PASI) >5 had significantly higher sodium

content in the skin compared to those with lower PASI or with healthy controls (17.73±1.52 vs. 14.32±1.54 vs. 14.30±2.59 AU, respectively); this elevation was water coupled. PASI significantly correlated with skin sodium content (Pearson's r=0.598, P<0.001). Additionally, patients with PASI>5 has higher sodium content in the affected skin compared to non-affected skin of the same patient.
Data from animal models and humans argue for higher sodium accumulation in the inflamed skin.

Disclosures: L. Markó: None. **J. Wild:** None. **N. Rakova:** None. **A. Balogh:** None. **E. Opitz:** None. **P. Linz:** None. **A. Birukov:** None. **N. Wilck:** None. **R. Dechend:** None. **J. Titze:** None. **M. Kleinewietfeld:** None. **A. Krause:** None. **G. Kokolakis:** None. **S. Philipp:** None. **F.C. Luft:** None. **M. Boschmann:** None. **M. Kelm:** None. **T. Kühne:** None. **S. Karbach:** None. **D.N. Müller:** None.

Funding: No

Funding Component:

P237

Elucidation of Blood Pressure Control Function of Essential Hypertension Candidate Gene Lpin1

Authors: koichiro sumida, Nobuhito Hirawa, Keisuke Yatsu, Yousuke Ehara, Yuki Oki, Megumi Fujita, Akira Fujiwara, Yusuke Kobayashi, Hiromichi Wakui, Satoshi Umemura, Kouichi Tamura, yokohama sity university, yokohama, Japan

We have found that the LPIN1 is the candidate gene for essential hypertension (EHT) by genome-wide association analysis in 2007. Mice characterized by loss of body fat, fatty liver, insulin resistance and peripheral neuropathy were discovered in nature and named fatty liver dystrophy (fld) mice in 2000. Recently, the fld mice were shown that they exhibit few LPIN1 genes, and the LPIN1 gene was proved as the cause of phenotype of fld mice. However, the blood pressure (BP) and the alteratios of the vasoactive factors in fld mice have not yet been elucidated. Therefore, to clarify the BP and the effects of LPIN1 gene defeciency, we analyzed the BP and regulatory factors of the fld mice. Furthermore, we transplanted the fat to fld mice, and clarify the importance of adipocytokine on BP in the model mice.
The BP were measured by the tail cuff method and the telemetry method. We performe fat grafting or sham surgery on fld mice, and systolic BP (SBP) was measured by tail cuff method 8 weeks after surgery.
We confirmed that the SBP of fld mice were higher than that of CO by the tail cuff method(fld 134±5 vs CO 111±4 mmHg P<0.05). Furthermore, SBP of the fld mice were higher than that of CO (fld 167.7±9.8 vs CO 133.5±10.9 mmHg P<0.01) by the telemetry method. It was confirmed that the adrenalin and noradrenalin excretions in the urine of fld mice were higher than those of CO(fld 0.133±0.067 vs CO 0.038±0.013 μg/day P<0.01)(fld 0.980 ±0.313 vs CO 0.364±0.080 μg/day P<0.001). There is no significant difference in plasma aldosterone concentration between two groups(fld 434±276.7 vs CO 781±469.5 pg/mL n.s).The SBP tended to decrease with the fat trasplantation in fld mice (fat transplanted 103.7±4.41 vs sham 114.7±7.67 mmHg P=0.056).
We confirmed that fld mouse exhibits higher SBP by both tailcuff method and telemetry method. Because the fld mice exhibit higher PR and high levels of CA concentration and excretion in urine, it was suggested that the sympathetic nervous systems of fld mice were activated. Sympathetic nervous system activity may be related to an increase in BP of fld mouse, and further research is under way. Since it showed that BP decreased by fat grafting, the visceral fat are essential for fld mice to BP control. The implications of adipokine and other fats are under consideration.

Disclosures: K. sumida: None. **N. Hirawa:** None. **K. Yatsu:** None. **Y. Ehara:** None. **Y. Oki:** None. **M. Fujita:** None. **A. Fujiwara:** None. **Y. Kobayashi:** None. **H. Wakui:** None. **S. Umemura:** None. **K. Tamura:** None.

Funding: No

Funding Component:

P238

Cerebral Ischemia With Amyloid-Beta Infusion Deteriorates Cognitive Decline ~Possible Amelioration of Cognitive Function by AT_2 Receptor Activation~

Authors: Li-Juan Min, Masaki Mogi, Hui-Yu Bai, Bao-Shuai Shan, Akinori Higaki, Jun Iwanami, Masatsugu Horiuchi, Ehime Univ Sch Of Med, Tohon, Ehime, Japan

Objectives: Amyloid-β (Aβ) deposition in brain and cerebral vessels accelerates the pathogenesis of Alzheimer's disease and vascular cognitive impairment. We examined possibilities that cerebral ischemia worsens Aβ infusion-mediated cognitive decline, and that angiotensin II type 2 (AT_2) receptor stimulation in vascular smooth muscle cells (VSMC) could ameliorate this cognitive impairment induced by cerebral ischemia and Aβ.
Methods: Adult male wild-type mice (WT) and the mice with VSMC-specific AT_2 receptor overexpression ($smAT_2$) were used. Mice were subjected to intracerebroventricular (ICV) injection of Aβ1-40. Cerebral ischemia was induced by 15 minutes of bilateral common carotid artery occlusion (BCCAO) 24 hours after Aβ injection. Cognitive function was evaluated by Morris water maze test 3 weeks after Aβ injection.
Results: ICV injection of Aβ 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 did not decline significantly cognitive function. In contrast, BCCAO following Aβ injection exhibited more marked cognitive impairment (84.27±8.00 sec) compared to Aβ injection alone in concert with the increases in superoxide anion production, NADPH oxidase activity, expressions of NADPH oxidase subunit p^{22phox}, p^{40phox} and inflammatory cytokines such as MCP-1, IL1-β in the hippocampus. BCCAO following Aβ injection significantly enhanced the expression of Aβ clearance factor, RAGE (receptor for advanced glycation end product). Aβ 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 increases in oxidative stress, inflammation markers and RAGE expression, pyknosis, which were induced by Aβ injection with/without BCCAO in WT.
Conclusion: Cerebral ischemia exaggerated Aβ-induced cognitive decline with possible involvements of enhanced oxidative stress, inflammation, neuronal degeneration, and breakdown of RAGE-mediated Aβ clearance. AT_2 receptor activation in VSMC could play preventive roles in this cognitive decline.

Disclosures: L. Min: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; JSPS Kakenhi Number 26860567. **M. Mogi:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; JSPS Kakenhi Number 25462220. **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); Modest; JSPS Kakenhi Number 15K19974. **M. Horiuchi:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Nippon Boehringer Ingelheim Co. Ltd..

Funding: No

Funding Component:

P239

Toll-Like Receptors 9 (TLR9) Play a Key Role in Cardiac and Vascular Dysfunction Associated With 2-Kydney 1-Clip (2K1C) Hypertension in Mice

Authors: Fernanda Luciano Rodrigues, Univ of Sao Paulo, Ribeirão Preto, Brazil; Ana Carolina Mieko Omoto, Univ of Sao Paulo, Ribeirao Preto, Brazil; Rafael Menezes da Costa, Univ of Sao Paulo, Ribeirão Preto, Brazil; Carlos Alberto Aguiar da Silva, Univ of Sao Paulo, Ribeirao Preto, Brazil; Gisele Facholi Bomfim, Federal Univ of Mato Grosso, Sinop, Brazil;

Fernando Silva Carneiro, Rita C. Tostes, Rubens Fazan Junior, Univ of Sao Paulo, Ribeirão Preto, Brazil; **Helio C Salgado**, Univ of Sao Paulo, Ribeirao Preto, Brazil

Introduction: Renovascular hypertension is the leading cause of secondary hypertension and is also involved in the physiopathogenesis of resistant hypertension. The activation of toll-like receptors 9 (TLR9) was demonstrated to contribute to the increase in arterial pressure (AP) in spontaneously hypertensive rats. We hypothesize that the TLR9 are also activated in two-kidney one- clip (2K1C) hypertensive mice, eliciting cardiac and vascular dysfunction; therefore, contributing to the increase in AP. **Methods and results:** C57BL (WT) and TLR9 knockout (TLR9_KO) mice were anesthetized with isoflurane and submitted to 2K1C hypertension by placing a silver clip (0.12 mm) around the left renal artery. After 4 weeks, the cardiac function of mice was evaluated by echocardiography in anesthetized subjects. Following, the direct AP measurement was carried out and the third-order mesenteric resistance arteries (MRA) were removed for vascular function evaluation. All data were compared to Sham-operated WT mice. The AP was remarkably elevated in 2K1C WT mice (133±2 vs 93 ± 4 mmHg), whereas this increase was partially prevented by the absence of the TLR9 (114±5 mmHg). 2K1C hypertension caused cardiac dysfunction in WT mice, displaying decreased ejection fraction (42.6±3.1 vs 52.7±1.5%), fractional shortening (10.1±0.7 vs 13.7±0.9%), stroke volume (25.9±2.2 vs 37.9±1.9 µL) and cardiac output (9.2±0.8 vs 14.6±0.8 mL/min); however, the 2K1K hypertension did not affect the cardiac function in TLR9_KO mice: ejection fraction (54.5±1.6%), fractional shortening (14.5±1.1%), stroke volume (43.4±4.6 µL) and cardiac output (15.2±1.6 mL/min). Vascular dysfunction, characterized by increased contractile response to phenylephrine (Emax: 167.2±2.2 vs 125.9±3.5%) and reduced relaxation to acetylcholine (Emax: 69.3±1.6 vs 87.6±1.4%) and sodium nitroprusside (pD2: 7.3±0.07 vs 7.9±0.05), was observed in 2K1C WT mice, but not in 2K1C TLR9_KO mice (Emax: 127.7±2.9%, 88.5±2.0% and pD2: 7.78±0.06, to phenylephrine, acetylcholine and sodium nitroprusside). **Conclusions:** TLR9 is involved in both cardiac and vascular dysfunctions of 2K1C mice and also in the increase of AP of this experimental model.

Disclosures: F. Rodrigues: None. **A. Omoto:** None. **R. Menezes da Costa:** None. **C. Aguiar da Silva:** None. **G. Bomfim:** None. **F. Carneiro:** None. **R. Tostes:** None. **R. Fazan Junior:** None. **H.C. Salgado:** None.

Funding: No

Funding Component:

P240

Identifying the Angiotensin AT2-Receptor Coupled Phosphoproteome in Human Aortic Endothelial Cells by Time-Resolved, Quantitative Phosphoproteomics

Authors: Augusto B Peluso, Stefan J Kempf, Univ of Southern Denmark, Odense C, Denmark; Thiago Verano Brega, Federal Univ of Minas Gerais Brazil, Belo Horizonte, Brazil; Michael Bader, Max Delbrück Ctr for Molecular Med, Berlin-Buch, Germany; Martin R Larsen, Univ of Southern Denmark, Odense C, Denmark; Robson A Santos, Federal Univ of Minas Gerais Brazil, Belo Horizonte, Brazil; **Ulrike Muscha Steckelings**, Univ of Southern Denmark, Odense C, Denmark

Objectives: The angiotensin AT2-receptor (AT2R) is a main component of the protective arm of the renin-angiotensin system. In order to get new and deeper insights into AT2R mediated signalling, this study mapped the AT2R-coupled phosphoproteome in human aortic endothelial cells (HAEC) by quantitative phosphoproteomics.
Methods: HAEC were stimulated with the AT2R agonist C21 (1 µM) for 1, 3, 5 or 20 minutes. Following protein digestion and TMT peptide labelling, samples were subjected to TiO2−SIMAC phospho-peptide enrichment and LC−MS/MS. Data were analysed using Proteome Discoverer and the human sequence libraries SwissProt and UniProt. For confirmation of phosphoproteomics results, expression of p53 and phosphorylation of HDAC-1 were determined by Western Blot. Cellular translocation of HDAC-1 and p53 was assessed by immunofluorescence and activity of HDAC-1 by deacetylation assay. HAEC proliferation was determined by resazurin assay and apoptosis by caspase 3/7 assay.

Results: AT2R stimulation of HAEC changed the phosphorylation status of 265 proteins at 471 sites (281 phosphorylations and 190 dephosphorylations). Gene Ontology and STRING analysis revealed the predominant phospho-modification of proteins involved in anti-proliferation, cell differentiation and apoptosis including HDAC-1, p53, BCL2 or HSP90. AT2R-mediated inhibition
of HDAC-1 and activation of p53 were confirmed by additional experiments demonstrating changes in cellular localisation of HDAC-1 and p53, in total expression of p53, and in enzymatic activity and phosphorylation of HDAC1. Conclusion: This study mapped the AT2R coupled phospho-signalling network. It identified dephosphorylation and deactivation of HDAC-1 as well as nuclear importation and increased expression of p53 as novel, potential mediators of AT2R-coupled anti-proliferation.

Disclosures: A.B. Peluso: None. **S.J. Kempf:** None. **T. Verano Brega:** None. **M. Bader:** None. **M.R. Larsen:** None. **R.A.S. Santos:** None. **U.M. Steckelings:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; undirected research grant from Vicore Pharma. C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; free drug supply of C21 by Vicore Pharma.

Funding: No

Funding Component:

P241

Involvement of Mitochondrial Telomerase Reverse Transcriptase in the Restoration of Nitric Oxide Levels by Angiotensin-(1-7) in the Dysfunctional Diabetic CD34$^+$ Cells

Authors: Shrinidh Joshi, NDSU, Fargo, ND; Sam Gomez, Wendy Cantu, Julio Quiroz, Museum District Eye Clinic, Houston, TX; Andreas Beyer, Medical Coll Wisconsin, Milwaukee, WI; Charles A Garcia, Museum District Eye Clinic, Houston, TX; Yagna P Jarajapu, NDSU, Fargo, ND

Telomerase reverse transcriptase (TERT) has been shown to translocate to mitochondria (mtTERT), and decrease the generation of mitochondrial reactive oxygen species (mtROS). Angiotensin (Ang)-(1-7) stimulates vasoreparative functions in diabetic CD34$^+$ stem/progenitor cells in part by decreasing ROS levels and increasing nitric oxide (NO) bioavailability. This study tested the involvement of mtTERT in mediating the effect of Ang-(1-7) on NO levels in diabetic CD34$^+$ cells. 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) (DB: HbA1C 6.5-11.2). Cells were treated with Ang-(1-7) (100nM) or Stromal derived factor-1α (SDF) and evaluated for NO, and mtROS levels by flow cytometry by using DAF-FAM and mitoSOX, respectively. Number of cells used was ≥5x10^4 per treatment. The Mean Fluorescence Intensity is expressed in arbitrary fluorescence units (AFIx10^5). Inhibitors of Mas receptor and TERT, A779 and BIBR-1532, respectively, and decoy peptides that inhibit mitochondrial translocation or nuclear transport of TERT, mtXTERT and nucXTERT, respectively, were used. SDF (100nM) failed to stimulate NO generation in DB-cells (1.2±0.07 vs basal 0.8±0.05, n=10) compared to ND-cells (3.5±0.2 vs basal 1.6±0.2) (n=10, P<0.0001). Ang-(1-7) induced NO levels in ND- (2.9±0.2) and restored NO generation in DB-cells (2.2±0.1) (n=10). DB-cells have increased mitoROS (3.6±0.4) compared to ND-cells (1.7±0.1, P<0.001, n=7) and Ang-(1-7) normalized the mitoROS in DB-cells (1.8±0.3, n=7). The effects of Ang-(1-7) on NO and mitoROS levels were reversed by A-779 or BIBR-1532 (n=5). Decoy peptide mtXTERT not nucXTERT increased mitoROS in ND- (4.4±0.7 vs basal 1.8±0.2, P<0.01) and in DB-cells (12±1 vs basal 6±0.4, P<0.001) (n=5). The decreasing effect of Ang-(1-7) on mitoROS (1.6±0.3) was reversed by mtXTERT (10±0.9, P<0.001, n=5) but not by nucXTERT. Along similar lines, restoration of NO levels by Ang-(1-7) in DB-cells was reversed by mtXTERT but not by nucXTERT. These observations provide compelling evidence for the involvement of TERT in stimulating the vasoreparative functions of diabetic stem/progenitor cells by Ang-(1-7)/Mas pathway.

Disclosures: **S. Joshi:** None. **S. Gomez:** None. **W. Cantu:** None. **J. Quiroz:** None. **A. Beyer:** None. **C.A. Garcia:** None. **Y.P.R. Jarajapu:** None.

Funding: Yes

Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

P242

Effects of Proton Receptor Gpr4 Deficiency on Angiotensin Receptors in Kidney and Hindbrain

Authors: Snezana Petrovic, Ellen Tommasi, Xuming Sun, Debra I Diz, Wake Forest Sch of Med, Winston-Salem, NC

The proton receptor GPR4, a G protein-coupled receptor with protons as ligands, is abundant in the brain [subfornical organ (SFO) and paraventricular nucleus (PVN) of the hypothalamus], lung, and kidney. The receptor is activated in physiological pH range and GPR4 deletion is associated with chronic mild acidosis. The GPR4-/- mice exhibit ~15 mm Hg lower systolic blood pressure accompanied by ~50% lower angiotensin lower AT_1 receptors in SFO and PVN. We now report data for angiotensin receptor binding in the kidney and hindbrain using *in vitro* receptor autoradiography with ^{125}I-Sarthran as the ligand in GPR4-/- (n = 3 kidney, n = 4 hindbrain) and wild type (GPR4+/+, n = 3 kidney, n = 4 hindbrain) mice. GPR4-/- mice have lower ^{125}I-Sarthran specific maximal binding in the renal medullary region (43 ± 16 vs. 127 ± 20 fmol/mg protein in GPR4+/+; p = 0.03), which is the region with highest expression of GPR4. There was a trend for lower binding, although not significant, in the vasa recta area (78 ± 17 vs. 177 ± 46 fmol/mg protein in GPR4+/+; p = 0.12), glomeruli (414 ± 48 vs. 522 ± 17 fmol/mg protein in GPR4+/+; p = 0.10) and tubules (250 ± 44 vs. 350 ± 28 fmol/mg protein in GPR4+/+; p = 0.13). There were no differences in binding maximal density in brain medullary regions such as nucleus tractus solitarii (NTS), area postrema (AP), dorsal motor nucleus of the vagus (DMV) or olivary complex (OC). Non-specific binding was similar in GPR4+/+ and -/- mice in both kidney and brain. AT_1 receptors as defined by competition with losartan account for the majority of binding in each of the regions in mouse kidney (>70%), while the brain medullary binding exhibits ~50% AT_1 and AT_2 sites. Thus, GPR4 deletion lowers AT_1 receptors in tissues involved in control of blood pressure and fluid and electrolyte balance, such as forebrain nuclei and renal medullary regions where there is high abundance of both AT_1 and GPR4 receptors. Whether the down-regulation of the AT_1 receptor at these sites is a direct effect in response to loss of activation of the GPR4 or is secondary to the acid-base balance alterations in these animals is unknown. However, the lower blood pressure in the GPR4-/- mice supports the concept that the GPR4 receptor is a potential therapeutic target for lowering of pressure.

Disclosures: **S. Petrovic:** None. **E. Tommasi:** None. **X. Sun:** None. **D.I. Diz:** None.

Funding: No

Funding Component:

P243

Vascular Drp1 Mediates Angiotensin II-Induced Cardiovascular Hypertrophy via Global as Well as Selective de novo Protein Synthesis.

Authors: Tatsuo Kawai, Hannah A Cooper, Temple Univ Sch of Med, Philadelphia, PA; Steven J Forrester, Emory Univ, Atlanta, GA; Michael J Boyer, Kathy J Elliott, Kyle Preston, Temple Univ Sch of Med, Philadelphia, PA; Yayoi Kimura, Yokohama City Univ, Yokohama, Japan; Hiromi Sesaki, Johns Hopkins Univ Sch of Med, Baltimore, MD; Rosario Scalia, Victor Rizzo, Satoru Eguchi, Temple Univ Sch of Med, Philadelphia, PA

Mitochondrial fission has been implicated in various cardiovascular and metabolic diseases but the role of the mitochondrial fission inducer Drp1 in regulating hypertension and cardiovascular remodeling remains unclear. To study the involvement of Drp1 in cardiovascular hypertrophy, rat aortic vascular smooth muscle cells (VSMCs) are infected with dominant negative (dn) Drp1 adenovirus and stimulated with 100 nM angiotensin II (AngII). In vivo, tamoxiphen-inducible SMMHC-Cre+/- Drp1 floxed mice and control mice are infused with AngII (1000ng/kg/min) for 2 weeks. In VSMCs, AngII-induced mitochondrial fission was attenuated with AT1 blocker RNH6270 (ARB) as well as with dnDrp1 (100 moi). ARB and dnDrp1 treatment also inhibited AngII-enhanced global as well as specific de novo protein synthesis evaluated by SunSet assay. To identify potential mediators downstream of Drp1-mitochondrial fission contributing to VSMC hypertrophy, shotgun proteomic analysis was performed on proteins extracted from AngII or vehicle treated VSMC with dnDrp1 or control adenovirus infection. 22 exclusively regulated proteins induced by AngII and attenuated by dnDrp1 were identified including previously known AngII targets, HMGB1 and PAIRB. In addition, ARB and dnDrp1 inhibited AngII-induced enhancement of mitochondrial ox-phosphorylation and oxidative stress (assessed with mito-timer adenovirus). In vivo, VSMC specific DRP1 silencing suppressed AngII-induced cardiac hypertrophy assessed with heart weight/body weight ratio Mean±SEM: 6.33±0.14 vs 5.25±0.07 ($p<0.001$) as well as with echocardiogram: LVPWd 1.46±0.02 vs 0.81±0.03 ($p<0.001$). However, both control and the Drp1 silenced mice developed hypertension when infused with AngII as assessed by DSI radio-telemetry system. In conclusion, these data suggest that Drp1 mediates AngII-induced global as well as specific de novo protein synthesis thereby contributing to cardiovascular hypertrophic remodeling.

Disclosures: T. Kawai: None. **H.A. Cooper:** None. **S.J. Forrester:** None. **M.J. Boyer:** None. **K.J. Elliott:** None. **K. Preston:** None. **Y. Kimura:** None. **H. Sesaki:** None. **R. Scalia:** None. **V. Rizzo:** None. **S. Eguchi:** None.

Funding: Yes

Funding Component: Great Rivers Affiliate (Delaware, Kentucky, Ohio, Pennsylvania & West Virginia)

P244

Hypoxic Stimulation of Vasoreparative Functions in Human CD34$^+$ cells is Mediated by Angiotensin Converting Enzyme-2 and Mas Receptor

Authors: Shrinidh Joshi, Estelle Leclerc, Yagna P Jarajapu, NDSU, Fargo, ND

CD34$^+$ stem/progenitor cells have the propensity of re-endothelialization and vascular regeneration. Angiotensin Converting Enzyme-2 (ACE2) generates Angiotensin (Ang)-(1-7), which produces vasoprotection by acting on Mas receptor (MasR). Hypoxic preconditioning has been shown to stimulate vascular repair-relevant functions of CD34$^+$ cells, which was impaired in MasR-deficient HSPCs. The current study tested the hypothesis that hypoxic stimulation of CD34$^+$ cell functions are mediated by ACE2 and MasR. CD34$^+$ cells were isolated from mononuclear cells (MNCs) derived from healthy volunteers (n=46). Cells were exposed to normoxia (20% O_2) or hypoxia (1% O_2). Protein and mRNA expressions of ACE2 and MasR were determined. ACE2 activity was determined by using an enzyme-specific fluorogenic substrate, and a selective inhibitor, MLN4760. CD34$^+$ cells were transduced with lentiviral particles carrying ACE2- or MasR- or scramble-3'-UTR (Scr) fused downstream to firefly luciferase reporter (flr) gene. Co-transfection luciferase assay was performed using MicroRNA, miR-421 or miR-143 with ACE-2 or MasR luciferase construct, respectively. Luciferase activity was determined by using luciferase assay kit. Hypoxia stimulated mRNA and protein expressions of ACE2 and MasR in CD34$^+$ cells (54.9±4.8% and 51.6±14.7%, respectively, higher than normoxia, n=5, $P<0.05$) but not in MNCs. No changes in ACE or AT1 receptor expressions were observed. Effects of hypoxia were blocked by 2-methoxyestradiol (0.5μM), an inhibitor of hypoxia-inducible factor-1α (n=5, $P<0.01$). Luciferase activity was increased by hypoxia in cells expressing ACE2- or MasR-FLR (258±23% and 214±19%, respectively, of Scr, n=6, $P<0.001$). Effect of hypoxia is recapitulated by vascular endothelial growth factor (100 nM) (n=5, $P<0.01$ vs untreated) or stromal-derived factor-1α (100 nM) under normoxia (n=5, $P<0.05$ vs untreated). Axitinib (30 nM), a nonspecific inhibitor of VEGFR, or AMD3100

(10 μM), a specific CXCR4 inhibitor, decreased the luciferase activity in ACE2- or MasR-FLR expressing cells (n=4, P<0.01). Collectively, these observations provide compelling evidence for the hypoxic upregulation of ACE2 and MasR in $CD34^+$ cells, which contribute to their vasoreparative functions.

Disclosures: S. Joshi: None. **E. Leclerc:** None. **Y.P. Jarajapu:** None.

Funding: No

Funding Component:

P245

The Angiotensin-(1-7) Agonist AVE0991 Attenuates Angiotensinogen Release from Human Proximal Epithelial Cells

Authors: Mark C Chappell, Wake Forest Sch of Med, Winston-Salem, NC

A local renin-angiotensin system (RAS) is evident within the renal proximal tubules and overexpression of the precursor angiotensinogen targeted to the tubules increases blood pressure and promotes renal injury. Tubular angiotensinogen is enhanced by the Ang II-AT1 receptor axis, oxidative stress and pro-inflammatory cytokines (IL-6, TGFβ), as well as hyperglycemic conditions. Stimulation of angiotensinogen by the Ang II-AT1 receptor pathway constitutes a positive-feedback loop that may promote a sustained increase in blood pressure and renal damage. Mediators that promote angiotensinogen also attenuate the expression of the ACE2-Ang-(1-7) axis. Downregulation of the Ang-(1-7) axis and its associated signaling pathways may contribute to greater expression of angiotensinogen within the kidney. To examine this potential pathway, we assessed the chronic effects of the Ang-(1-7) agonist AVE0991 on angiotensinogen release in human HK-2 proximal epithelial cells maintained under mild hyperglycemic conditions. HK-2 cells were placed in serum-free media and then treated with varying doses of the AVE0991 agonist (0.1 nM to 1 uM) for up to 48 hours. Human angiotensinogen was quantified in the cell media by ELISA with a sensitivity of 30 picogram (pg). Basal release of angiotensinogen at 48 hrs was 1.47 ± 0.28 ng/ml (n=4). Addition of 0.1 nM AVE0991 attenuated angiotensinogen release by 23 ± 4%, p<0.05 with a maximal inhibitory effect of 48 ± 9%, p<0.05 [n=3]. AVE0991 at the maximal dose had no detrimental effect on cell viability. The NOS inhibitor LNAME (100 uM) failed to block the inhibitory effect of AVE0991 and LNAME alone did not influence release. Addition of the AT2R agonist C21 (1 uM) had no effect on angiotensinogen release. The Ang-(1-7) receptor antagonist [D-Pro^7]-Ang-(1-7) blocked the inhibitory effects of AVE0991; however, [D-Ala^7-Ang-(1-7)(A779), losartan, PD123319 or the kinin B2 receptor HOE140 had no effect. We conclude that the Ang-(1-7) axis may exert negative feedback on the tubular angiotensin system and that the renoprotective effects of AVE0991 may reflect attenuation of angiotensinogen release. Furthermore, the inhibitory actions of AVE0091 on the tubular release of angiotensinogen appear to be independent of the NOS-NO pathway.

Disclosures: M.C. Chappell: None.

Funding: Yes

Funding Component: Mid-Atlantic Affiliate (Maryland, North Carolina, South Carolina, Virginia & Washington, DC)

P246

Increased Urinary Prorenin and sPRR Excretion in Early Type 1 Diabetes

Authors: Nirupama Ramkumar, Deborah Stuart, Donald E Kohan, Univ of Utah, Salt Lake Cty, UT

Activation of the intra-renal, rather than systemic renin angiotensin system (RAS) has been suggested to play a role in the development of diabetic kidney disease. In this study, we examined plasma and urinary prorenin/renin levels and

urinary soluble prorenin receptor (sPRR) levels in a murine model of Type 1 diabetes. Male C57BL/6 mice were injected with streptozotocin (STZ) 50mg/kg at 3 months of age and metabolic balance studies conducted at 6 and 10 weeks post-injection. STZ injected mice developed hyperglycemia within 4 weeks of injection (STZ: 335 ± 35 vs control: 145 ± 22 mg/dL) and remained hyperglycemic at 10 weeks. At 10 weeks post-injection, STZ injected mice had significant polyuria (STZ: 20.9 ± 5.9 vs controls 1.8 ± 0.4 ml/day), increased water intake (STZ: 26.2 ± 5.9 vs controls: 6.2 ± 0.9 ml/day) and lower body weight (STZ: 28.3 ± 1.3 vs controls: 23.6 ± 1.4 grams) despite similar food intake. Compared to controls, urinary angiotensinogen, total prorenin/renin and sPRR levels were increased in STZ injected mice; these increases were proportionally greater than the trend in increased urinary microalbumin excretion (Table 1). In particular, urinary sPRR excretion was markedly increased in STZ injected mice compared to controls. In a separate experiment, 10 week old male Akita mice bearing a mutated *Ins2* gene, demonstrated a 4.8 fold increase in renal medullary renin mRNA expression and a 1.5-fold increase in renal medullary PRR expression. Taken together, these results demonstrate early activation of the intra-renal RAS in Type 1 diabetes. These changes occur independent of the systemic RAS.

Table 1. Plasma and urine RAS parameters at 10 weeks post-STZ injection

	Control (N=6)	STZ injected (N=5)	P value
Plasma renin concentration (ng/ml/hr Ang-I)	134.6 ± 36.7	75.6 ± 12.6	0.09
Urine microalbumin (µg/day)	32.7 ± 7.3	69.5 ± 26.1	0.07
Urine angiotensinogen (ng/day)	133.6 ± 9.8	556.2 ± 160	0.005
Urine total prorenin/renin (ng/day)	5.7 ± 2.2	19.0 ± 4.8	<0.001
Urine sPRR (µg/day)	ND	67.1 ± 22.3	<0.001

Disclosures: N. Ramkumar: None. **D. Stuart:** None. **D.E. Kohan:** None.

Funding: No

Funding Component:

P247

ACE2/ACE Imbalance in the Placental Circulation in an Ovine Model of Intrauterine Growth Restriction

Authors: Shrinidh Joshi, Caleb O Lemley, Kimberly A Vonnahme, Yagna P Jarajapu, NDSU, Fargo, ND

Intrauterine growth restriction (IUGR) has been linked to the development of cardiovascular complications in the offspring. We have previously shown that in an ovine model of IUGR placental blood flow, in both umbilical and uterine artery, was decreased in diet-restricted dams. Angiotensin-converting enzyme (ACE), its metabolite Ang II and the receptor AT1 constitute the vascular-detrimental axis or renin angiotensin system, while ACE2, its metabolite Ang-(1-7) and Mas receptor constitute vasoprotective axis. This study tested the hypothesis that IUGR induces ACE2/ACE imbalance in the placental circulation. In ewe lambs, IUGR was accomplished by providing 60% of the nutrient requirement (RES) while the control group received adequate nutrients (CON) from day 50 to day 130 of gestation. At day 130, plasma samples were collected from umbilical artery (Um-A) and vein (Um-V), uterine artery (Ut-A) and vein (Ut-V), and jugular vein (J-V). ACE and ACE2 enzyme activities were determined by using enzyme-specific fluorogenic substrates, and enzyme-selective inhibitors, captopril and MLN-4760, respectively. Activities were expressed as percent inhibition by the respective inhibitors. ACE2 activity is decreased in the plasma obtained from Um-A (16±2), Um-V (29±4) and Ut-V (31±3) but not in Ut-A of RES group, compared to the CON group (Um-A 25±3, Um-V 42±2 and Ut-V 43±2, $P<0.05$, $P<0.02$ and $P<.01$, respectively, n=5). This resulted in decreased ACE2/ACE (CON: Um-A 0.43±0.03, Um-V 0.84±0.08 and Ut-V 0.82±0.05, and RES: Um-A 0.26±0.02, Um-V 0.46±0.05 and Ut-V 0.5±0.03, $P<0.002$, $P<0.004$, $P<0.001$, respectively). ACE activity was unchanged (n=5) in all four samples of placental circulation. In the J-V plasma ACE2 was decreased (24±2 vs 31±6, NS) and ACE was increased (68±4 vs 54±6, $P<0.05$) in RES group compared to CON (n=5). This resulted in decreased ACE2/ACE (CON: 0.58±0.04 and RES: 0.3±0.02). These observations suggest that IUGR induces ACE2/ACE imbalance. In the maternal systemic circulation it is largely due to increased ACE, while in the

placental circulation it is due to decreased ACE2. Hyperactivity of ACE/Ang II/AT1 receptor pathway due attenuated opposing activity of ACE2 may restrict blood flow to the placental tissues during development.

Disclosures: S. Joshi: None. **C.O. Lemley:** None. **K.A. Vonnahme:** None. **Y.P. Jarajapu:** None.

Funding: No

Funding Component:

P248

Overexpression of an Intracellular Angiotensin II Fusion Protein Selectively in the Mitochondria of Mouse Proximal Tubule Cells Induces Mitochondrial Stress and Glycolytic Responses: Roles of Nitric Oxide and Superoxide

Authors: Xiao C Li, Univ Mississippi Medical Ctr, Jackson, MS; Julia L Cook, Ochsner Clinic Fndn, New Orleans, LA; **Jia L Zhuo**, Univ Mississippi Medical Ctr, Jackson, MS

Endocrine and paracrine angiotensin II **(**ANG II) activates cell surface AT_1 receptors to induce mitochondrial superoxide (O_2^-) production and uncoupling of eNOS, leading to ANG II-induced renal and hypertensive injury. Little is known, however, whether ANG II is internalized to the mitochondria to activate AT_1 or AT_2 receptors. We tested the hypothesis that ANG II in the mitochondria exerts important dual roles on mitochondrial stress and glycolytic responses via the AT_{1a}/NADPH oxidase/O_2^- and AT_2/eNOS/NO signaling pathways. To test the hypothesis, a mitochondria-targeting ANG II protein (mito-ECFP/ANG II) was expressed selectively in the mitochondria of mouse proximal tubule cells, and treated with or without AT_1 receptor blocker losartan (10 μM), AT_2 receptor blocker PD123319 (10 μM), a non-selective NOS inhibitor L-NAME (10 mM), or a scavenger of mitochondrial superoxide, mito-TEMPO (10 μM) for 48 h, respectively. The mitochondrial stress and glycolytic responses were then determined using Seahorse XF^e24 Extracellular Flux Analyzer. Expression of mito-ECFP/ANG II alone significantly increased oxygen consumption rate (OCR) (Control: 259.8 ± 52.2 vs. mito-ECFP/ANG II: 512.5 ± 75.6 pmol/min; $p<0.01$, n=8) and extracellular acidification rate (ECAR) (Control: 13.5 ± 2.2 vs. mito-ECFP/ANG II: 19.3 ± 2.4 mpH/min; $p<0.01$, n=8), respectively. These mito-stress responses were blocked by losartan ($p<0.01$, n=8), but not by PD123139 (*n.s.*). Furthermore, the mito-stress response of mito-ECFP/ANG II was significantly attenuated by mito-TEMPO (288.1 ± 10.3 pmol/min; $p<0.01$, n=8), and augmented by L-NAME (623.5 ± 8.7 pmol/min, $p<0.05$, n=8). Finally, the mito-stress response to mito-ECFP/ANG II expression was associated with significant increases in mitochondrial redox carries, Complex I (NADH coenzyme Q reductase), Complex II (succinate dehydrogenase), Complex III (cytochrome bc_1 complex) and Complex IV proteins (cytochrome c oxidase) ($p<0.01$). We concluded that ANG II in the mitochondrial induces important mitochondrial stress and glycolytic responses primarily via the AT_{1a}/NADPH oxidase/O_2^- signaling pathways in mouse proximal tubule cells.

Disclosures: X.C. Li: None. **J.L. Cook:** None. **J.L. Zhuo:** None.

Funding: No

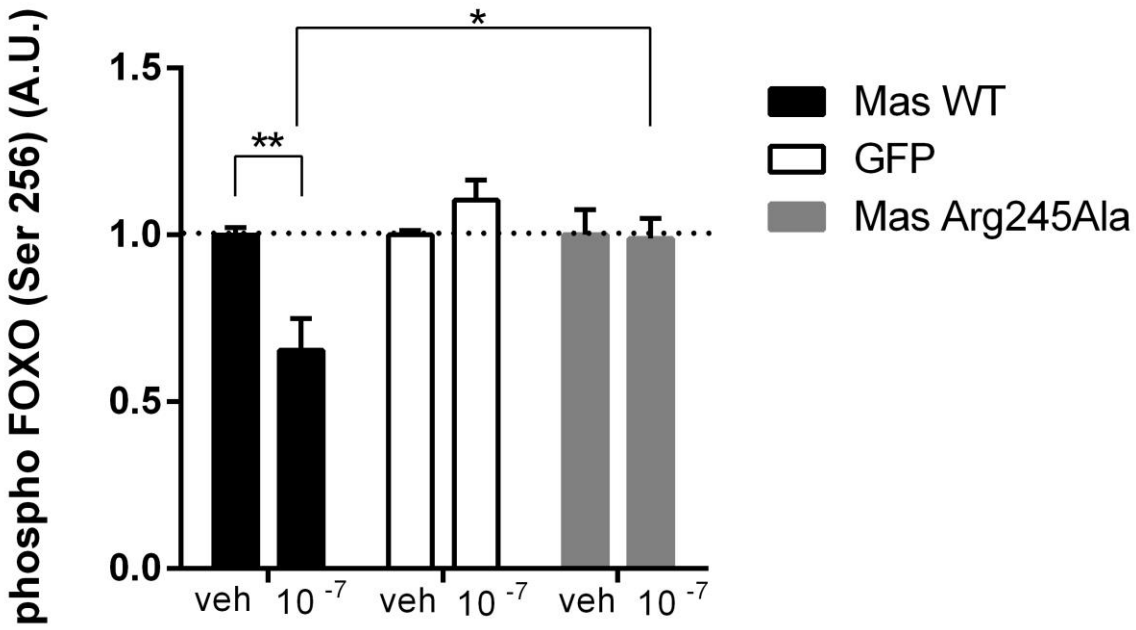
Funding Component:

P250

Unveiling Binding Pocket Structure Of Mas Receptor And Its Interaction With Angiotensin-(1-7)

Authors: Maiia E. Bragina, EPFL STI IBI-STI LHTC, Lausanne, Switzerland; Nuria Cirauqui, EPFL SV IBI UPDALPE, Lausanne, Switzerland; Matteo Dal Peraro, EPFL SV IBI1 UPDALPE, Lausanne, Switzerland; Robson A. S. Santos, Dept de Fisiologia e Biofísica, Insto de Ciências Biológicas, Univ Federal de Minas Gerais,, Belo Horizonte, Brazil; Rodrigo A. Fraga-Silva, Nikos Stergiopulos, EPFL STI IBI-STI LHTC, Lausanne, Switzerland

Substantial amount of preclinical data points out Mas receptor as a promising pharmacological target to treat cardiovascular diseases. Here, we aimed to characterize Mas binding pocket and its interaction with Angiotensin-(Ang)-1-7, in order to provide insights for potential structure-based drug discovery. Homology modeling based on AT1 and AT2 crystal structures, combined with peptide docking (Schrodinger^{TM}) and molecular dynamics simulations (Gromacs) were employed to generate hypothesis about crucial interactions in protein-ligand complex. Proposed model was further validated *in vitro* using HEK cells transiently transfected with wild-type Mas or mutant Mas (Arg245Ala). Mas-receptor activation was assessed by FOXO1 dephosphorylation at Ser256 after 5 minutes incubation with Ang-1-7 (10^{-7}M). Model of Mas/Ang-(1-7) complex revealed electrostatic interaction between Ang-(1-7) C-terminus and Mas Arg245. Interestingly, while Ang-1-7 incubation reduced FOXO1 phosphorylation by 35 % in HEK-Mas wild type (1.000 vs. 0.654 A.U., $p<0.01$, n=8), this effect was abolished in HEK-Mas mutant (1.000 vs. 0.990 A.U., *n.s.*, n=5). In sum, Arg245 of Mas is essential for the receptor activation, which is in accordance with A-779 antagonism (C-terminus modification of Ang-(1-7)), endorsing our proposed structure model. Further elucidation of the ligand-receptor interactions may drive rational drug discovery and lead to development of improved strategies for Mas targeting.



Disclosures: M.E. Bragina: None. **N. Cirauqui:** None. **M. Dal Peraro:** None. **R.A. Santos:** None. **R.A. Fraga-Silva:** None. **N. Stergiopulos:** None.

Funding:

Funding Component:

P251

Angiotensin Based Biomarkers for Molecular Profiling and Drug Efficacy Monitoring in Hypertension

Authors: Marko Poglitsch, Attoquant Diagnostics, Vienna, Austria; Ashraf H Ahmed, Endocrine Hypertension Ctr, Univ of Queensland, Sch of Med, Princess Alexandra Hosp, Brisbane, Australia; Andrea Stoller, Univ Hosp Basel, Clinical Pharmacology and Toxicology, Basel, Switzerland; Oliver Domenig, Attoquant Diagnostics, Vienna, Austria; Manuel Haschke, Univ Hosp Basel, Clinical Pharmacology and Toxicology, Basel, Switzerland; Michael Stowasser, Endocrine Hypertension Ctr, Univ of Queensland, Sch of Med, Princess Alexandra Hosp, Brisbane, Australia

RAAS 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). RAAS 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.

Disclosures: **M. Poglitsch:** A. Employment; Significant; Attoquant Diagnostics. **A.H. Ahmed:** None. **A. Stoller:** None. **O. Domenig:** None. **M. Haschke:** None. **M. Stowasser:** None.

Funding:

Funding Component:

P252

Angiotensin II Type 1 Receptor Autoantibody Inhibited Bk Channels In Vascular Smooth Muscle Cells

Authors: Xiaochen Yin, Suli Zhang, Meili Wang, Yue Qu, Huirong Liu, Capital Medical Univ, Beijing, China

Aims: The angiotensin II type 1 receptor autoantibody (AT1-AA) leads pathological sustainable vasoconstriction. Large-conductance Ca^{2+}-and voltage-activated potassium (BK) channel, mainly consist by functional α subunits and modulatory β1 subunits in vascular smooth muscle cells (SMCs), plays a vital role on vascular relaxation. Recently, it has been found that AT1-AA inhibited BK channel's function. However, the specific mechanisms remain unclear. In this study, we further investigated the open probability, Ca^{2+} sensitivity and β1 subunits of BK channel after AT1-AA stimulation.
Methods and **Results:** BKα subunit has several splicing variants. Immunofluorescence and PCR results showed that BK channels widely distributed among mesenteric artery (MA), most of them belong to zero-type. SMCs from MA were isolated freshly and used for patch clamp within 6 hours. AT1-AA downregulated the open probability of BK channel to 57.41±21.36% (the corresponding data of baseline was considered as 100%), specifically reduced the number of simultaneous opened channels (level 4 to level 2) instead of dwell time (T_1=0.45 ms, T_2= 3.39 ms to T_1=0.58 ms, T_2=4.99 ms) and amplitude (187.4 ±16.23 to 187.3 ±13.80 pS). This phenomenon could be reversed by BK channel agonist (NS1619) (enhanced to 124%). Noteworthy, different from Ang II, AT1-AA suppressed BK channel's open probability consistently even after 15min (Ang II: recovered to 164.8%; AT1-AA, kept at 66.0%). The activated effect of Ca^{2+} on BK channel nearly disappeared after bathing with AT1-AA in inside-out recording mode (vehicle: 100% to 471.6%; AT1-AA: 100% to 163.9%). Furthermore, to explore whether AT1-AA's inhibitory effect on BK channel was independent from β1 subunit, only ZERO-BKα-GFP subunits were transfected into HEK293T cells. AT1-AA still diminished BK channels' function to 57.35±5.4%. However, the inhibition of AT1-AA on BK channels was almost non-existent (to 92.1±12.81%) in AT1

receptor knock out rats.
Conclusion: These results demonstrated that AT1-AA sustainably decreased open probability and Ca^{2+} sensitivity of BK channels even if β1 subunit were absence. This effect was AT1 receptor dependent. It remains us that BK channel may become a novel target for improving AT1-AA related hypertension.

Disclosures: X. Yin: None. **S. Zhang:** None. **M. Wang:** None. **Y. Qu:** None. **H. Liu:** None.

Funding:

Funding Component:

P253

Forty-Two Year Followup in a Patient With Conn's Syndrome

Authors: Clarence Ezra Grim, High Blood Pressure Consulting, Stateline, NV

This case report relies on detailed hospital and clinic records from the UK provided by the patient for review and synthesis. In 1977 a 24 y/o M college student in the UK was found to have asymptomatic HTN (245/125) during a routine exam and was admitted one day later for evaluation. He had no FH of HTN or low K. FH + for CAD before age 60. A renal arteriogram was normal. Hypokalemic alkalosis was noted as was an elevated plasma aldosterone (PALDO), but normal urine Aldo. Adrenal CT showed a normal L adrenal but the R was not seen. Adrenal venography was negative but the R adrenal could not be cannulated. The interpretation was bilateral disease. Spironolactone (S) was started at 300 mg/d. Admitted for surgery 2 months later the BP 160/100 and K was normal. At surgery, the L adrenal was estimated to be 2x normal size and was excised as was 1/2 of the R adrenal. Adrenal steroid coverage was used for 2 months, then ACTH gel was given for 2 months and stopped. BP was lowered for about 2 months but then returned to 200/110. S was started at 400 mg/d with good control but gynecomastia developed. BP control was attempted with S and hydrazine (H) for several years but despite being normal at home was always high in the office. Age 33 was noted to have a K or 3.2 and muscle "fascillcuations". Age 51, atrial fibrillation developed/converted with Amiodarone. BP continued to be difficult to control until he began DASH eating plan at age 56 when he joined the Yahoo Group hyperaldostreronism@yahoogroups.com which I have managed for 16 years. He retired from teaching math at age 58 early to care for his wife. Age 62 PALDO was 1300 and Renin was 10 on S and DASH diet (ENa 55, EK 139 mM/d) and then 600/1 off S and on high salt intake (ENa, 125 EK 100 mM/d). Age 60 MRI showed adenoma L adrenal. Rx with S and enalapril continued with home BPs 122/84. At age 65 non-ST MI was diagnosed and PTA and stenting was performed. BP has been stable for last year with home BP 120/80 on DASH Diet, Spiro 100 mg/d and enalapril 30 mg/d. Statin myalgia has prevented their use but lipids have improved on DASH. This case illustrates that long-term survival after 3/4 adrenalectomy for Conn's syndrome is possible but that BP management can be difficult.

Disclosures: C.E. Grim: None.

Funding: No

Funding Component:

P254

Synergistic Effect of Angiotensin II and Amyloid-Beta on Brain Vascular Smooth Muscle Cell Senescence

Authors: Li-Juan Min, Hui-Yu Bai, Bao-shuai Shan, Harumi Kan-no, Akinori Higaki, Masaki Mogi, Jun Iwanami, Masatsugu Horiuchi, Ehime Univ Sch of Med, Tohon, Ehime, Japan

Objectives: Cerebrovascular damage has been known to contribute to cognitive impairment and dementia. Amyloid-β (Aβ) induces cerebrovascular damage and is reported to stimulate endothelial cell senescence. We previously demonstrated that angiotensin II (Ang II) plays an important role in enhancing the vascular senescence. Here, we examined the possible cross-talk between Ang II and Aβ in regulating brain vascular smoot muscle cell (BVSMC) senescence.
Methods: BVSMCs were prepared from adult male C57BL/6 mice by enzymatic digestion method. Cells were stimulated with Ang II with/without Aβ 1-40. Cellular senescence was evaluated by senescence associated β-galactosidase staining. Oxidative stress was determined by measuring superoxide anion production. Signal transduction was examined by Western blotting and luciferase activity assay.
Results: Treatment with Ang II (100nM) or Aβ (1uM) at a relatively higher dose for 6 days significantly increased the percentage of senescent cells (25±1.43, 16±0.53 respectively) compared with control (13±2.22, 11±0.76 respectively). Treatment with Ang II less than 10nM or Aβ less than 0.5uM had no significant effect on cellular senescence. Interestingly, combination treatment of lower doses of Ang II (10nM) and Aβ (0.5uM) for 6 or 8 days markedly increased the percentage of senescent cells compared with control (control, 9±1.11; Ang II+Aβ, 15±0.77 for 6 days, control, 8±0.83; Ang II+Aβ, 15±1.14 for 8 days). Moreover, this lower dose combination of Ang II and Aβ induced significant increases in superoxide anion production, p-IκB, p16 and p53 expression, NF-κB activity for 4 days, p-ERK and p-IKKα/β expression for 2 days, and obvious decrease in pRb expression. These increases in senescent cells, superoxide anion production, expressions of p-ERK, p16, pRb by lower dose combination Ang II and Aβ were significantly inhibited by the treatment with U0126, an ERK inhibitor. However, U0126 had no significant effect on NF-κB activity.
Conclusion: Ang II and Aβ synergistically promoted BVSMC senescence at least due to the enhancement of p-ERK-p16-pRb signaling pathway and oxidative stress, and the increase in NF-κB/IκB activity would be involved in this synergistic effect.

Disclosures: **L. Min:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; JSPS Kakenhi Number 26860567. **H. Bai:** None. **B. Shan:** None. **H. Kan-no:** None. **A. Higaki:** None. **M. Mogi:** None. **J. Iwanami:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; JSPS Kakenhi Number 15K19974. **M. Horiuchi:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Nippon Boehringer Ingelheim Co. Ltd..

Funding: No

Funding Component:

P255

Differential Regulation of Blood Pressure and Renal Injury by HDAC Inhibitor Mocetinostat in *Npr1* Gene-targeted Male and Female Mutant Mice

Authors: Prerna Kumar, Meaghan Bloodworth, Christian Nguyen, Venkateswara R. Gogulamudi, Kailash N. Pandey, Physiology, Tulane Univ Health Sciences Ctr and Sch of Med, New Orleans, LA

The objective of the present study was to elucidate the systolic blood pressure (SBP) lowering effect of class I histone deacetylase (HDAC) inhibitor, mocetinostat (MGCD0103) by enhanced expression of natriuretic peptide receptor-A (NPRA) in gene (*Npr1*). Adult male and female *Npr1* heterozygous (1-copy; $Npr1^{+/-}$), wild-type (2-copy; $Npr1^{+/+}$), and gene-duplicated (3-copy; $Npr1^{++/+}$) mice were intraperitonealy injected with MGCD (2 mg/kg) for 2-weeks. The renal NPRA protein levels were significantly increased in the treated male (1-copy, 2.1-fold; 2-copy, 2.3-fold; and 3-copy, 1.5-fold; $p < 0.05$) and female (1-copy, 3.6-fold; 2-copy, 2.1-fold; and 3-copy, 1.7-fold; $p < 0.05$) mice compared with untreated animals. HDAC activity was significantly higher in male (1-copy, 24.4 ± 2.8; 2-copy, 15.9 ± 1.2; and 3-copy, 6.4 ± 0.7; ng/min/mg protein) than female (1-copy, 13.7 ± 1.4; 2-copy, 8.5 ± 0.9; and 3-copy, 4.7 ± 0.9, $p < 0.05$) mice; however, treatment with MGCD significantly attenuated HDAC activity in both male (~40%, $p < 0.05$) and female (~50%,

$p < 0.05$) animals. Male mice exhibited significantly higher SBP than female mice and treatment with MGCD decreased SBP in both 1-copy male (106 ± 0.6 vs. control, 129 ± 1.9; mm Hg) and female (97 ± 2.2 vs. control, 110 ± 2.1, $p < 0.001$) mice. Significantly high urinary protein to creatinine ratio was detected in male compared with female mice which was attenuated by MGCD treatment in male mice (1-copy, 3.2 ± 0.4 vs. untreated 5.5 ± 04; 2-copy 2.5 ± 0.4 vs. untreated 4.6 ± 0.2; and 3-copy, 1.6 ± 0.3 vs. untreated 3.1 ± 0.4; $p < 0.05$). Lower creatinine clearance (µl/min) was observed in both male (1-copy, 51 ± 11 vs. 2-copy, 130 ± 14.0; $p < 0.05$) and female (1-copy, 55 ± 2.7 vs. 2-copy, 129 ± 6.4; $p < 0.01$) mice. However, treatment with MGCD increased creatinine clearance in male (105 ± 13; $p < 0.05$) and female (97 ± 8.4; $p < 0.05$) vs. control mice. The present results demonstrate that class I HDAC inhibitor, MGCD upregulates NPRA expression *in vivo*, lowers SBP, and repairs renal injury differentially in male vs. female mice. These findings will have important implications for treatment of hypertension and renal injury in humans in a sex-related manner.

Disclosures: P. Kumar: None. **M. Bloodworth:** None. **C. Nguyen:** None. **V.R. Gogulamudi:** None. **K.N. Pandey:** None.

Funding: No

Funding Component:

P256

Development of an Automated High Throughput Screen for GRK4 as Potential Novel Therapeutics for Treatment of Hypertension and/or Salt Sensitivity

Authors: Michael G Daley, John J Gildea, Peng Xu, Katie A Schiermeyer, Wei Yue, Stephen G Marshall, Pedro A Jose, Robin A Felder, Univ of Virginia, Charlottesville, VA

G protein coupled kinase type 4 (GRK4) reduces renal sodium excretion by deactivating the renal dopamine type 1 receptor (D_1R) through serine phosphorylation. At least 3 polymorphisms in GRK4 have been shown to be associated with the expression of hypertension and/or salt sensitivity in humans and in mice. GRK4 is an ideal therapeutic target for treating hypertension since putting human GRK4 variants in mice results in hypertension which can be reversed by reducing the expression of GRK4. Inhibitors of GRK4 could serve as potent and selective agents to promote sodium excretion in salt sensitive and/or hypertensive individuals. Therefore, we developed an automated high throughput homogeneous time resolved fluorescent resonance energy transfer assay (TR-FRET) to identify small molecule inhibitors of GRK4 isoenzymes. The assay relies on the close proximity (90 A) transfer of 337 nm energy from a europium anti-phosphoserine antibody (Eu-pSer) to an allophycocyanin labeled streptavidin acceptor (streptavidin-APC), producing FRET at 665 nm. The assay was optimized through serial dilutions of streptavidin-APC, Eu-pSer, GRK4, and a peptide consisting of serine sites in the D_1R, serving as a substrate. To validate the assay and determine its suitability for a large scale high throughput screen, three inhibitors, GRKA, GRKB, and GRKC were selected and concentration response assays were performed to determine IC50 values and variability analysis. The IC50 and (R^2) values of GRKA, GRKB, and GRKC were 4.99 µM (0.99), 26.52 µM (0.99), and 179.8 µM (0.82), respectively. GRKA is the most efficient GRK4 inhibitor, while GRKC is the least. This assay will be used to test congeners of compounds A, B, and C to identify more selective candidates, as well as for screening available small molecule compound libraries.

Disclosures: M.G. Daley: None. **J.J. Gildea:** None. **P. Xu:** None. **K.A. Schiermeyer:** None. **W. Yue:** None. **S.G. Marshall:** None. **P.A. Jose:** None. **R.A. Felder:** None.

Funding:

Funding Component:

P257

Angiotensin Ii Type 1 Receptor Autoantibodies Decrease Angiotensin Ii Type 1 Receptor Internalization Through Attenuating The Recruitment Of β-arrestin1/2

Authors: Jingwei Bian, Suli Zhang, Jinghui Lei, Xiaochen Yin, Pengli Wang, Huirong Liu, Capital Medical Univ, Beijing, China

Introduction: Angiotensin II type 1 receptor autoantibody (AT1-AA) can continuously activate angiotensin II type 1 receptor (AT_1R), which is related to cardiovascular disease, but the exact mechanism remains obscure. The activated AT_1R signaling is adjusted or terminated effectively via the internalization, which is mediated by β-arrestin1/2 dependent-endocytic pathways. The hypothesis is that AT1-AA can attenuate β-arrestin1/2 recruitment, leading to the decreased AT_1R internalization and the sustained AT_1R activation. **Methods and Results:** Firstly, subcellular protein fractionation and Total Internal Reflection Fluorescence were used to examine the AT_1R internalization induced by AT1-AA. The results confirmed that AT1-AA limited the AT_1R internalization. Then, by co-expressing YFP-labeled AT_1R and RFP-labeled β-arrestin1/2 in HEK293 cells with different stimulant for 10 min, the image showed that AT_1R and β-arrestin1/2 were co-localized in the cytoplasm with Ang II. However, there were rare co-localization with AT1-AA. To further investigate the AT1-AA-induced recruiting of β-arrestin1/2, we recorded the relative change of BRET ratio of the interaction between YFP-labeled AT_1R with Rluc-labeled β-arrestin1/2. The relative change of BRET ratio was significantly elevated with the time [(the BRET ratio with Ang II for 10 min were considered as 100%; 0 (β-arrestin1/2: 0.33 ±0.58/0.00 ±1.46), 2 (89.10 ±19.36/69.27 ±4.47), 5 (107.08 ±12.17/89.27 ±1.46), 10 (100.22 ±0.38/100.00 ±13.12), 20 (93.45 ±11.59/102.93 ±10.38) and 30 min (101.45 ±16.93/114.15±8.15)] and with the concentration ($EC50_{\beta\text{-arrestin1/2}}$=6.69 ±4.09/6.34 ±2.29 nmol/L) after Ang II treatment. However, AT1-AA could not induce the recruitment of β-arrestin1/2. Furthermore, pre-incubation with an inhibitor of β-arrestin1/2 dependent-endocytic pathways prolonged the duration of AT_1R downstream PKC, ERK1/2 phosphorylation, elevate intracellular Ca^{2+} and vasoconstriction caused by Ang II, which simulated AT1-AA-caused persistent AT_1R activation. **Conclusions:** Our data suggested that reduced AT_1R internalization caused by AT1-AA was attributed to the inhibition of β-arrestin1/2 recruitment, which played a key role in how AT1-AA prolonged receptor activation.

Disclosures: J. Bian: None. **S. Zhang:** None. **J. Lei:** None. **X. Yin:** None. **P. Wang:** None. **H. Liu:** None.

Funding:

Funding Component:

P258

What Clinic Blood Pressure Best Protects Renal Function in Children With Chronic Kidney Disease?

Authors: Joseph T Flynn, Seattle Childrens Hosp, Seattle, WA; Megan Carroll, Derek Ng, Johns Hopkins Sch of Public Health, Baltimore, MD; Brad Warady, Children's Mercy Hosp, Kansas City, MO; Susan Furth, Children's Hosp of Philadelphia, Philadelphia, PA

Background: No data are available regarding what office blood pressure (BP) is protective against progression of chronic kidney disease (CKD) in children. In this study, we examined the association between achieved office BP and CKD outcomes among 688 hypertensive children in the Chronic Kidney Disease in Children Cohort study.
Methods: BP categories were defined as the maximum systolic/diastolic BP percentile (<50th, 50th to 75th, 75th to 90th and ≥90th percentile) with time-updated classifications corresponding to annual study visits. The outcome was time to renal replacement therapy or a 30% decline in estimated glomerular filtration rate from first visit with HTN or high BP. Cox hazard models described the putative protective effect of each lower BP category compared to those with BP ≥90th percentile as non-proportional relative hazards.
Results: Median age was 11.3 years, 62% were male, median duration of CKD was 8y; 213 had glomerular [G] CKD and

475 had nonglomerular [NG] CKD. For both G and NG groups, the highest risk of CKD progression was observed for those with BP ≥90th percentile, and BP 50 to 75th percentile was associated with the lowest risk from 0 to 2 years for G (time-varying RH between 0.09 to 0.24, p<0.001) and over 8 years for NG (RH= 0.45, p<0.001); figure. Those with BP <50th had lower risk than those with BP between the 75th to 90th percentiles (time-varying RH between 0.16 and 0.26 from 0 to 2 years for G and 0.67 for NG), but not as low as in the 50th to 75th percentile group.
Conclusion: Achieved office BP between the 50th-75th percentiles appeared to offer the greatest protection against CKD progression in this cohort. This observation should be confirmed prospectively.

Figure 1a. Time to renal replacement therapy or 30% decline in eGFR among those with a glomerular CKD diagnosis.

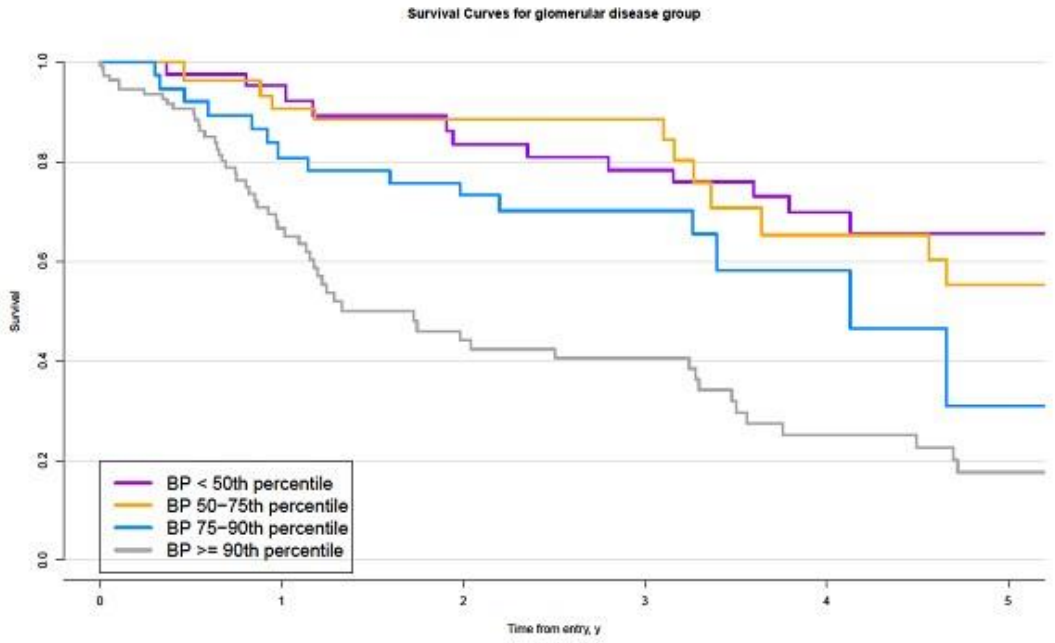
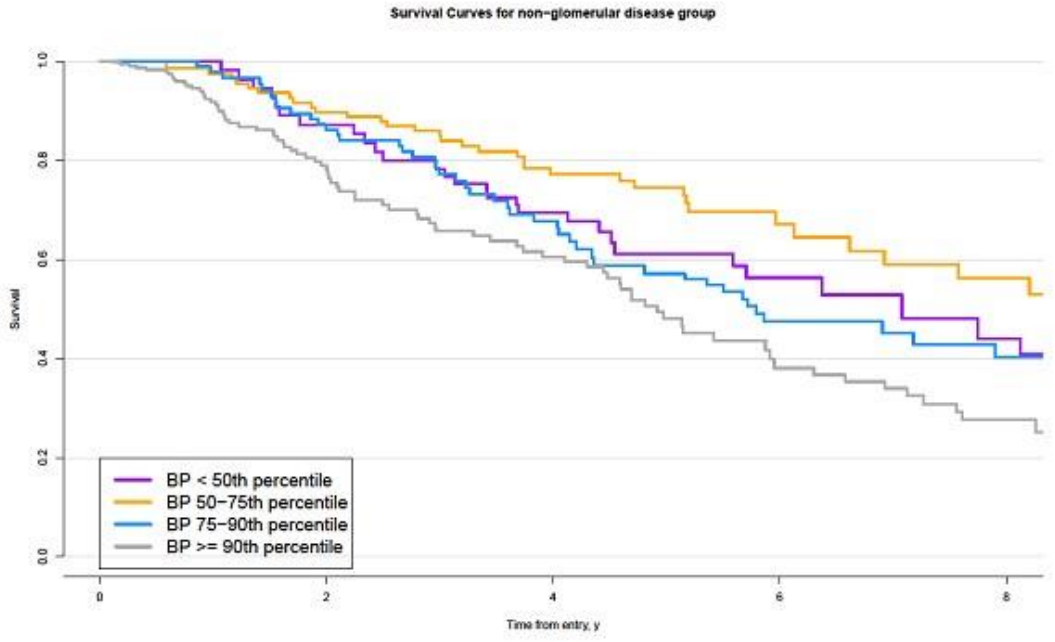


Figure 1b. Time to renal replacement therapy or 30% decline in eGFR among those with a non-glomerular CKD diagnosis.



Disclosures: **J.T. Flynn:** G. Consultant/Advisory Board; Modest; Ultragenyx, Inc, Silvergate Pharaceuticals, Inc. **M. Carroll:** None. **D. Ng:** None. **B. Warady:** None. **S. Furth:** None.

Funding: No

Funding Component:

P260

Utilizing Real-World Big Data in the Search for New Renoprotective Drugs

Authors: Yuya Horinouchi, Yasumasa Ikeda, Keijo Fukushima, Masaki Imanishi, Hirofumi Hamano, Yuki Izawa-Ishizawa, Yoshito Zamami, Hiromichi Fujino, Keisuke Ishizawa, Koichiro Tsuchiya, Toshiaki Tamaki, Inst of Biomedical Sciences, Tokushima Univ Graduate Sch, Tokushima, Japan

[BACKGROUND] The incidence of chronic kidney disease (CKD) has been increasing globally. Because CKD worsens morbidity and mortality, elucidation of its mechanism and discovery of novel therapeutic strategies are imperative.
[PURPOSE] We utilized real-world big data to search for potential novel drugs for the treatment of CKD.
[METHOD] Signal detection by reported odds ratios (RORs) was performed using the Food and Drug Administration Adverse Events Reporting System (FAERS), one of the largest global databases. We then analyzed the relevance between existing drugs and nephritis. Drugs with low reported rates of nephritis were identified as candidate drugs for CKD; their renoprotective effects were verified by basic research.
[RESULTS] Analysis of the FAERS database revealed a significant inverse association between tubulointerstitial nephritis and factor Xa (FXa) inhibitors (ROR, 0.65; 95% CI, 0.49-0.85), and between nephritis and cholinesterase inhibitors (ROR, 0.23; 95% CI, 0.07-0.71).
The FXa inhibitor (FXa-I) suppressed unilateral ureteral obstruction (UUO)-induced tubulointerstitial fibrosis (UUO, 5.20 ± 0.73%; UUO+FXa-I, 3.36 ± 0.32%; $p < 0.05$) and extracellular matrix expression (collagen I, collagen III, and fibronectin; $p < 0.01$). The FXa-I also attenuated UUO-induced macrophage infiltration (UUO, 9.59 ± 0.62%; UUO+FXa-I; 6.70 ± 0.98%; $p < 0.05$) and inflammatory molecule upregulation (monocyte chemoattractant protein 1 [MCP-1], interleukin 1 beta [IL-1β], and tumor necrosis factor alpha [TNF-α]; $p < 0.01$). Furthermore, the FXa-I significantly reduced the UUO-induced increase in plasma creatinine in mice (UUO, 0.163 ± 0.005 mg/dL; UUO+FXa-I, 0.136 ± 0.011 mg/dL; $p < 0.05$).
The cholinesterase inhibitor significantly mitigated the increased expression of collagen I and MCP-1 in aristolochic acid-induced CKD mice ($p < 0.05$). That also inhibited elevated expression of IL-1β and TNF-α in lipopolysaccharide-stimulated J774 mouse cells.
[CONCLUSION] We believe that the anti-inflammatory effects of FXa inhibitors and cholinesterase inhibitors make them potentially useful renoprotective drugs. Thus, real-world big data might be utilized for the discovery of drug repositioning candidates for the treatment of CKD.

Disclosures: **Y. Horinouchi:** C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; edoxaban. **Y. Ikeda:** C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; edoxaban. **K. Fukushima:** None. **M. Imanishi:** None. **H. Hamano:** None. **Y. Izawa-Ishizawa:** None. **Y. Zamami:** None. **H. Fujino:** None. **K. Ishizawa:** None. **K. Tsuchiya:** None. **T. Tamaki:** C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; edoxaban.

Funding: No

Funding Component:

P261

GPER is Required for Age-dependent Albuminuria and Glomerulosclerosis: Evidence for its Role in Podocyte Injury and Mesangial Nox1 Regulation

Authors: Matthias R Meyer, Univ of New Mexico, Albuquerque, NM; Christoph Daniel, Friedrich-Alexander-Univ of Erlangen-Nürnberg, Erlangen, Germany; Christine D. Woods, Geetanjali Sharma, Natalie C. Fredette, Univ of New Mexico, Albuquerque, NM; Kerstin Amann, Friedrich-Alexander-Univ of Erlangen-Nürnberg, Erlangen, Germany; **Matthias Barton**, Univ of Zurich, Zurich, Switzerland; Eric R Prossnitz, Univ of New Mexico, Albuquerque, NM

We recently found that the G-protein coupled estrogen receptor (GPER) exerts constitutive effects on cell proliferation and fibrosis in heart failure and arterial hypertension via the NADPH oxidase isoform Nox1 (Sci Signal 2016; 9(452): ra105). Whether GPER affects glomerulosclerosis or podocyte function is unknown. Thus, the present study investigated the effects of GPER in a model of age-dependent spontaneous focal segmental glomerulosclerosis and studied effects of GPER inhibition in mesangial cells and podocytes. Albuminuria and kidney histology were studied in male wild-type (WT) and GPER-deficient (*Gper*$^{-/-}$) mice at 4 and 24 months of age. Aged *Gper*$^{-/-}$mice were largely protected from albuminuria (albumin/creatinine-ratio, 0.9±0.4 vs. 3.5±1.0, -74 %, p<0.05 vs. WT). *Gper*deficiency had no effect at 4 months of age, but largely prevented age-dependent increases in kidney weight (306±11 vs. 554±94 mg), and glomerulosclerosis index (1.3±0.2 vs. 2.9±0.4, p<0.05 vs. WT). All changes were independent of blood pressure. In human podocytes exposed to TGFβ-1, treatment with the selective GPER blocker G36 markedly reduced mRNA expression of injury markers nephrin, collagen-4, and Wilms-tumor-1 (all p<0.01). *Gper* knock-down in rat mesangial cells reduced Nox1 protein expression by approx. 50% (p<0.05) while Nox2 and Nox4 expression remained unchanged. These results indicate that constitutive activity of *Gper*maintains Nox1 expression and contributes to podocyte injury in vitro and that *Gper*is essential for age-dependent podocyte injury, subsequent albuminuria, fibrosis and glomerulosclerosis in vivo. Nox1 downregulators such as G36 represent a new class of drugs that may offer therapeutic potential for patients with chronic renal diseases and other forms of chronic non-communicable diseases involving inflammation and fibrosis.

Disclosures: M.R. Meyer: F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; US patent pending. **C. Daniel:** None. **C.D. Woods:** None. **G. Sharma:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; US patent pending. **N.C. Fredette:** None. **K. Amann:** None. **M. Barton:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; US patent pending. **E.R. Prossnitz:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH CA163890, CA194496; Dialysis Clinic, Inc.. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; US patent 7875721.

Funding:

Funding Component:

P262

Repression of Genes Involved in Fatty Acid Metabolism during Acute Renal Failure

Authors: Mohd Shahid, Lina Kishta, Dana Mdanat, Chicago State Univ, Chicago, IL; Zivar Yousefipour, Coll of Pharmacy and Health Sciences, Texas Southern Univ, Houston, TX; Mohammed Newaz, Chicago State Univ, Chicago, IL

Peroxisome proliferator-activated receptors (PPARs) are highly expressed in the kidney, but their role in acute renal failure (ARF) remains elusive. Previously, we have shown PPARγ-ligand attenuated renal injury and improved renal function in ARF. In this study, we explored specific downstream targets of PPARγ that may be involved in reno-protection. Male Sprague-Dawley rats (250-300 gm) were randomly divided into two groups, control, and ARF: where they received GW1929 (PPARγ ligand), apocynin (NADPH oxidase inhibitor), or vehicle. After 7 days of pretreatment, ARF was induced by injecting glycerol (50% v/v, 8 ml/kg; i.m.). After 24 hours, blood and kidneys were collected and processed for mRNA extraction. The expression of PPARγ-responsive genes was determined by a PCR array using qRT-PCR. Markers for renal injury and oxidative stress and NADPH oxidase activity were determined by ELISA. The data revealed that fatty acid (FA) transporter genes (*Slc27a2, ApoE, FABP2, FABP3,* and *CD36)*, were strikingly suppressed (>100-folds, p<0.001) in ARF compared to control. Genes involved in FA metabolism such as *FABP, PGC1α, Cpt1b, Creb1* were also markedly inhibited (>20-folds, p<0.001). In addition, expressions of key survival genes *PCK2, Pten, Crebbp*, and *SRC*, were concurrently diminished (>25-folds, p<0.001). However, this attenuation was restored to normal levels with GW1929 treatment. Pretreatment with GW1929 reduced the activity of KIM1 (14.8±1.5 vs. 18.7±0.3 ng/μg; p<0.05) and NGAL (129±6 vs. 233±5 ng/μg; p<0.01), isoprostane levels (73.3±3.7 vs. 110±8 pmol/mg), and NADPH oxidase activity (0.96±0.04 vs. 1.7±0.04 nmol/mg protein), indicating reversal of renal injury and oxidative stress. Interestingly, apocynin treatment also inhibited ARF-induced downregulation of genes involved in FA metabolism and growth signals, demonstrating the ROS-dependency of ARF-induced repression of PPARγ-responsive genes. As expected, apocynin reduced isoprostane levels but did not impact KIM1 expression in ARF, suggesting no improvement in glomerular injury with this treatment. Collectively, the data suggest that renal damage in ARF is preceded by impairment in FA metabolism and growth signals via a ROS-dependent manner.

Disclosures: M. Shahid: None. **L. Kishta:** None. **D. Mdanat:** None. **Z. Yousefipour:** None. **M. Newaz:** None.

Funding:

Funding Component:

P263

Renal Proximal Tubule Cells from Inverse Salt Sensitive Individuals Have Lower Basal but Higher Sodium Stimulated ENaC Compared to Salt Resistant Individuals

Authors: Peng Xu, John J. Gildea, Wei Yue, Univ of Virginia, Charlottesville, VA; Pedro A Jose, George Washington Univ, Washington, DC; Robin A Felder, Univ of Virginia, Charlottesville, VA

Our previous studies on salt sensitivity has demonstrated that approximately 11% of study participants had a paradoxical increase in blood pressure (BP) (greater than 7-mm Hg) on a low NaCl diet and an increased basal renal proximal tubule cells (RPTC) Na^+ transport, which we define as inverse salt sensitivity (ISS). We hypothesized that the amiloride blockable Na^+ channel (ENaC) could be involved in ISS, particularly in the RPTC where up to 75% of renal nephron Na^+ reabsorption occurs. Previous investigators have localized αENaC in the distal nephron; however, we found αENaC is also present in the human RPTCs, as confirmed by tissue and cellular immunostaining, RT-PCR, and western blot with two selective αENaC antibodies. The presence of αENaC in human kidney tissue was confirmed by the Human Protein Atlas. Urine derived RPTCs from 3 ISS and 3 salt resistance (SR) clinical study participants were treated overnight with normal salt and high salt (140mM Na^+ and 170mM Na^+ respectively). High salt increased αENaC expression to a greater extent in ISS versus SR cells; however, ISS cells have significantly less αENaC at basal level (αENaC/actin: SR 1.41±0.248 VS ISS 0.592±0.157, n=3, P<0.05). Patch clamp ramp currents were recorded using a voltage ramp from -100 to +45 mV within 200 ms (N≥5 for each cell line). Na^+ current was measured at both 140 and 170 mM Na^+ with and without Bensamil (ENaC antagonist) and expressed as a ratio of current (170 mM/ 140 mM Na^+). Bensamil blocked ENaC activity in ISS cells, but not in SR cells (Bensamil ratio/primary ratio: SR 1.149±0.026 VS ISS 0.818±0.017, n=3, t-test, P<0.001; final ratio/primary ratio: SR 1.088±0.022 VS 0.705±0.1, n=3, t-test, P<0.05). αENaC knockdown using siRNA

significantly decreased Na^+ current in ISS cells (0.763±0.029, n=3, t-test, P<0.05) but not in SR cells (1.046±0.097, n=3, t-test, P<0.05). In conclusion, RPTC from ISS study participant urine demonstrated increased Bensamil Na^+ current compared to SR RPTC when exposed to increased sodium. This data could provide a novel functional assay for the diagnosis of ISS in the clinical setting and allow for an appropriate personalized medical intervention.

Disclosures: P. Xu: None. **J.J. Gildea:** None. **W. Yue:** None. **P.A. Jose:** None. **R.A. Felder:** None.

Funding: No

Funding Component:

P264

Ghrelin-Induced Sodium Reabsorption is Mediated by PKA and a Microtubulin-Dependent Network In Rats

Authors: Brandon A. Kemp, Nancy L. Howell, Shetal H. Padia, Univ of Virginia, Charlottesville, VA

Previous studies have shown that intrarenal ghrelin infusion activates ghrelin receptors in the collecting duct to increase $\alpha E_{Na}C$-dependent sodium (Na^+) reabsorption *in vivo*, but the underlying mechanisms are unknown. 72h following uninephrectomy, 12-week-old female Sprague-Dawley rats received the following renal interstitial (RI) infusions for 1h after a 1h control period in which they received RI infusions of vehicle (5% dextrose in water, D_5W): (1) vehicle (N=7), (2) ghrelin (3 µg/min, N=6), (3) ghrelin + PI3K inhibitor, LY-294002 (0.1 µg/kg/min, N=6), (4) LY-294002 alone (N=4), (5) ghrelin + PKA inhibitor, Rp-cAMPS (10 µg/kg/min, N=7), (6) Rp-cAMPs alone (N=5), (7) ghrelin + microtubule polymerization inhibitor nocodazole (3 µg/kg/min, N=7), (8) nocodazole alone (N=6), (9) ghrelin + actin polymerization inhibitor, cytochalasin D (0.3 µg/kg/min, N=6), and (10) cytochalasin D alone (N=6). Compared to baseline, RI ghrelin significantly decreased $U_{Na}V$ to 52.2 ± 9.9% (P<0.001), but this effect was abolished during concomitant PKA and microtubulin inhibition (128.6 ± 15.9% and 123.5 ± 12.2%, respectively, both P<0.01 from ghrelin alone). Ghrelin-induced antinatriuresis persisted in the presence of PI3K and actin inhibition (62.2 ± 4% and 59.7 ± 10.8% respectively, both P<0.01 from baseline and P=NS from ghrelin alone). RI infusion of inhibitors alone had no effect on $U_{Na}V$ compared to vehicle and mean arterial pressures did not change following any RI infusion. Taken together, these studies highlight the importance of PKA and microtubulin polymerization in $\alpha E_{Na}C$-mediated Na^+ reabsorption.

Disclosures: B.A. Kemp: None. **N.L. Howell:** None. **S.H. Padia:** None.

Funding:

Funding Component:

P265

Early Renal Hyperfiltration In Obese Dahl Salt-Sensitive Leptin Receptor Mutant Rats is Associated With Glomerular Leukocyte Extravasation and Renal Disease

Authors: Jan M Williams, Kasi C McPherson, Corbin A Shields, Bibek Poudel, Denise C Cornelius, Michael R Garrett, Univ of Mississippi Medi, Jackson, MS

Hypertension and diabetes are the major causes of chronic kidney disease (CKD). However, epidemiological studies within the last few decades have revealed obesity as an independent risk factor for CKD. Recently, we reported that the obese Dahl salt-sensitive leptin receptor mutant (SS^{LepR}mutant) strain displays proteinuria and podocyte injury by 6 weeks of age independent of hyperglycemia and elevations in arterial pressure. The current study examined whether the development of renal injury in the SS^{LepR}mutant strain is associated with elevations in glomerular filtration rate

(GFR). During the study, the SS^{LepR}mutant strain developed hyperinsulinemia and dyslipidemia but not hyperglycemia. Baseline MAP (via carotid catheter) at 6 weeks of age was similar between SS_{WT} (n=8) and SS^{LepR}mutant (n=8) rats and averaged 124 mmHg. However, by 18 weeks of age, MAP increased significantly in the SS^{LepR}mutant strain compared to the values measured in SS_{WT} rats (192±4 vs 149±6 mmHg, respectively). At baseline, protein excretion was 4-fold higher in the SS^{LepR}mutant strain compared to SS_{WT} rats and remained elevated over the course of the study (778±96 vs 137±25 mg/day, respectively). At 6 weeks of age, GFR was 34% higher in the SS^{LepR}mutant strain compared to age-matched SS_{WT} rats indicating renal hyperfiltration (2.92±0.23 vs 2.18±0.25 mL/min/kwt, respectively). While we observed only a 40% reduction in GFR in SS_{WT} rats (1.30±0.07 mL/min/kwt), GFR markedly decreased by 70% in the SS^{LepR}mutant strain (0.87±0.08 mL/min/kwt). Over time, kidneys from the SS^{LepR}mutant strain displayed more glomerulosclerosis, mesangial expansion, and renal fibrosis in comparison to SS_{WT} rats. Glomeruli were isolated from the renal cortex of both strains at 6 and 18 weeks of age and RNA sequencing was performed to identify genes and pathways driving glomerular injury. The major, most consistent signaling pathways that changed at 6 and 18 weeks of age were involved in leukocyte extravasation. In conclusion, these data provide evidence that renal hyperfiltration may contribute to glomerular capillary leukocyte extravasation leading to the early development of proteinuria during obesity in the absence of hypertension and hyperglycemia.

Disclosures: J.M. Williams: None. **K.C. McPherson:** None. **C.A. Shields:** None. **B. Poudel:** None. **D.C. Cornelius:** None. **M.R. Garrett:** None.

Funding: No

Funding Component:

P266

Western Diet Impairs Small Vessel Relaxation and Initiates Kidney Endothelial Stiffening, Fibrosis and Tubulointerstitial Fibrosis Through the Endothelial Mineralocorticoidreceptor

Authors: Annayya Aroor, Javad Habibi, Francisco I Ramirez-Perez, Luis Martinez-Lemus, Iris Z Jaffe, James R Sowers, Guanghong Jia, **Adam Whaley-Connell**, Univ Missouri, Columbia, MO

Obesity enhances mineralocorticoid receptor (MR) activation, development of vascular stiffness and end organ injury. In this context, western diet (WD) activation of the endothelial mineralocorticoid receptor (ECMR) contributes to endothelial cell stiffening and promotes maladaptive inflammatory responses and fibrosis in cardiovascular tissue of female mice. However, the role of ECMR on kidney endothelial stiffening, inflammation and fibrosis remains unknown. We hypothesized that deletion of the ECMR would prevent WD-induced increases in endothelial cell stiffness, reductions in bioavailable nitric oxide (NO), increased perivascular and tubulointerstitial inflammation oxidant stress, and fibrosis in females. Four-week-old female ECMR knockout and wild-type mice were fed either a mouse chow or a WD high in saturated fat and refined carbohydrates for 16 weeks. Without blood pressure changes between groups, WD-feeding increased body weight and fat mass as well as indices of vascular stiffness (pulse wave velocity and kidney endothelial cell stiffness) and impaired endothelial-dependent vasodilatation. The WD-induced kidney endothelial cell stiffness was associated with attenuated endothelial NO synthase activation, increased oxidative stress, along with pro-inflammatory immune responses, alterations in extracellular matrix degradation pathways and tubulointerstitial fibrosis. ECMR deletion prevented these abnormalities through improvements in endothelial NO synthase and reductions in macrophage polarization, LARP6, TG2 and MMP2. Our data support that activation of ECMR contributes to endothelial dysfunction, increased permeability and stiffening in the kidney which, in turn, promotes macrophage infiltration, M1 polarization, inflammation and oxidative stress, resulting in alterations in matrix degradation that promote tubulointerstitial fibrosis in females consuming a WD.

Disclosures: A. Aroor: None. **J. Habibi:** None. **F.I. Ramirez-Perez:** None. **L. Martinez-Lemus:** None. **I.Z. Jaffe:** None. **J.R. Sowers:** None. **G. Jia:** None. **A. Whaley-Connell:** None.

Funding: No

Funding Component:

P267

Aging & Endothelial Cell Senescence: Does Prolylcarboxypeptidase Predict Endothelial Dysfunction?

Authors: Zia Shariat-Madar, Nicholas Boullard, Cody Cissom, Univ of Mississippi, University, MS

Changes due to cellular senescence may lead to the formation of a unique cellular morphology such as the transformation of endothelium from an anticoagulant to a potentially prothrombotic surface. Investigations were performed to determine whether the ratio of prolylcarboxypeptidase (PRCP) gene expression to the rate of telomere shortening in vascular endothelial cells would predict endothelial dysfunction and subsequent endothelial surface alteration from anticoagulant to potentially prothrombotic surface. Here, we present evidence for a novel mechanism of senescence-induced cell-surface expression of PRCP involving activation of the plasma kallikrein-kinin system (KKS). We found that the abilities of human pulmonary vein endothelial cells (HPVECs) to express PRCP first incrementally increased at early passages and then decreased after passage 18, in contrast to changes in average human telomerase reverse transcriptase (hTERT) mRNA expression levels. In agreement with the changes in average PRCP expression, incubation of prekallikrein (PK) and high molecular weight kininogen (HK) plasma proteins resulted in increased cleavage of HK, higher enzymatic activities of kallikrein, and increased liberation of bradykinin (BK). Furthermore, the alterations in PK and BK generation was prevented using PRCP-siRNA or PRCP inhibitor (z-Pro- prolinal) that blocked PK activation in passage 18 by 60% and 80% respectively. SDD31 that interferes with the binding of PK on HK reduced kallikrein generation by 90%. There was a direct correlation between PRCP-dependent BK generation and cell permeability. Our results were supported when we found that nitric oxide (NO), a downstream product of the PRCP-catalyzed HK-PK system, also shared the same peak in formation as PRCP. In contrast, increased eNOS expression remained unchanged after passage 12. While β-Gal rose after passage 18, calnexin expression like PRCP dropped significantly in passage 30. These data are the first to demonstrate the inverse relationship between hTERT and PRCP expression prior to an apparent onset of EC replicative senescence, which could have profound implications as a prognostic tool for predicting disease progression and cardiovascular event rates.

Disclosures: Z. Shariat-Madar: None. **N. Boullard:** None. **C. Cissom:** None.

Funding: No

Funding Component:

P268

Extracellular Vesicles from Wistar Kyoto and Spontaneously Hypertensive Rats Have Differential Vasodilatory Effects on Resistance Arteries

Authors: Uta Erdbruegger, Miranda Good, Luca Musante, Sabrina LaSalvia, Nancy Howell, Robert M Carey, Brant Isakson, Thu H Le, Univ of Virginia Health System, Charlottesville, VA

Extracellular vesicles (EVs) have been described as novel bio-markers and bio-activators in vascular dysfunction in HTN. However, the exact mechanism how EVs affect vascular function is not known. We hypothesized that hypertensive and normotensive EVs have differential vasodilatory effects on resistance arteries. To examine the effects of EVs on

acetylcholine (ACh)-mediated vasodilation, we freshly isolated 3rd/4th order mesenteric arteries and circulating EVs from 6 and 12-week-old normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats. Arteries were cannulated on a pressure myograph and pressurized to 80 mmHg. EVs (~$6x10^7$ EV/mL) from hypertensive and normotensive 6 and 12 week old rats were added to the vessel lumen and circulating bath solutions and equilibrated for 10 min. We also added EVs after delipidation with Chloroformmethanol. Inner diameter was measured as cumulative concentrations of ACh were applied to the bath following a preconstruction with 10 µM phenylephrine (PE). No significant difference in ACh vasodilation was observed in arteries from WKY and SHR rats, although SHR arteries were more vasoconstrictive to PE. Interestingly, EVs from WKY had an anti-vasodilatory effect on WKY arteries, whereas EVs from SHR had no effect compared to control condition without EVs. This differential effect was not observed in arteries from SHR rats treated with WKY or SHR EVs. The anti-vasodilatory effect on WKY arteries was also achieved by infusing either delipidated EVs from 12 week-old SHR, or intact EVs from 6 week old SHR rats that have not yet developed hypertension. Together, these data suggest that EVs from normotensive WKY rats and pre-hypertensive SHR rats have anti-vasodilatory effects on healthy resistance arteries. Additionally, the anti-vasodilatory effect of pre-hypertensive SHR EVs is dependent on intact vesicles, but not that of WKY EVs. The effect of normotension on EV cargo might be more important to vasodilatory response than EV species source. This data supports the functional role of EVs in vascular regulation in HTN. Further studies are needed to delineate the effector cargo of these functional EVs.

Disclosures: U. Erdbruegger: None. **M. Good:** None. **L. Musante:** None. **S. LaSalvia:** None. **N. Howell:** None. **R.M. Carey:** None. **B. Isakson:** None. **T.H. Le:** None.

Funding: No

Funding Component:

P269

Chronic Cerebral Ischemic Injury Worsens Vascular Dysfunction

Authors: Jun Iwanami, Masaki Mogi, Kana Tsukuda, Akinori Higaki, Moe Kawakami, 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

Introduction: Distant organ dysfunction has been highlighted whereby acute or chronic damage in one organ may induce acute or chronic dysfunctions of the other organs such as cardiorenal syndrome. Vascular remodeling is an important process in various vascular diseases. However, the relationship cerebral ischemic damage and peripheral vascular disorder is still unclear. Therefore, we have investigated the possible pathophysiological interactions between brain ischemia and vascular remodeling. **Methods:** Eight-week-old male C57BL/6 mice underwent permanent cerebral brain ischemia by permanent occlusion of the left middle cerebral artery occlusion (MCAO) by electrocoagulation using a subtemporal approach. Four weeks after MCAO, vascular injury was induced by polyethylene cuff placement on the femoral artery. Neointima formation was determined 14 days after cuff placement by evaluating intima/media ratio. Macrophage fractions in spleen two weeks after MCAO were examined and M1 and M2-polarized macrophages were separated by flow cytometry and analyzed. **Results:** Body weight after MCAO and cuff placement did not differ among all groups. Cerebral ischemia did not influence the intima/media ratio 6 weeks after MCAO (MCAO-Cuff (-), 0.123) compared with sham-operated mice (sham-Cuff (-), 0.120). Neointima formation in the injured artery was significantly increased 2 weeks after cuff placement (sham-Cuff (+), 0.258). Interestingly, the neointima formation 2 weeks after cuff placement was more marked in MCAO-operated mice (MCAO-Cuff (+), 0.369) compared with sham-Cuff (+). Next, we examined the macrophage fractions in spleen 2 weeks after MCAO. The macrophage fraction in SVF evaluated by F4/80 staining did not differ between sham- (7.5%) and MCAO-operated (7.6%) mice. There was no significant difference in M1 and M2 fraction with/without MCAO. **Conclusion:** These results suggest that cerebral ischemic injury aggravate peripheral vascular disorders, and we are addressing the possible roles of renin-angiotensin system and oxidative stress

etc. involved in these interesting results, which could provide us with the discovery of new pathological mechanisms concerning the distant organ dysfunctions between brain and peripheral vasculature.

Disclosures: **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. Mogi:** None. **K. Tsukuda:** None. **A. Higaki:** None. **M. Kawakami:** 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..

Funding: No

Funding Component:

P270

Increase of Total Peripheral Vascular Resistance and Arterial Stiffness in Non-Diabetic Hypertensive Patients With Overweight, Compared With Hypertensive Patients of the Same Age With Normal Body Mass Index

Authors: Ricardo Cabrera-Sole, Caridad Turpin Lucas, Santiago Garcia Ruiz, Liliana Urrego Rivera, Erik Stephan Luekpe, Manuel Aguilera Saldaña, Univ General Hosp, Albacete, Spain

INTRODUCTION: it is known that obese hypertensive patients (HTAO) have more difficulties for the better control of blood pressure, being involved many factors that try to explain this situation. However, little has been said regarding total vascular resistance and arterial stiffness (AS) in overweight patients (OWHTA), so we present this work that could provide data of interest in these P OBJECTIVES: to assess the relationship between adequate control of blood pressure, AS and TPR in patients with OWHTA compared with hypertensive patients with a normal body mass index (BMI). MATERIAL AND METHODS: cross-sectional and prospective study of a cohort of 1700 male patients (P) with hypertension, we have 290 P divided into two groups: OWHTA Group 190 P with BMI between 25 and 29 (65 ± 8 years) and Group HTA: 100 P with BMI <25 (66 ± 6 years) who have received treatment from their GP, to keep the BP below 140/80 and apparently were well controlled. A 24 hours blood pressure holter (ABPM) was performed in all patients, and augmentation index (AI) ,pulse wave velocity (PWV) as well as the TPR were measured. The results were compared in both groups and are presented in the following table:RESULTS:Table of results:

DATA	Sistolic BP ABPM	Diastolic BP ABPM	AI	PWV	TPR
OWHTA	138±3*	89±4*	28±3*	9±4	1.9±0.7*
HTA	124±4	79±3	19±2	7±3	1.1±0.2

*means p value less than 0.05.
CONCLUSIONS: according to our data, in hypertensive males with overweight there are already aggravating factors such as the difficulty for better hypertension control , increase in AS and a significant increase in the TPR that has been seen in obese patients. All of these factors make the cardiovascular risk of these patients much greater, and forces the physician to be more demanding with the therapeutic measures.

Disclosures: **R. Cabrera-Sole:** None. **C. Turpin Lucas:** None. **S. Garcia Ruiz:** None. **L. Urrego Rivera:** None. **E. Stephan Luekpe:** None. **M. Aguilera Saldaña:** None.

Funding: No

Funding Component:

P271

Relationship of Resting Heart Rate and Subclinical Arteriosclerosis Markers in Apparently Normal Participants From China

Authors: Xiuqin Wu, Yongqiang Hong, Mindong Hosp Affiliated to Fujian Medical Univ, Fujian, China; Huan Liu, Hongyu Wang, Hongwei Zhao, Peking Univ Shougang Hosp, Beijing, China

Objective To evaluate the relationship between resting heart rate and arterial stiffness in natural and apparently normal population of She minority from Fujian province in China. Method 6 natural village 511 participants were enrolled into our study using cluster sampling method in 2009. Eventually 325 subjects with full data were analyzed. All subjects carried on detection of carotid femoral pulse wave velocity(CF-PWV), carotid radial pulse wave velocity (CR-PWV), cardio ankle vascular index(CAVI), ankle brachial index(ABI), carotid intima media thickness(CIMT) by Doppler ultrasound and blood test (fasting plasma glucose[FPG], blood lipids, blood uric acid(UA), high sensitive C reactive protein(hs-CRP). Resting heart rate(RHR) was the mean of three values measured by VS-1000 vascular detection equipment and ultrasound equipment. Results In different quartiles of RHR, that were RHR1≤61beats/min, RHR2:62-69beats/min, RHR3:70-77beats/min, RHR4≥78beats/min, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol to high density lipoprotein cholesterol ratio (TC/HDL-C), HDL-C, CF-PWV and CR-PWV were significantly different between RHR groups ($p<0.05$); SBP, DBP, TC/HDL-C, CF-PWV and CR-PWV in RHR4 group were significantly higher than RHR1 group, and HDL-C was lower than RHR1 group (all $p<0.05$). However, there were no statistical significance of CAVI, ABI and CIMT in different RHR groups ($p>0.05$). Logistic regression analysis indicated in the model without adjustment, the RHR4 was associated with higher CF-PWV(CF-PWV>9m/s) with odds ratios (ORs) was 3.074 (95% confidence interval was 1.555-6.075, $p<0.05$) compared with RHR1; After adjustment of age and gender, the OR of RHR4 was 4.542 (95% confidence interval was 2.078-9.928, $p<0.05$); And after further adjustment of SBP, DBP, plasma lipids, FPG and hs-CRP, the OR of RHR4 was 5.336 (95% confidence interval was 1.512-18.831, $p<0.05$). Conclusion In natural population of She minority of Fujian province in China, high resting heart rate was followed by a high level of blood pressure, plasma lipids and arterial stiffness. Furthermore, a higher rest heart rate was independently associated with arterial stiffness.

Disclosures: X. Wu: None. **Y. Hong:** None. **H. Liu:** None. **H. Wang:** None. **H. Zhao:** None.

Funding: No

Funding Component:

P272

Gender Features of Angiological Screening in Young People

Authors: Maria Evsevyeva, Stavropol State Medical Univ, Stavropol, Russian Federation; Mikhail Eremin, Stavropol Regional Clinical Hosp, Stavropol, Russian Federation; Anjelika Rusidi, Elena Fursova, Vladimir Koshel, Vladimir Baturin, Stavropol State Medical Univ, Stavropol, Russian Federation

Relevancy. The wide occurrence of risk factors (RF) necessitates rapid introduction of angiological screening. Purpose. To determine the results of mass instrumental evaluation of vascular stiffness in students taking into account gender and cases of EVA-syndrome. Methods and materials. It was surveyed 224 students on base of Student Health Centre of StSMU as a part of project " The University of healthy life style" for study of FR profile, including fat metabolism by using rapid diagnosis "Lipid Panel" (USA). It was valued the condition of the vessel wall according to Cardio-Ankle Vascular Index (CAVI) from left (L) and right (R) sides thanks to device Vasera VS-1500('Fukuda Danshi', Japan). The device also gives an opinion on vascular age, which allows to identify cases of early vascular aging or EVA syndrome. Statistics analyses were made with programme ' Statistica 10.0'(StatSoft Inc, USA). Results. Among boys in the upper quartile of

CAVI-R and CAVI-L there were 18 (25,4 %) and 17 (23,9%) , but among the female - 30 (19,6%) and 26 (17,0%). Signs of syndrome EVA also were recorded more often in boys than in girls - 9 (12,7 %) versus 12 (7,8%). Noteworthy that there were some differences in lipidogram in carriers of this syndrome, depending on sex. Thus, girls' total cholesterol and low density lipoprotein levels were 4.4±0.34 and 2.76±0.24 versus 3.7±0.11 and 2.3±0.32 mmol/l in males. But at same time, high density lipoproteins were also higher in the fairer sex - 1,3±1,2 against 0,98±0,06 mmol/l in boys. In girls triglycerides were registered at lower level - 0,72±0,1 against 0,98±0,25 mol/l. However, all described differences did not reach a reliable level. The average value of CAVI-R and CAVI-L for male were 5,80 ± 0,09 and 6,01 ± 0.09, but for female - 5,58 ± 0,06 and 5,90 ± 0,05. The median of CAVI-R and CAVI-L for men was 5, 81 and 6,02, but for the women - 5,73 and 5,81. Conclusion. In upper quartile of CAVI values boys fall almost a third more than girls. Carriers of EVA syndrome are also almost half the most common among representatives of the stronger sex. At the same time, not all indicators of lipid status in boys with EVA syndrome are worse than in their girl-peers with same syndrome. These data can be useful in forming groups of increased cardiovascular risk among young people.

Disclosures: M. Evsevyeva: None. **M. Eremin:** None. **A. Rusidi:** None. **E. Fursova:** None. **V. Koshel:** None. **V. Baturin:** None.

Funding: No

Funding Component:

P273

Association Between Arterial Stiffness and Vascular Related Diseases- Arterial Stiffness Increases in Participants Exposing in Combined Vascular Related Diseases: The Best Study

Authors: Hongyu Wang, Huan Liu, Jinbo Liu, Hongwei Zhao, Peking Univ Shougang Hosp, Beijing, China

Background Arterial stiffness has been confirmed the predictive value on first cardiovascular diseases, and many parameters could reflect arteriosclerosis. However, few studies evaluated the condition of artery in patients with various cardiovascular diseases and the combined influences on vascular function. The present study was designed to explore the association of arterial stiffening reflected by two different parameters in participants exposing in combined vascular related diseases from China. Methods A total of 12576 participants were enrolled into the study from year 2010 to 2016, with males 6041 females 6535, mean age 58.75±13.23 years. All participants were analyzed according to the exposing and number of hypertension, diabetes mellitus (DM), coronary artery disease (CAD), stroke and peripheral artery disease (PAD). Arterial stiffness was assessed by carotid femoral pulse wave velocity (CF-PWV) and cardio-ankle vascular index (CAVI). Age- and sex-adjusted analyses by covariance and regressions were used. Results Arterial stiffness reflected by CF-PWV and CAVI was significantly higher, and ankle brachial index (ABI) was lower in patients with hypertension, DM, CAD, stroke and PAD compared with no these diseases (all $p<0.05$). CF-PWV and mean CAVI increased, mean ABI decreased gradually with the increase of the number of combined diseases, the β for each 1 increase were 0.404 m/s, 0.106 and -0.022 respectively (all $p<0.001$). With the increase of the number of diseases, the proportion for arterial stiffness reflected by CF-PWV>12m/s, RCAVI>9 or LCAVI>9 all increased, with a β for each 1 increase were 1.40, 1.26 and 1.22 ($p<0.001$). Conclusions Participants with hypertension, DM, CAD, stroke or PAD, the degree and proportion of arterial stiffness were higher. With the increase of the combined number of diseases, the degree and proportion for arterial stiffness all increased. CAVI showed a similar effect to CF-PWV in various vascular related diseases.

Disclosures: H. Wang: None. **H. Liu:** None. **J. Liu:** None. **H. Zhao:** None.

Funding: No

Funding Component:

P274

Endothelial Dysfunction of Conduit Arteries in Amyloid Precursor Protein-Deficient Mice

Authors: Livius V. d'Uscio, Zvonimir S. Katusic, Mayo Clinic, Rochester, MN

Amyloid precursor protein (APP) is an integral membrane protein expressed in the peripheral arteries. However, the exact vascular physiological function of APP is unknown. Male APP-deficient ($APP^{-/-}$) and their wild-type littermates (WT) mice were used to characterize the phenotype of APP in the control of vascular function. Isometric force of isolated aortic rings was recorded in organ chambers. Circulating levels of norepinephrine and epinephrine were significantly enhanced in $APP^{-/-}$ mice (4723±566 pg/mL and 854±98 pg/mL, respectively $P<0.05$ vs. WT: 1999±319 pg/mL and 429±71 pg/mL, respectively; n=13). The efficacy of phenylephrine induced contractions were significantly reduced in the aorta of $APP^{-/-}$ mice (21±3%, $P<0.05$ vs. WT: 47±4%; n=10) while contractions to prostaglandin $F_{2\alpha}$ were unchanged (135±4%, P=n.s. vs. WT: 133±3%; n=9). Western blot analysis revealed that protein expression of alpha1D adrenergic receptors was significantly downregulated in $APP^{-/-}$ mice aortas (0.21±0.05 O.D.; $P<0.05$ vs. WT: 0.48±0.11 O.D.; n=6). In contrast, endothelium-dependent relaxations to β-agonist isoproterenol were significantly enhanced in $APP^{-/-}$ mice aortas ($P<0.05$; n=10) while endothelium-dependent relaxations to acetylcholine were unaltered (P=n.s.; n=12). Incubation of aortic rings with indomethacin significantly impaired relaxations to isoproterenol as well as acetylcholine in $APP^{-/-}$ mice ($P<0.05$; n=8) while concomitant treatment with NOS inhibitor L-NAME completely abolished relaxations to both agonists ($P<0.05$; n=6-7). Incubation of aortic rings with isoproterenol significantly increased cAMP in the aortas of $APP^{-/-}$ mice (16.2±4.1 pmol/mg; $P<0.05$ vs. WT: 6.6±4.1 pmol/mg; n=7). Furthermore, cAMP levels were significantly enhanced by acetylcholine in $APP^{-/-}$ mice aortas (38±9 pmol/mg; $P<0.05$ vs. WT: 14±3 pmol/mg; n=8) while acetylcholine stimulated cGMP levels were reduced (59±5 pmol/mg; $P<0.05$ vs. WT: 83±7 pmol/mg; n=12). Our results suggest that increased circulating levels of catecholamines in $APP^{-/-}$ mice are responsible for observed vascular phenotype. These findings indicate that under physiological conditions, APP expression plays an important role in control of vascular endothelial function**.**

Disclosures: L.V. d'Uscio: None. **Z.S. Katusic:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH.

Funding: No

Funding Component:

P275

Transglutaminase-2 Contributes to Angiotensin II-Induced Reactive Oxygen Species Production in Mice

Authors: Sara Ucci, Sergio Chiandotto, Cecilia Verga Falzacappa, Allegra Battistoni, Massimo Volpe, **Carmine Savoia**, Sapienza Univ Rome, Rome, Italy

Transglutaminase type II (TG2) is a pleiotropic enzyme that exhibits various activities and it is involved in diverse biological functions, including cell signaling, cytoskeleton rearrangements, displaying enzymatic activities. We previously demonstrated that TG2 may contribute to angiotensin II-induced reduction of NO bioavailability as well as to the impaired vascular functional and structural alterations induced by angiotensin II. Here we hypothesized that TG2 may contribute to increased production of reactive oxygen species (ROS) in the vasculature of angiotensin-II-treated mice. TG2-knockout mice (TG2-K/O, 8 weeks old, n=6) and age-matched wild type (WT) control mice were treated or not with angiotensin-II (400ng/kg/min) for 14 days. TG2 activity in aorta was measured by ELISA. ROS production in aorta was evaluated by dihydroethidium staining. The expression of angiotensin type1 receptor (AT1R), TG2, NOX-1, and ERp72 (the positive modulator of NOX-1) was evaluated in aorta by immunoblotting, coimmunoprecipitation analysis was also performed.

As expected, TG2-K/O lacked TG2 expression and activity. Angiotensin-II significantly increased (2-fold) TG2 expression and activity only in WT. AT1R expression in aorta was not influenced by Angiotensin II treatment in both WT and TG2K/O mice. ROS production was similar in WT and TG2-K/O and increased only in angiotensin-II-treated WT (+9%, $p<0.01$). NOX-1 and ERp72 expression was similar in WT and TG2-K/O. Angiotensin-II significantly increased NOX-1 (+23%, $p<0.01$) and ERp72 (+29%, $p<0.01$) only in WT. Only in aorta from WT and not from TG2-K/O, TG2 was successfully immunoprecipitated by AT1 and ERP72, indicating that TG2 is able to interact with both proteins, and suggesting that it may be involved in angiotensin II- induced NOX modulation and ROS production.
In conclusion, Angiotensin-II increased ROS production and NOX-1 expression and activation only in presence of TG2 in WT. TG2 interacts with both AT1R and ERp72. Thus, TG2 may contribute to NOX-induced ROS production in mice treated with angiotensin-II.

Disclosures: S. Ucci: None. **S. Chiandotto:** None. **C. Verga Falzacappa:** None. **A. Battistoni:** None. **M. Volpe:** None. **C. Savoia:** None.

Funding: No

Funding Component:

P276

Hydrogen Sulfide Regulates Local Blood Flow in Conscious, Free-Moving Rats

Authors: Juan H Loredo, Adelaeda Barrera, Joshua Garcia, Jay Naik, **Nancy L Kanagy**, Univ New Mexico, Albuquerque, NM

Inhibiting cystathionine gamma-lyase (CSE) to prevent H_2S synthesis augments constriction in isolated mesenteric and renal arteries, but H_2S regulation of resistance in these vascular beds in vivo is unknown. This study evaluated regulation of blood flow and resistance in the mesenteric and renal vascular beds in chronically instrumented rats. Based on studies in isolated arteries, we hypothesized that endogenous production of H_2S by CSE decreases vascular resistance more in mesenteric than in renal arteries. Rats were anesthetized with inhaled isofluorane (2%) and instrumented with femoral vein catheters, a telemeter blood pressure device and a pulsed Doppler flow probe (Crystal Biotech) on either the superior mesenteric artery or the renal artery (after denervation of the renal artery by removing all visible nerves and painting the surface of the artery with 10% phenol). After five to seven days of recovery from surgery, arterial pressure (AP), heart rate (HR) and relative blood flow (Q) was recorded. Rats then received a daily injection of the irreversible CSE inhibitor, propargylglycine (PAG, 50 mg/kg/day). After five injections, AP was significantly increased in rats with mesenteric flow probes (110±3 start to 122±4 mmHg post PAG, $p<0.05$ n=7 rats per group). Relative mesenteric resistance (AP/Q) normalized to starting values was increased in PAG rats (150±7% starting value) compared to no change in rats receiving saline (98±3% starting value) ($p<0.05$, n=7 rats per group). In the rats instrumented with renal flow probes, AP was also increased in the PAG but not in the saline rats (115±6 start to 143±5 mmHg post PAG, $p = 0.0014$) and calculated renal resistance increased (198% ± 14% versus 103 ± 5% starting value, $p=0.0003$, n = 5 rats). Heart rate was not changed by any of the treatments. Our results suggest that endogenous H_2S is an important regulator of vascular resistance in both the mesenteric and renal circulations and that the contribution may be larger in the renal bed than in the mesenteric bed.

Disclosures: J.H.M. Loredo: None. **A. Barrera:** None. **J. Garcia:** None. **J. Naik:** None. **N.L. Kanagy:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH HL 123301.

Funding: No

Funding Component:

P277

Ceramide Modulates GPCR And TK Receptor Signaling To Impact Endothelial Functions And Blood Pressure

Authors: Anna Cantalupo, Antonella Gargiulo, Linda Sasset, Luisa Rubinelli, Ilaria Del Gaudio, WEILL CORNELL MEDICAL COLLEGE, New York, NY; Maria Rosaria Bucci, Univ of Naples "Federico II", Naples, Italy; **Annarita Di Lorenzo**, WEILL CORNELL MEDICAL COLLEGE, New York, NY

Ceramides are sphingolipids that modulate a variety of cellular processes, via two major modes of actions: by functioning as second messengers and by regulating the formation of lipid raft, important signaling platform. Receptor-mediated signaling orchestrates multiple pathophysiological processes, hence more than 50% of drugs clinically prescribed target mainly GPCRs. Alterations of ceramide levels are implicated in endothelial dysfunction, a key event in many cardiovascular diseases, including hypertension and atherosclerosis. However, specific molecular mechanisms of how ceramides modulate the structure and the function of GPCRs and TK receptors to impact endothelial functions remains unknown and unexplored. Thus, we generated mice lacking the endothelial sphingolipid *de novo* biosynthesis, by deleting the serine palmitoyltransferase long subunit 2, of the first enzyme of the pathway, named ECKO-Sptlc2. Systolic blood pressure was markedly increased in ECKO-Sptlc2 vs. control mice (122.1± 1.9 vs. 103.6±0.7 mmHg, n=9). Vasodilation of mesenteric arteries to acetylcholine and histamine (Gq-coupled receptors) and the NO-signaling downstream was preserved suggesting that altered membrane sphingolipid levels did not affect Gq-coupled receptor activation. On the contrary, vasodilation induced by the activation of tyrosine kinase receptors, i.e. VEGFR2 (Emax 32.8±3.5 vs. 55.7±3.6 % vasodilation, n=5), Gi-coupled GPCRs, i.e. S1PR1 (Emax 9.0±11.0 vs. 58.7 ±3.5 % vasodilation, n=5) and flow were markedly reduced. C16-, C24- and C24:1-ceramide are the most abundant ceramides in the endothelium and were markedly reduced by the loss of Sptlc2. Interestingly, the treatment of the mice and endothelial cells in culture with C16-, C24- and C24:1-ceramides, showed that C16-ceramide play a specific role in VEGFR2, IR, S1PR1 and flow mediated vasodilation. Mechanistically, C16-ceramide modulates VEGFR2 phosphorylation and downstream signaling in a concentration-dependent manner. The finding that C16-ceramide affects specific the function of Gi-coupled, TK but not Gq-coupled receptor is of great significance considering that alterations in C16-ceramide strongly correlate with CV conditions, such as CAD and heart failure.

Disclosures: A. Cantalupo: None. **A. Gargiulo:** None. **L. Sasset:** None. **L. Rubinelli:** None. **I. Del Gaudio:** None. **M. Bucci:** None. **A. Di Lorenzo:** None.

Funding:

Funding Component:

P278

Flow-Induced NO Deficiency in Thick Ascending Limbs of Dahl Salt-Sensitive Rats Fed a High Salt Diet is Due to Decreased NOS3 Expression

Authors: Fara Saez, Agustin Gonzalez-Vicente, Nancy J Hong, **Jeffrey L Garvin**, Case Western Reserve Univ, Cleveland, OH

The effects of medullary NO on Na excretion are reduced in Dahl salt-sensitive (SS) compared to salt-resistant rats (SR). We have shown that flow-induced NO production by thick ascending limbs (THALs) of SS is reduced when rats are on a normal salt diet although NO synthase 3 protein expression was the same. In Sprague-Dawley rats a high-salt diet markedly increase NOS3 expression. It is unknown whether this is also the case for both SS and SR fed a high-salt diet. We hypothesized that flow-induced NO production by SS THALs is blunted in part due to a failure to increase NOS3

expression in response to dietary salt. SS and SR were fed normal and high-salt diets for 7-9 days. We measured systolic blood pressure (SBP); flow-induced NO in isolated THALs; and NOS3 protein expression in renal medullary lysates. SBP was 164.5±3.2 mm Hg (n = 9) in SS and 130.1±4.8 in SR when fed a high-salt diet (n = 7; p < 0.0001). Flow-induced NO production by SS THALs from rats fed high salt was 9 ± 2 arbitrary units (AU)/min (n = 6) while it was 44 ± 10 AU/min by SR tubules (n = 6), significantly greater (p < 0.005). NOS3 expression was 0.74 ± 0.08 AU (n = 5) in THALs from SS on high salt while it was 1.26 ± 0.08 AU in tubules from SR (n = 5), significantly greater (p < 0.003). THAL NOS3 expression was unchanged by a high-salt diet in SS (1.01 ± 0.08 vs 1.11 ± 0.11; n = 5) but increased in SR. We conclude that decreased flow-induced NO production in high-salt-fed SS is at least partially due to failure to increase NOS3 expression.

Disclosures: F. Saez: None. **A. Gonzalez-Vicente:** None. **N.J. Hong:** None. **J.L. Garvin:** None.

Funding: No

Funding Component:

P279

Regulation Of H_2S Production By Cystathionine Gamma-lyase In Endothelial Cells.

Authors: Perenkita J Mendiola, Laura V Gonzalez Bosc, Jay Naik, Nancy L Kanagy, Univ of New Mexico, Albuquerque, NM

Hydrogen sulfide (H_2S) is the most recently described endothelium-derived vasodilator. In the endothelium, H_2S is predominantly produced by cystathionine γ-lyase (CSE). We and others have previously shown CSE inhibition diminishes acetylcholine (ACh)-induced dilation in aortic ring segments. However, the mechanism of CSE regulation is not well-defined. The goal of this study was to identify endogenous regulators of CSE in endothelial cells. Previous reports suggest that [Ca^{2+}] and phosphorylation regulate CSE activity. We previously reported that there was an increase in H_2S production in rat aortic endothelial cells loaded with an H_2S fluorescence indicator, sulfide fluor-7 acetoxymethylester (SF7-AM, 10 μM) after exposure to either ACh (10 μM) or an H_2S donor (NaHS, 10 μM). Pretreatment with the CSE inhibitor, β-cyanoalanine (BCA, 100 μM) prevented the ACh-induced but not the NaHS-induced increases in SF_7 flourescence. Our current study tested the hypothesis that increasing intracellular calcium stimulates CSE-dependent H_2S production. Cultured rat aortic endothelial cells were loaded with SF_7 and a nuclear dye (Nuclear-ID Red DNA stain, to correct for cell number) and fluorescence was measured at baseline and in response to a calcium ionophore (ionomycin, 100 nM). Ionomycin increased H_2S production (Control: 0.8293, +ionomycin: 1.0365, p = 0.0001, n=3 of 7-15 wells per replicate). Pretreatment with BCA significantly decreased ionomycin-induced H_2S production (+ionomycin: 1.0365, +BCA: 0.6600, p < 0.0001, n=3 of 7-15 wells per replicate). Preliminary ongoing studies in pressurized mesenteric arteries suggest a tendency of CSE inhibition to reduce ACh-induced dilation (Control: 77.06%, +BCA: 42.04%, p=0.08, n=3-4 arteries per group). However, a larger sample size is needed. Taken together, these studies suggest that ACh elicits vasodilation through a Ca^{2+}-dependent activation of CSE.

Disclosures: P.J. Mendiola: None. **L.V. Gonzalez Bosc:** None. **J. Naik:** None. **N.L. Kanagy:** None.

Funding:

Funding Component:

P280

Type V Collagen is a Lung-Associated Self-Antigen Uncovered by Chronic Hypoxia

Authors: Laura V Gonzalez Bosc, Juan Humberto Morales-Loredo, Levi Maston, Tamara Howard, Univ of New Mexico, Albuquerque, NM; Ewa Jankowska-Gan, Matthew P Arvedson, Jeremy A Sullivan, William J Burlingham, Univ of Wisconsin Sch of Med and Public Health, Madison, WI

Hypoxic pulmonary hypertension (PH) is a progressive and often fatal consequence of chronic hypoxia (CH) exposure (chronic lung diseases, high altitude and sleep apnea). We recently demonstrated that T helper 17 (T_H17) cells are localized in the perivascular region of pulmonary arteries and contribute to CH-induced PH, at least in part, by causing pulmonary arterial (PA) remodeling. Naturally occurring "nT_H17" cells, can be detected as early as week 17 of fetal life in humans, and are in homeostatic equilibrium with natural T regulatory (nTregs) cells. nT_H17 cells are specific for a limited range of self-antigens, including type V collagen (colV). ColV is normally sequestered within type I col in the extracellular matrix of the lungs hidden from the immune system. Activation of matrix metalloproteinase-2, which is induced by CH, can expose colV. Therefore, we tested the hypothesis that ColV is a lung-associated self-antigen uncovered by CH. We found colV immunoreactivity only in lungs from mice exposed to hypobaric CH for 5 days vs. controls and colV mRNA expression is significantly increased in isolated PA (log fold change mean±SEM, normoxia= 0.22±0.08 vs. CH=0.48±0.02, n=3, p=0.02) suggesting that CH uncovers hidden colV. We then, determined cellular autoimmunity to colV using a trans-vivo delayed-type hypersensitivity assay (TV-DTH). Splenocytes obtained from 5 days CH-exposed mice displayed a TV-DTH colV response significantly higher than that from normoxic mice (footpad swelling [10^{-4}"] normoxia=11.8±1.1 vs. CH= 35.6±3.0, n=14, p=0.0001), while there was minimal response to col I. To determine that reactivity to colV contributes to CH-induced PH, we induced peripheral tolerance to colV by administering 25 µl of 0.08 µg/µl of bovine colV (Sigma) in PBS in each nare or PBS every two days for two weeks prior and during CH (21 days). Right ventricular systolic pressure (RVSP) was higher in vehicle-treated compared to colV-treated mice (mmHg CH vehicle= 32.4±0.3 vs. CH colV= 27.8±0.9, n=4, p=0.0085) suggesting that oral tolerance to col V attenuated PH.
These results are ground-breaking suggesting that acute autoreactivity to colV develops after exposure to hypoxia. Next, we will determine whether a disrupted balance of nTh17/nTreg mediates this response.

Disclosures: L.V. Gonzalez Bosc: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; AHA 15GRNT25090039. **J.H. Morales-Loredo:** None. **L. Maston:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NHI F30HL123109. **T. Howard:** None. **E. Jankowska-Gan:** None. **M.P. Arvedson:** None. **J.A. Sullivan:** None. **W.J. Burlingham:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH R01AI119140.

Funding: Yes

Funding Component: South Central Affiliate (Arkansas, New Mexico, Oklahoma & Texas)

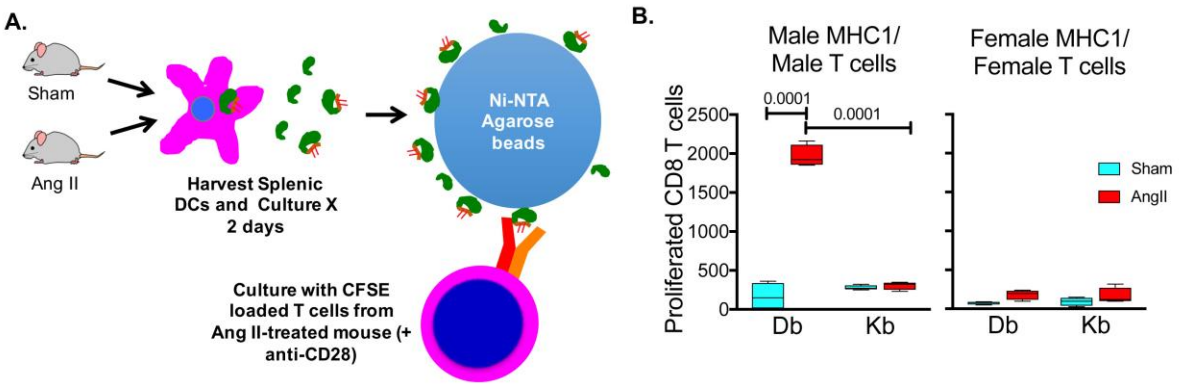
P281

Artificial Dendritic Cells Identify a Major Histocompatibility Complex Class 1 Subset that Activates T Cells in Hypertension

Authors: Wei Chen, David M. Patrick, Natalia R. Barbaro, Vanderbilt, Nashville, TN; Kenneth E. Bernstein, Cedars Sinai, Los Angelos, CA; Kenneth E. Bernstein, Cedars Sinai, Los Angeles, CA; Daniel Roeth, Markus Kalkum, City of Hope, Duarte, CA; Liang Xiao, Annet Kirabo, David G. Harrison, Vanderbilt, Nashville, TN

We and others have shown an important role CD8+ cells in both experimental and human hypertension. CD8+ T cells are activated by antigens presented by major histocompatibility complex 1 (MHC1). C57BL/6 mice express two MHC1, referred to as H2-Kb and H2-Db. To identify antigenic peptides responsible for hypertension, we made two transgenic mice lacking the transmembrane domains of MHC1 and an added His-tag to the modified MHC1. These transgenes are driven by CD11c promoter, allowing expression in antigen presenting cells. The soluble Kb and Db (sKb and sDb) mice received 2 week infusions of sham or angiotensin II and their splenocytes placed in culture for two days. Ni-NTA beads

were then used to bind the shed MHC-1 and these beads mixed with 10^6 T cells from other ang II infused mice (Figure panel A). CFSE dilution was used to monitor T cell proliferation. We found that sDb from ang II infused male mice, but not sham infused mice, potently stimulated proliferation of CD8+ T cells from ang II infused male mice. This degree of stimulation was significantly greater than that observed by sKb (Figure panel B). The shed MHC1 from female ang II-treated mice caused significantly less stimulation of female CD8+ T cells compared to male MHC1, in keeping with prior observations that female mice have less immune activation in hypertension. In summary, these data strongly suggest that unique antigens, presented in the context of H2-Db, are generated in hypertension. These data also suggest that there may be unique human lymphocyte antigens (HLAs), the analog of mouse MHC, that predispose to hypertension and related end-organ damage.



Disclosures: W. Chen: None. **D.M. Patrick:** None. **N.R. Barbaro:** None. **K.E. Bernstein:** None. **K.E. Bernstein:** None. **D. Roeth:** None. **M. Kalkum:** None. **L. Xiao:** None. **A. Kirabo:** None. **D.G.G. Harrison:** None.

Funding: Yes

Funding Component: National Center

P282

Knockout of Transient Receptor Potential Ankyrin 1 Exacerbates Renal Ischemia-Reperfusion Injury

Authors: Shuangtao Ma, Donna H. Wang, Michigan State Univ, East Lansing, MI

Transient receptor potential ankyrin 1 (TRPA1) is a newly identified oxidant sensor and has anti-oxidative properties. The role of TRPA1 in acute kidney injury is largely unknown. In this study, we test the hypothesis that dysfunctional TRPA1 leads to aggravated renal inflammation and injury after renal ischemia-reperfusion injury using Trpa1 gene knockout and wild-type mice. Male 8-week-old mice were subjected to renal ischemia for 30 minutes by clamping bilateral renal pedicles under isoflurane anesthesia; the mice were sacrificed at 24 hours after surgery. Renal functional impairment, tubular injury, oxidative stress, and renal inflammation were measured at 24 hours after reperfusion. TRPA1 was found to be expressed in the kidney of wild-type mice and significantly downregulated after ischemia-reperfusion injury ($p<0.01$). In Trpa1 knockout mice, plasma creatinine (1.58±0.08 *vs.* 1.19±0.07 mg/dl, $p<0.01$) and blood urea nitrogen (171.1±6.5 vs. 143.2±3.9 mg/dl, $p<0.01$) levels after ischemia-reperfusion injury were significantly higher than those in wild-type mice. Similarly, tubular injury score, calculated based on hematoxylin- and eosin-stained kidney sections, was significantly elevated in Trpa1 knockout mice compared with wild-type mice ($p<0.05$). Dihydroethidium-stained sections showed that superoxide production was enhanced in Trpa1 knockout mice than wild-type mice after ischemia-reperfusion injury ($p<0.01$). In renal injured mice, immunofluorescence staining showed that F4/80-positive macrophages were elevated in Trpa1 knockout mice than wild-type mice ($p<0.01$). Moreover, inflammatory cytokines, including tumor necrosis factor-alpha, interleukin-1β, and interleukin-6 in the kidneys, were increased in Trpa1 knockout mice compared to wild-type mice (all $p<0.05$). These results show that knockout of TRPA1 exacerbates renal oxidative

stress, inflammation, and injury after ischemia-reperfusion, indicating that dysfunctional TRPA1 may lead to impaired protective mechanisms for the kidney while TRPA1 activation may serve as therapeutic means preventing renal injury.

Disclosures: S. Ma: None. **D.H. Wang:** None.

Funding: No

Funding Component:

P283

Gamma Delta T Cells Drive CD4$^+$ and CD8$^+$ T Cell Activation in Hypertension

Authors: Pierre Paradis, Antoine Caillon, Lady Davis Institute for Medical Res, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada; Ernesto L. Schiffrin, Lady Davis Institute for Medical Res, Dept of Med, SMBD-Jewish General Hosp, McGill Univ, Montreal, QC, Canada

Objective: Both innate (monocyte/macrophages) and adaptive immune cells (T lymphocytes) have been shown to play a role in hypertension and vascular injury. Recently, we demonstrated that a small subset of "innate-like" T lymphocytes, expressing the γδ 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: C57BL/6 male mice were infused with Ang II (490 ng/kg/min, SC) for 7 and 14 days (n=5-7). All mice were 14-15 week-old at the end of the study. Spleen T cell profile was determined by flow cytometry. γδ and αβ T cells were isolated using magnetic beads from peripheral lymph nodes and spleen of 8 to 9-week-old C57BL/6 male mice. αβ T cells were cultured alone or with γδ T cells (5:1) in presence of anti-CD3 antibodies plus or minus Ang II, and αβ T cell activation was evaluated by flow cytometry.
Results: Close correlations were demonstrated between the number (#) of activated CD69$^+$γδ T cells and CD4$^+$CD69$^+$ T cells (r^2=0.74, P<0.01) and CD8$^+$CD69$^+$ T cells (r^2=0.64, P<0.01) after 7-day Ang II. These correlations decreased after 14-day Ang II. Correlations were also shown between the # of CD27$^+$CD69$^+$γδ T cells and CD4$^+$CD69$^+$ T cells (r^2=0.76, P<0.001) and CD8$^+$CD69$^+$ T cells (r^2=0.65, P<0.01) after 7-day Ang II. *In vitro*, Ang II increased the fraction of CD69$^+$αβ T cells when αβ T cells were co-cultured with γδ T cells (% of αβ T cells: 19.9±2.9 vs. 16.4±2.6, P<0.001) but not when cultured alone (% of αβ T cells: 13.7±0.7 vs. 12.7±0.6). A correlation between the fraction of CD69$^+$γδ T cells and CD69$^+$αβ T cells was also observed (r^2=0.69, P<0.001).
Conclusion: These results suggest that γδ T cells mediate activation of αβ T cells in Ang II-induced hypertension. Targeting γδ T cells may contribute to reduce the low-grade inflammation found in hypertension.

Disclosures: P. Paradis: None. **A. Caillon:** None. **E.L. Schiffrin:** None.

Funding: No

Funding Component:

P284

Increased Blood Pressure in Interleukin-6 Infused Mice is Secondary to Reduced Urinary Sodium Excretion and Not Vascular Dysfunction

Authors: Gillian G Hecht, Emory Univ Sch of Med, Atlanta, GA; Cam G McCarthy, Camilla F Wenceslau, Medical Coll of Georgia at Augusta Univ, Augusta, GA; Hayley C Moyer, Ashwini Krishnamurthy, Emory Univ Sch of Med, Atlanta, GA; R. Clinton Webb, Medical Coll of Georgia at Augusta Univ, Augusta, GA; Robert S. Hoover, **Brandi M Wynne**, Emory Univ Sch of Med, Atlanta, GA

Hypertension (HTN) is associated with increased renal sodium (Na^+) reabsorption, vascular and endothelial dysfunction and an increase in cytokines, such as interleukin 6 (IL-6). We have previously shown that IL-6 increases blood pressure (BP), and increases the expression/activity of distal nephron Na^+ transport proteins via the mineralocorticoid receptor. We hypothesized that IL-6 increases Na^+ reabsorption, and induces endothelial dysfunction, leading to HTN. All mice were fed high salt food, and osmotic minipumps were implanted containing IL-6 (16 ng/hr, 0.01% BSA/saline), or vehicle/sham surgery (Ctl). To allow for early urine collection, IL-6 infusion was delayed by adding catheters to the minipumps during metabolic cage studies. Tail cuff plethysmography was used to measure systolic BP. Concentration response curves (CRC) to phenylephrine (Phe) and acetylcholine (ACh) were performed on aorta (Ao) and mesenteric arteries (Mes) from IL-6 or Ctl mice. IL-6 infused mice had increased BP by day 3 (D3), as compared to Ctl. Total urine volume (U_{Vol}) on D1 of infusion was reduced in IL-6 mice (U_{Vol} 17.3±4 *vs.* 29.5±3 mL/day/per 100g body weight (BW); $p<0.05$), as compared to Ctl. This corresponded with a reduction in total urinary Na^+ excretion (U_{Na} 81±19 IL-6 *vs*. 139±13 mg/day/BW Ctl; $p<0.05$). By D2, no differences in U_{Na} were observed, and by D3 U_{Na} levels were increasing in IL-6 infused mice. Similar trends for U_{Vol} were observed. These results corresponded with our observed increases in BP. CRCs (D3) to Phe were not changed in Ao. However, Mes from IL-6 mice exhibited a surprising reduction in Phe-mediated contraction (136±7% *vs.* 155±5%; $p<0.05$), compared to Ctl. Similarly, Mes from IL-6 mice exhibited an increase in ACh-mediated relaxation responses (EC_{50} -7.5±0.12 *vs*. -6.9±0.15; $p<0.05$). These data suggest that IL-6 increases BP via an early (D1) reduction in U_{Na}/U_{Vol}, followed by steady state recovery and pressure natriuresis (D3). Additionally, the increased relaxation and reduced contractility observed in the resistance arteries may suggest a compensatory response to the increased BP. Together, our studies suggest that in the early stages of HTN, cytokines may cause changes in renal Na^+ reabsorption via activation of distal Na^+ transporters.

Disclosures: G.G. Hecht: None. **C.G. McCarthy:** None. **C.F. Wenceslau:** None. **H.C. Moyer:** None. **A. Krishnamurthy:** None. **R. Webb:** None. **R.S. Hoover:** None. **B.M. Wynne:** None.

Funding: No

Funding Component:

P285

Carotid Sinus Nerve Stimulation Attenuates Alveolar Bone Loss in Rats With Induced Periodontitis.

Authors: Aline B Ribeiro, Patricia G Fernandes, Fernanda Brognara, Jaci A Castania, Carlos A Silva, Michel R Messora, **Helio C Salgado**, Univ of Sao Paulo, Ribeirao Preto, Brazil

Introduction. Few studies have focused on the impact of the progression of periodontitis on hypertension. Baroreflex Activation Therapy (BAT) has been used to treat patients with resistant hypertension. Our laboratory has investigated the role of electrical carotid sinus nerve (CSN) stimulation in unanesthetized rats, on local and systemic inflammation. Since periodontitis is a chronic inflammatory disease, the aim of this study was to evaluate the role of the baro- and chemoreflex activation, by means of electrical stimulation of the CSN, on alveolar bone loss in rats with periodontitis. **Methods and results:** Under ketamine/xylazine anesthesia Wistar Hannover rats were implanted with electrodes around the CSN and a catheter inserted into the abdominal aorta for blood pressure recording. After 48h periodontitis was induced by the ligation of the bilateral mandibular first molar, followed by the electrical stimulation of the CSN (1,5-4V, 1ms, 30Hz for 10 min); which was continued during the next eight days. At the 8th day after the induction of periodontitis, the rats were euthanized and the jaws were resected; besides, microtomographic analysis was performed

by bi and three-dimensional quantification using micro-computerized tomography. As compared to baseline results the electrical stimulation of the CSN (N=7) promoted a decrease in mean arterial pressure (108± 8 vs. 91± 5 mmHg; p<0.05) and heart rate (374±10 vs. 319±15 bpm; p<0.05). The CSN stimulated rats (N=7) with periodontitis showed greater bone volume and bone surface than the non-stimulated control rats (N=4) with periodontitis, evaluated by three-dimensional analysis (0.5±0.005 vs. 0.7±0.03 mm^3; 16.54±0.22 vs. 23.30±0.66 mm^2, respectively; p<0.05). Moreover, the CSN stimulated rats with periodontitis presented a decrease of the furcation area and interproximal region as compared to the non-stimulated control rats (1.14±0.06 vs. 0.5±0.07 mm; 3.25±0.03 vs. 2,48±0,13 mm, respectively; p<0.05). **Conclusions:** Overall, these results demonstrate that stimulation of the CSNs promotes a protective effect on alveolar bone loss elicited by periodontitis involving the activation of the baro- and chemoreflex.

Disclosures: A.B. Ribeiro: None. **P.G. Fernandes:** None. **F. Brognara:** None. **J.A. Castania:** None. **C.A.A. Silva:** None. **M.R. Messora:** None. **H.C. Salgado:** None.

Funding: No

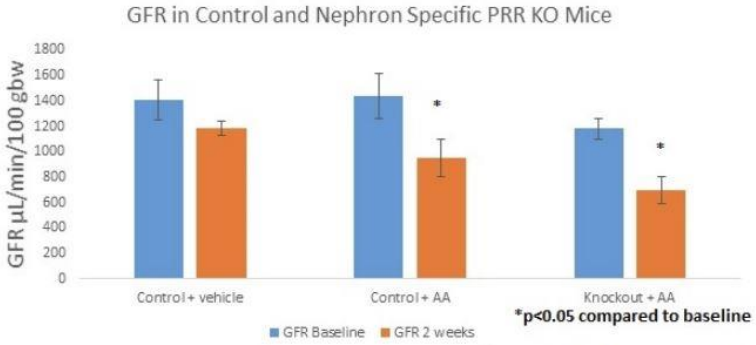
Funding Component:

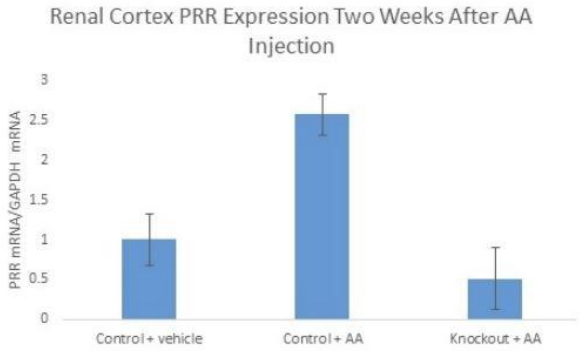
P286

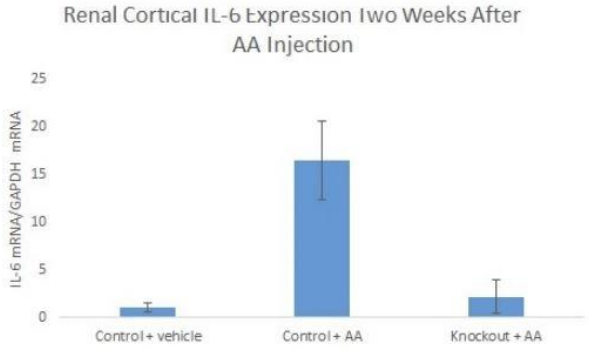
(Pro)renin Receptor Mediates Inflammation Induced by Renal Injury

Authors: Silas Allen Culver, Rebecca McDevitt, Safia Akhtar, Caixia Li, Helmy Siragy, Univ of Virginia, Charlottesville, VA

(Pro)renin Receptor (PRR) expression in the kidney is reported to increase during injury from diabetic nephropathy, and ischemia. PRR is also reported to play a role in promoting renal inflammation. Aristolochic acid (AA) is a nephrotoxin which is known to target the proximal tubule. However, it is not known whether PRR plays a role in cortical inflammation caused by AA. We hypothesized that PRR promotes renal cortical inflammation in the setting of AA induced injury. This study utilized an inducible nephron specific PRR knockout (KO) mouse with KO induced at 6 weeks of age. Ten week old wild type and KO mice received intraperitoneal injection with 10mg/kg AA or vehicle. At baseline and two weeks after injection, glomerular filtration rate (GFR) was measured by FITC-sinistrin contrast injection. Two weeks after injection, kidneys were harvested, and RNA extracted from cortical tissue. PRR and IL-6 mRNA expression was measured by RT-PCR. Two weeks after injection, GFR was reduced in the Control +AA (1436 vs. 948 ul/min/100g body weight p<0.05) and KO + AA (1181 vs. 697 ul/min/100g body weight p<0.05) groups but not in control + vehicle group (1407 vs. 1186 ul/min/100g body weight). Renal cortical PRR expression increased by 157% in the Control + AA group compared to Control + vehicle (2.57 vs. 1) but was not increased in the KO + AA group (0.54). IL-6 expression similarly increased in the Control + AA group compared to Control + vehicle 1643% (16.43 vs. 1) and this response was attenuated in the KO compared to Control + vehicle (2.15 vs. 1). PRR promotes the inflammation of the renal cortex in response to AA injury. Further studies are needed to determine the long term role of PRR in renal injury.







Disclosures: **S.A. Culver:** None. **R. McDevitt:** None. **S. Akhtar:** None. **C. Li:** None. **H. Siragy:** None.

Funding: No

Funding Component:

P287

Time Course of Hemodynamic Responses to Different Doses of Lipopolysaccharide Administration in Unanesthetized Rats

Authors: Fernanda Brognara, Jaci A Castania, Univ of São Paulo, Ribeirão Preto, Brazil; Daniel P Dias, Barão de Mauá Univ Ctr, Ribeirão Preto, Brazil; Alexandre Kanashiro, **Helio C Salgado**, Univ of São Paulo, Ribeirão Preto, Brazil

Introduction: Intravenous administration of lipopolysaccharide (LPS) from *Escherichia coli* is an experimental model of systemic inflammation that has been widely used for investigating new therapeutic strategies for sepsis, which is characterized by clinical manifestations such as hypo or hyperthermia, tachycardia, hypotension and tachypnea. However, there is a number of doses of LPS used in several studies, and the hemodynamic responses were not well characterized. Thus, the aim of the present study was to evaluate the hemodynamic (arterial pressure and heart rate) responses from unanesthetized rats to different doses of LPS over time. **Methods and results:** Femoral artery and vein catheters were inserted into anaesthetized Wistar Hannover male rats for arterial pressure recording and LPS administration, respectively. On the next day, the arterial pressure was recorded before (baseline) and after (90, 180 and 360 min) LPS injection in the unanesthetized subjects. Four different doses of LPS were tested: 0.06 mg/kg (n = 8), 20 mg/kg (n = 4), 30 mg/kg (n = 4), and 40 mg/kg (n = 5). As compared to baseline, the dose of 0.06 mg/kg of LPS decreased the mean arterial pressure at 360 min after its administration (113 ± 4 vs. 103 ± 7 mmHg, $p = 0.007$). However, the doses of 20, 30 and 40 mg/kg increased the mean arterial pressure at 360 min after LPS, compared to baseline values (*20 mg/kg:* 101 ± 2 vs. 121 ± 4 mmHg, $p < 0.001$; *30 mg/kg:* 115 ± 6 vs. 130 ± 0.4 mmHg, $p = 0.023$; *40 mg/kg:* 92 ± 2 vs. 107 ± 3 mmHg, $p = 0.005$). In addition, all doses tested increased the heart rate at 360 min after LPS administration (*0.06 mg/kg:* 342 ± 19 vs. 435 ± 11 bpm, $p < 0.001$; *20 mg/kg:* 388 ± 6 vs. 588 ± 24 bpm, $p < 0.001$; *30 mg/kg:* 366 ± 15 vs. 569 ± 8 bpm $p < 0.001$; *40 mg/kg:* 426 ± 27 vs. 606 ± 24 bpm, $p < 0.001$). Nevertheless, the dose of 0.06 mg/kg promoted a smaller tachycardia as compared to the other doses evaluated (*0.06 mg/kg:* 435 ± 11 bpm; *20*

mg/kg: 588 ± 24 bpm; *30 mg/kg:* 569 ± 8 bpm; *40 mg/kg:* 606 ± 24 bpm, $p < 0.001$). **Conclusions:** These findings indicate that it is important to choose the correct dose of LPS, depending on the aim of the study. The current data indicate that each dose of LPS may promote different hemodynamic (mean arterial pressure and heart rate) responses, affecting the therapeutic strategy, for instance, for sepsis.

Disclosures: F. Brognara: None. **J.A. Castania:** None. **D.P.M. Dias:** None. **A. Kanashiro:** None. **H.C. Salgado:** None.

Funding: No

Funding Component:

P288

Bortezomib Reduces Blood Pressure and Proteinuria in the Dahl Salt Sensitive (S) Rat

Authors: Kenji J Maeda, Erin B Taylor, Courtney A Ross, Michael J Ryan, Michael R Garrett, Jennifer M Sasser, Univ of Mississippi Medical Ctr, Jackson, MS

Hypertension is a major health care disorder affecting up to one in three adults in the United States and is a major risk factor for multiple diseases including coronary artery disease, stroke, congestive heart failure, and end-organ damage. Previous studies have proposed that hypertension is partly driven by an increase in autoantibodies and immunological changes that lead to increased blood pressure. In this study, we tested the hypothesis that treatment with Bortezomib (BTZ), a proteasome inhibitor approved for the treatment of multiple myeloma and mantle cell lymphoma, would attenuate the increase in blood pressure and accompanying rise in urinary protein excretion in the Dahl S and Angiotensin II (Ang II) infused Sprague Dawley (SD) rat models of hypertension. Telemetry implants were placed in the femoral artery for continuous monitoring of mean arterial pressure (MAP) in male Dahl S and SD rats (~14 weeks old, maintained on a normal salt diet), and rats were allowed to recover for one week before baseline urine and MAP were collected. Then, Ang II (400ng/kg/hr) osmotic mini-pumps were implanted in SD rats, and BTZ treatment (0.2 mg/kg twice weekly, s.c.) was started in both Dahl S and SD rats. Ang II mini-pumps were replaced at 2 weeks, and urine was collected at weeks 2 and 4. MAP was reduced by BTZ in Dahl S rats, but no effect was observed in control or Ang II treated SD rats (Table 1). BTZ had no effect on urinary protein excretion (Bradford assay) in any group. These data show that administration of Bortezomib attenuates hypertension in Dahl S but not Ang II hypertension.

Table 1: MAP and Urinary Protein Excretion after 4 weeks of BTZ treatment
* p = 0.0035 Dahl S vs BTZ Dahl S

	MAP (mm Hg) n = 4 - 5	Urinary Protein Excretion (mg/day) n = 7 - 9
Control SD	108.06 ± 8.13	39.22 ± 6.40
BTZ SD	110.26 ± 2.11	42.35 ± 6.71
ANG II SD	143.65 ± 14.11	64.60 ± 15.48
BTZ ANG II SD	140.78 ± 10.09	62.98 ± 13.70
Dahl S	137.26 ± 1.48*	330.36 ± 43.03
BTZ Dahl S	128.25 ± 1.46	241.61 ± 41.99

Disclosures: K.J. Maeda: None. **E.B. Taylor:** None. **C.A. Ross:** None. **M.J. Ryan:** None. **M.R. Garrett:** None. **J.M. Sasser:** None.

Funding:

Funding Component:

P289

Effect of Abaptacept on Blood Pressure in Essential Hypertensive Subjects with Rheumatological Disorders

Authors: Cheryl L Laffer, Megan M Shuey, Jonathan D Mosley, Meenakshi S Madhur, David G Harrison, Fernando Elijovich, Vanderbilt Univ Sch Medicin, Nashville, TN

We previously showed that abatacept, an inhibitor of T cell co-stimulation, prevents and reverses experimental hypertension. We now investigated whether it lowers blood pressure (BP) in humans. Of 1624 subjects receiving this drug in our de-identified electronic health record, 320 had hypertension and adequate baseline and follow up data (1 month to 3 years). Use of glucocorticoids (GC), non-steroidal antiinflammatory agents (NSAIDs), and antihypertensive drugs (HTND) was scored with the WHO system. ΔBPs at 1, 3 and 6 months and 1, 2 and 3 years after abatacept were adjusted for concomitant changes in medications and weights (Wt) by multivariate regression. Subjects were 80.6% female, 88.4% white, age 62±1 y, BMI 30.9±0.4 Kg/m^2 and BP 128.2±0.8/74.6±0.5 mmHg. Significant changes in confounders over time included decreases in GC, NSAID and HTND over the initial months followed by rebound beyond baseline at 2 and 3 years, and increases in Wt in these late periods. Changes in adjusted BP were limited to an increase in systolic BP at 2 years (1.75±1.04, $p<0.05$) and a decrease in diastolic BP at 3 years (-1.67±0.80, $p<0.02$). 91 subjects (28.4%) had a decrease of MAP ≥5 mmHg in at least half of the periods. ΔBP in these "responders" vs all other subjects was -10.8±0.7/-7.6±0.4, MAP -8.6±0.4 vs +5.0±0.4/+2.3±0.2, MAP +3.2±0.2, but these groups did not differ in sex, age, race, obesity, use of concomitant immunomodulators, pressors or blockers of the renin-angiotensin system (RASB). However, pooled adjusted ΔBP differed between women and men (diastolic -0.72±0.26 vs +1.48±0.48, $p=0.0002$; MAP -0.29±0.26 vs +1.58±0.55, $p=0.003$); between subjects younger and older than 65 y (systolic -0.05±0.48 vs +1.88±0.58, $p=0.021$; MAP -0.39±0.33 vs +0.64±0.37, $p=0.037$); and between those with BMI≤25 and >25 Kg/m^2 (systolic -1.31±0.79 vs +1.37±0.42, $p<0.008$). Finally, Δsystolic BP was -0.15±0.53 in subjects not taking and +1.42±0.50 in those taking RASB, $p=0.037$. In summary, long term use of abatacept mildly reduces BP in lean women with essential hypertension and rheumatological disorders. The effect is attenuated by obesity and perhaps also by aging and treatment with RASB. Further studies of abatacept in resistant essential hypertension are warranted, particularly in women.

Disclosures: C.L. Laffer: None. **M.M. Shuey:** None. **J.D. Mosley:** None. **M.S. Madhur:** None. **D.G. Harrison:** None. **F. Elijovich:** None.

Funding:

Funding Component:

P290

Central Angiotensin II and Interleukin-1©¬ Upregulate Brain Tumor Necrosis Factor-¥á Converting Enzyme in the Rat

Authors: Yiling Cao, Yang Yu, Balyssa B. Bell, Univ of Iowa Carver Coll of Med, Iowa City, IA; Robert B. Felder, Univ of Iowa Carver Coll of Med & VA Medical Ctr, Iowa City, IA; Shun-Guang Wei, Univ of Iowa Carver Coll of Med, Iowa City, IA

Tumor necrosis factor-α (TNF-α) converting enzyme (TACE/ADAM17) proteolytically cleaves the extracellular domain of transmembrane TNF-α to release the soluble form of TNF-α that contributes to augmented sympathetic nerve activity in heart failure (HF) and hypertension (HTN). Our previous study indicated that TACE is upregulated in the subfornical organ (SFO) and hypothalamic paraventricular nucleus (PVN), two key cardiovascular and autonomic brain centers, in rats with ischemia-induced HF, and is associated with TNF-α –induced sympathetic excitation in that setting. However, the mechanisms regulating TACE activity in the brain remain a mystery. The present study sought to determine whether angiotensin II (ANG II) or interleukin-1β (IL-1β), which increase in SFO and PVN in HF, upregulate TACE expression. Urethane anesthetized male SD rats underwent a 4- hour intracerebroventricular (ICV) infusion of artificial cerebrospinal fluid (aCSF), ANG II (100 ng/hr) or IL-1β (50 ng/hr). Some rats were euthanized to collect SFO and PVN tissues to measure

TACE mRNA and protein; others were transcardially perfused with 4% paraformaldehyde to immunostain for TACE in SFO and PVN. ICV infusion of ANG II (n=6) or IL-1β (n=6) significantly (*p<0.05) increased TACE mRNA level (fold change) in SFO (2.54 ± 0.24* and 2.36 ± 0.21*, respectively) and PVN (2.39* ± 0.25 and 2.23 ± 0.20*, respectively) compared with ICV aCSF infusion. Western blot analysis also revealed a significant increase in the TACE protein (normalized to β-actin) in ANG II or IL-1β infused rats in SFO (0.31±0.06* and 0.26±0.05* respectively) and PVN (0.27 ± 0.04*, 0.24 ± 0.03*, respectively) compared with ICV aCSF-infused rats (SFO: 0.12±0.05; PVN: 0.09±0.04). Confocal images exhibited that TACE-like immunoreactivity was markedly higher in all four subdivisions of PVN and in SFO in rats treated with ICV ANG II or IL-1β, compared with aCSF-infused rats. These data indicate that ANG II and IL-1β upregulate TACE expression in SFO and PVN, suggesting that activation of the renin-angiotensin system and augmented inflammation may upregulate TACE expression in the SFO and PVN in HF and HTN. TACE-mediated ectodomain shedding in SFO and PVN may contribute to ANG II- and cytokine-driven sympathoexcitation in HF and HTN.

Disclosures: Y. Cao: None. **Y. Yu:** None. **B.B. Bell:** None. **R.B. Felder:** None. **S. Wei:** None.

Funding:

Funding Component:

P292

Gene Editing Rat Resource Center: Rat Models for Heart, Lung, Blood, and Sleep Disorder Studies

Authors: Aron M Geurts, Rebecca Schilling, Michael Grzybowski, Anne Temple, Allison Zappa, Lynn Lazcares, Jessica Niebuhr, Shawn Kalloway, Jamie Foeckler, Akiko Takizawa, Melinda R. Dwinell, Medical Coll of Wisconsin, Milwaukee, WI

Transgenesis and gene editing in the rat has produced gene-modified strains which can be used to validate a gene underlying a quantitative trait locus or human association, or to design follow up studies to define function and physiological roles of genes or sequence variants. We are currently in the fifth year of developing rat models for investigators to validate human variation and study function and mechanism for genes implicated in heart, lung and blood disorders. The Gene Editing Rat Resource Center (GERRC, http://rgd.mcw.edu/wg/custom_rats/gerrc) is an NHLBI-funded R24 resource program to generate, distribute, and cryopreserve novel rat models to the research community. Investigator-initiated applications to produce novel genetically modified rat strains were received from laboratories across the world and reviewed by an external advisory board for scientific merit and potential broad interest to the heart, lung, blood, and sleep disorder research community. Custom knockout, knockin, and transgenic rat models continue to be developed. To date, we have transferred over 19,570 microinjected embryos, resulting in 4000+ live-born pups of which 3400+ have been screened for transgenesis or mutagenesis of the target gene. Of these, we have successfully generated ~630 pups containing transgenes or targeted mutations in 95 genes, distributed among 16 inbred, outbred, consomic and congenic rat strains frequently used in cardiovascular research. After confirming germline transmission, heterozygous breeders are distributed to the requesting investigator and then each model is made available to the rat research community. Sperm is cryopreserved to maintain a permanent source of these models. Collectively, the GERRC resource represents the largest collection of genetically modified rat models which are distributable to any investigator through a standard materials transfer agreement at the cost of rearing and shipping. We will discuss the general progress of genetic engineering in rats, current challenges for the field, and opportunities for future developments.

Disclosures: A.M. Geurts: None. **R. Schilling:** None. **M. Grzybowski:** None. **A. Temple:** None. **A. Zappa:** None. **L. Lazcares:** None. **J. Niebuhr:** None. **S. Kalloway:** None. **J. Foeckler:** None. **A. Takizawa:** None. **M.R. Dwinell:** None.

Funding: No

Funding Component:

P293

Chronic Pressure Overload Impacts Cardiac Endothelial Cell and Macrophage Gene Expression

Authors: Achim Lother, Heart Ctr Freiburg Univ, Freiburg, Germany; Lisa Deng, Inst of exp. and clin. Pharmacology and Toxicology, Univ of Freiburg, Freiburg, Germany; Ingo Hilgendorf, Theresa Kehl, Christoph Bode, Heart Ctr Freiburg Univ, Freiburg, Germany; Lutz Hein, Inst of exp. and clin. Pharmacology and Toxicology, Univ of Freiburg, Freiburg, Germany

Introduction
The heart is composed of various different cell types that show distinct differences in gene expression. Inflammation is a key driver of adverse cardiac remodeling and endothelial cells and macrophages are crucially involved in that process. Thus, the aim of this study was to investigate cell type-specific changes in gene expression in cardiac endothelial cells and macrophages after left ventricular pressure overload.
Methods and results
Wildtype mice underwent transverse aortic constriction (TAC) to induce cardiac remodeling. After pressure overload, the number of macrophages in the heart increased about 3-fold (TAC 1755 ± 214 vs. CTRL 533 ± 76 macrophages / mg tissue, $P < 0.001$) as determined by flow cytometry. Lectin^{+} endothelial cells and CD45^{+} CD11b^{+} F4/80^{high} Ly6C^{low} macrophages from CTRL and pressure overloaded hearts were isolated by fluorescence-assisted cell sorting. Cell type-specific gene expression was determined by RNAseq. We found 207 genes differentially expressed in cardiac endothelial cells isolated from pressure overloaded vs. CTRL hearts (n = 3-4 per group, $q < 0.05$). Approximately 25 % (52 genes) of these genes were highly enriched in endothelial cells vs. heart tissue (> 8-fold, $q < 0.05$). In cardiac macrophages, 1.359 genes were differentially expressed after pressure overload (n = 4-5 per group, $q < 0.05$). Bioinformatics analysis were performed to identify affected gene networks and putative key transcription factors involved in the regulation of these genes during cardiac remodeling. Integration of these datasets allowed to determine common, distinct and potentially interacting features of gene expression in both cell types.
Conclusion
Chronic pressure overload induces distinct changes in gene expression in isolated cardiac endothelial cells and macrophages. Ongoing epigenetic analysis will provide insight into mechanisms of cell type-specific transcriptional regulation in these cells during cardiac remodeling.

Disclosures: A. Lother: None. **L. Deng:** None. **I. Hilgendorf:** None. **T. Kehl:** None. **C. Bode:** None. **L. Hein:** None.

Funding: No

Funding Component:

P294

Intergenerational Obesity: Impact on Cardiac Function and Reserve in Mice Fed a High Fat Diet

Authors: Fabio N. Gava, John E. Hall, Alexandre A. da Silva, **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. However, the impact of intergenerational obesity on cardiac function and reserve during increased stress is still unknown. In this study we examined if intergenerational obesity alters cardiac function and responses to a stress test induced by dobutamine in 22-week-old male lean mice fed a control diet (n=5) and obese mice fed a high fat diet (HFD) after birth and that were offspring of mothers who were fed a HFD (F1-HFD, n=7). Mice were instrumented with venous catheters 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 (VEVO3100). Baseline heart rate (HR) was similar in F1-HFD (388±15 bpm) and control (426±22 bpm). Compared to controls, F1-HFD mice exhibited impaired

diastolic function (E'/A' ratio: 0.9±0.1 vs. 1.4±0.1 mm/s and isovolumetric relaxation time: 30±2 vs. 21±1 ms) but increased baseline ejection fraction (EF) (84±1 vs. 64±4 %). Dobutamine infusion increased HR by 6±3 and 41±11 bpm, and EF (84±1 to 93±2 and 64±4 to 94±2 %) in F1-HFD and controls, respectively. These results indicate that intergenerational obesity is associated with diastolic dysfunction, but no major alterations in cardiac reserve in response to a cardiovascular stress test. (NHLBI-PO1HL51971, NIGMS P20GM104357 and U54GM115428)

Disclosures: F.N. Gava: None. **J.E. Hall:** None. **A.A. da Silva:** None. **J.M. do Carmo:** None.

Funding: No

Funding Component:

P295

Ambulatory Blood Pressure And Heart Rate Variability In Dietary Sodium And Weight Intervention

Authors: John S LEE, Princeton Univ; Univ of Minnesota Sch of Med, Minneapolis, MN; Mary S. Lee, Northwestern Univ Feinberg Sch of Med; Univ of Minnesota Sch of Med, Minneapolis, MN; Jong Y. Lee, Richard Gillum, Franz Halberg, Univ of Minnesota Sch of Med, Minneapolis, MN

Blood pressure (BP) fluctuates due to complex interactions between genetic factors and environmental stimulation. BP variability (BPV) is a physiological phenomenon, as a measure of hemodynamic conditions reflected in the autonomic nervous system. Daily (circadian) rhythms as biological time structures are considered essential parameters for recognizing and treating BP risk factors. Thus, ambulatory BP and heart rate (HR) were monitored (ABPM) in 11 normotensive (NT) in a free living, and 13 borderline hypertensive (BH) adults with Na and weight (WT, Kg) interventions in a freely moving lab environments, around the clock at 30-minute to hourly intervals. In the intervention study, circadian BP, HR, urinary aldosterone (Aldo, µg/h), creatinine (Cr), Na, K in mEq/h and Na/K ratio were compared. Data was analyzed by the linear least square rhythmometry method. All subjects showed significant circadian fluctuations in BP (mm Hg) and HR (beats/min). The circadian response peaked in the noon to late afternoon hours in the NT and in the reference stage of the intervention study. In NT, SBP: 111±2 with variability 14.6% and peak hr at 16:00; DBP: 74.0±1 with variability 16.0% and peak hr at 15:40; and HR: 64.3±2 with peak hr at 16:96 (-254°). In the intervention study with reference (I), Na restriction (II) and weight (WT) reduction (III) stages, SBP: 130±3.7; 130±1.7; 116±1.7 with variability (SBPV), 27.5, 31.4, and 25.4%; peak hrs at 16:26, 13:52; 12:24, respectively; DBP: 85±2.3; 83±2.2; 77±1.9 with variability (DBPV), 21.1, 26.4, 33.5%; peak hrs at 16:15, 18:29, 07:52, respectively; and HR: 64±2.1; 61±2.1; 66±2.3 with HRV, 30.2, 34.6, 28.3%; peak hrs at 15:07; 14:64, 14:55, respectively. Circadian UAldo: 0.63, 0.73, 0.58; UNa: 6.3, 2.9, 3.5; UK: 3.1, 3.1, 2.9; and WT: 89.9, 87.8, 83.2 in I, II, and III study stages, respectively. Thus, BP and HR, and their variabilities are the factors readjusted in the Na and WT reductions, and the peak shifted to earlier hours in the intervention. The variability, in the reference stage already higher than the NT levels, was not favorably changed with Na and WT interventions, although BP was normalized, especially following the WT reduction. Increased or shallow amplitudes may be predictive risk factors of vascular disease.

Disclosures: J.S. Lee: None. **M.S. Lee:** None. **J.Y. Lee:** None. **R. Gillum:** None. **F. Halberg:** None.

Funding:

Funding Component:

P296

High Salt (NaCl) Induces Metabolic Modifications and Affects Classic and Alternative Macrophage Activation

Authors: Sabrina Yasmin Geisberger, ECRC & Max Delbruck Ctr for Molecular Med & DZHK, Berlin, Germany; Christin Zasada, Max Delbruck Ctr for Molecular Med & BIMSB, Berlin, Germany; Jonathan Jantsch, Inst of Clinical Microbiology and Hygiene, Univ Hosp of Regensburg, Regensburg, Germany; Ralf Dechend, ECRC & Charite Univsmedizin & DZHK & BIH & Helios Hosp, Berlin, Germany; Stefan Kempa, Max Delbruck Ctr for Molecular Med & BIH & BIMSB, Berlin, Germany; Dominik N. Mueller, ECRC & Max Delbruck Ctr for Molecular Med & Charite Univsmedizin & DZHK & BIH, Berlin, Germany

High intake of dietary sodium is a risk factor for cardiovascular disease. Previously, we have shown that an increase in extracellular hypertonic sodium (+40 mM Na^+; HS) inhibits alternatively-activated M2 macrophage (M(IL4+IL13)) gene signature and function, while boosting classic M1 macrophage polarization (M(LPS)) and bacterial killing capacity. M1 macrophages predominantly rely on glycolysis, whereas M2 macrophages use oxidative phosphorylation, fueled by the oxidation of fatty acids, at a late phase of activation (24 h).
The aim of this study was to elucidate the role of HS on the metabolic signature of M1 and M2 macrophages. Therefore, we quantified ^{13}C incorporation into intermediates of central carbon metabolism via GC-MS based pulsed stable isotope resolved metabolomics (pSIRM), and performed Seahorse analyses at varying time points during M1/M2-activation. After 24 h, LPS-induced increase in basal ECAR (extracellular acidification as surrogate parameter for glycolysis) and lactate production were further boosted under HS compared to isotonic salt conditions (NS), whereas mitochondrial respiration (OCR) was not affected. On the other hand, IL4+IL13-induced increase in OCR was decreased under HS compared to NS, while ECAR was not affected at this stage. Regarding TCA-cycle, we have found an early (1 h and 3 h post-stimulation) increase in citrate levels in M1 and M2 macrophages independent of HS, which further increased after 24 h in M1 and M1+HS. Interestingly, both in early and late phase of activation, glucose-derived ^{13}C incorporation into citrate was lower in HS-treated compared to NS-treated M0, M1 and M2 macrophages, suggesting an alternative replenishing source. Consistently, other TCA-cycle intermediates increased after 24h (especially in M1 and even further in M1+HS), whereas glucose-derived ^{13}C incorporation was minimal. Our data suggests that under hypertonic sodium macrophages re-direct TCA-cycle intermediates into the production of pro-inflammatory mediators (as described for late-phase M1 macrophages), whereas TCA-cycle itself is replenished by other alternative metabolic substrates. These HS-induced metabolic modifications might play an important role for macrophage activation and function.

Disclosures: S. Geisberger: None. **C. Zasada:** None. **J. Jantsch:** None. **R. Dechend:** None. **S. Kempa:** None. **D.N. Mueller:** None.

Funding: No

Funding Component:

P297

Hypertensive Crisis in an Inner-City Population: A Retrospective Case Control Study

Authors: Frederick Waldron, Newark Beth Israel Medical Ctr, Newark, NJ; Irina Benenson, Rutgers Univ, Newark, NJ

Hypertensive crisis (HTNC) is a potentially life-threatening condition that can develop de novo or as a complication of hypertension. HTNC is characterized by a severe and an acute increase in blood pressure (BP) ≥ 180/120 mmHg, which may progress to end organ damage (EOD), and premature death. HTNC is divided into hypertensive emergency (HTNE), in which there is evidence of EOD, and hypertensive urgency (HTNU) with no EOD. We conducted a 3-year retrospective case controlled study, to determine the prevalence and the risk factors for HTNE and HTNU in an inner city population. Cases (1784) were adult patients visiting the ER with a BP ≥ 200/120 mmHg. Controls had a diagnosis of HTN defined by

a BP >140/90, but < 200/120 mmHg. Controls were matched 1:1 for age, gender and race using the SAS program. The total population of subjects with a diagnosis of hypertension was 15631. African Americans accounted for 89% (1585/1784) of the cases, other races for 9% (159/1784) and Caucasians for 2% (40/1784). The prevalence of HTNC was 11.4 % (1784/15631) and HTNE 3.2 % (505/15631). Twenty eight percent (28%) of the cases (505/1784) had EOD. Cases had significantly increased odds of developing the following EOD: acute kidney injury (OR 1.54, p=0.022), acute or worsening congestive heart failure (OR 4.91, p<0.0001), non ST elevation myocardial infarction (OR 2.39, p<0.0001), ischemic stroke (OR 3.27, p<0.0001), hemorrhagic stroke (OR 4.55, p<0.001). The predictors for EOD were: age > 65, male gender, anemia, chronic kidney disease and a history of stroke and cardiovascular comorbidities (hyperlipidemia, coronary artery disease, congestive heart failure). Insurance status and access to primary care were not associated with an increased odds of EOD. The study highlights the high morbidity of poorly controlled HTN, and the disparity in the prevalence of HTNC in the African American community, which is 5 times the national average.

Disclosures: F. Waldron: None. **I. Benenson:** None.

Funding: No

Funding Component:

P298

Combination of Systolic Blood Pressure and Non-HDL Cholesterol Categories Increased the Risk of Incident Latent Heart Failure: The Suita Study

Authors: Yoshihiro Kokubo, Makoto Watanabe, Aya Higashiyama, Yoshihiro Miyamoto, Natl Cerebral and Cardiovasc Ctr, Suita, Osaka, Japan

Objective: There are few population based prospective studies on heart failure (HF) in non-Western populations. We hypothesized that the combination of systolic blood pressure (SBP) and non-HDL cholesterol (non-HDLC) categories affect the risk of incident latent HF (LHF) in a general Japanese population. **Methods**: We prospectively followed-up 2,774 Suita Study subjects (average age 66.7±10.4 years, initially free of LHF) for incident LHF. BP were taken as the average of the first and second measurements. Each subject was classified into an SBP category: normal SBP, <120 mmHg; systolic prehypertension (preHT), 120-139 mmHg; or systolic hypertension (HT), ≥140 mmHg or antihypertensive drug use. The subjects' non-HDLC levels were calculated by subtracting the HDLC from the total cholesterol. B-type natriuretic peptide (BNP) was measured by the CLEIA method. LHF was defined as BNP ≥100 pg/mL or HF medication from medical records. Each subject's health status and BNP were checked in biannual medical examinations, and all subjects completed annual questionnaires. The endpoint of the follow-up period for incident LHF was whichever occurred first: the date of the first diagnosis of LHF, the date of the last examination, or December 31, 2017. We analyzed Cox proportional hazard ratios (HRs) and 95% confidence intervals (CIs) for incident LHF after adjusting for cardiovascular risk factors. **Results**: In 18,134 person-years of follow-up, 294 incident LHF events occurred. Compared to the normal SBP subjects, the adjusted HR (95% CIs) of incident LHF in the systolic HT subjects was 1.64 (1.16-2.30). The adjusted HR (95% CIs) of incident LHF per 20 mmHg of SBP was 1.18 (1.04-1.34). Compared to the normal non-HDLC (130-179 mg/dL) subjects, the adjusted HR (95% CIs) of incident LHF in the low non-HDLC (<130 mg/dL) subjects was 1.47 (1.15-1.88). Compared to the normal non-HDLC subjects with normal SBP, the adjusted HRs (95% CIs) of incident LHF in the normal non-HDLC subjects with systolic HT and low non-HDLC subjects with systolic preHT and HT were 1.53 (1.01-2.31), 1.76 (1.02-3.04), and 2.44 (1.58-3.76), respectively. **Conclusion**: This is the first demonstration that systolic preHT and low non-HDLC were positively associated with incident LHF in a general Japanese population.

Disclosures: Y. Kokubo: None. **M. Watanabe:** None. **A. Higashiyama:** None. **Y. Miyamoto:** None.

Funding: No

Funding Component:

P299

Evaluation of the Knowledge of Risk Factors Related to Diabetes Mellitus in a Cross High-Risk Population Sample

Authors: Maria Clara Duarte Dal Pai, Odara da Costa, Laura Martins Peçanha, Beatriz Glielmo Saraiva, UNESA, Rio de Janeiro, Brazil; Bernardo Pires de Freitas, FTESM, Rio de Janeiro, Brazil; Andressa Regina da Fonseca Wolff, UNESA, Rio de Janeiro, Brazil; Caroline Campos Garcia, FTESM, Rio de Janeiro, Brazil; Renan Alves Garcia, Nicole Ceccon de Castro, Fernando Ribas Brustolin, UNESA, Rio de Janeiro, Brazil; Bruno Vasconcelos Coimbra, FTESM, Rio de Janeiro, Brazil; **Lilian Soares da Costa**, FTESM and UNESA, Rio de Janeiro, Brazil

Introduction: Risk factors (RF) for the development of diabetes mellitus (DM) are highly prevalent in high-risk cardiovascular populations. Objective: To evaluate the knowledge of RF in the development of DM in a cross-sectional sample of patients attended at an outpatient clinic of a Cardiology Hospital in Rio de Janeiro, Brazil. Methods: There were 147 participants (53.7% women), mean age of 63 ± 11.9 years and education level divided into illiteracy (2%), elementary school (44.2%), average (38.1%) and higher school (15.7%). Among the participants, 85% reported hypertension (HY), 25.9% DM and 60.5% previous cardiovascular events, myocardial infarction and/or stroke. Other RF found were: dyslipidemia (46.9%); smoking (10.2%); physical inactivity (66.7%); family history of cardiovascular diseases (52.4%); overweight (25.2%) and obesity (37.4%). A structured questionnaire was applied, evaluating the following question: "In your opinion, what are the FIRST, SECOND and THIRD factor of importance in the development of diabetes? ". Results: The first, second and third factors of importance were the following distribution, respectively: excessive sugar intake (68.5%, 23.3% and 8.2%), excessive carbohydrate intake (23.3%, 43.4% (2.8%, 10.9% and 29.3%), stress (2.7%, 6.2% and 17.2%), obesity (0.7%, 7% , 8% and 17.2%), smoke (0.7%, 3.1% and 5.2%), dyslipidemia (uncalculated, 4.7% and 1.7%) and HY (uncalculated, 8% and 1.7%). Thus, the eight RF most cited were inadequate food in sugar (100%) and carbohydrates (85.7%), sedentary lifestyle (44.3%), stress (26.1%), obesity (25.7%), smoking (9%), dyslipidemia (6.4%) and HY (2.5%). It should be noted that the excessive intake of sugar and carbohydrate, sedentary lifestyle, stress and obesity were the most frequently cited RF in a population with a high cardiovascular risk, with a high percentage of sedentary lifestyle and overweight. There was no difference in the percentage of knowledge distribution of RF between DM and non-DM, and between HY and non-HY (p = 0.326). Conclusion: Although participants have demonstrated knowledge of RF, it is evident the need for better strategies to reduce the discrepancy between the knowledge and the high percentage of RF aggregation in this population.

Disclosures: M.D. Dal Pai: None. **O. da Costa:** None. **L.M. Peçanha:** None. **B.G. Saraiva:** None. **B.P. de Freitas:** None. **A.D. Wolff:** None. **C.C. Garcia:** None. **R.A. Garcia:** None. **N.C.C. de Castro:** None. **F.R. Brustolin:** None. **B.V. Coimbra:** None. **L.S. da Costa:** None.

Funding: No

Funding Component:

P300

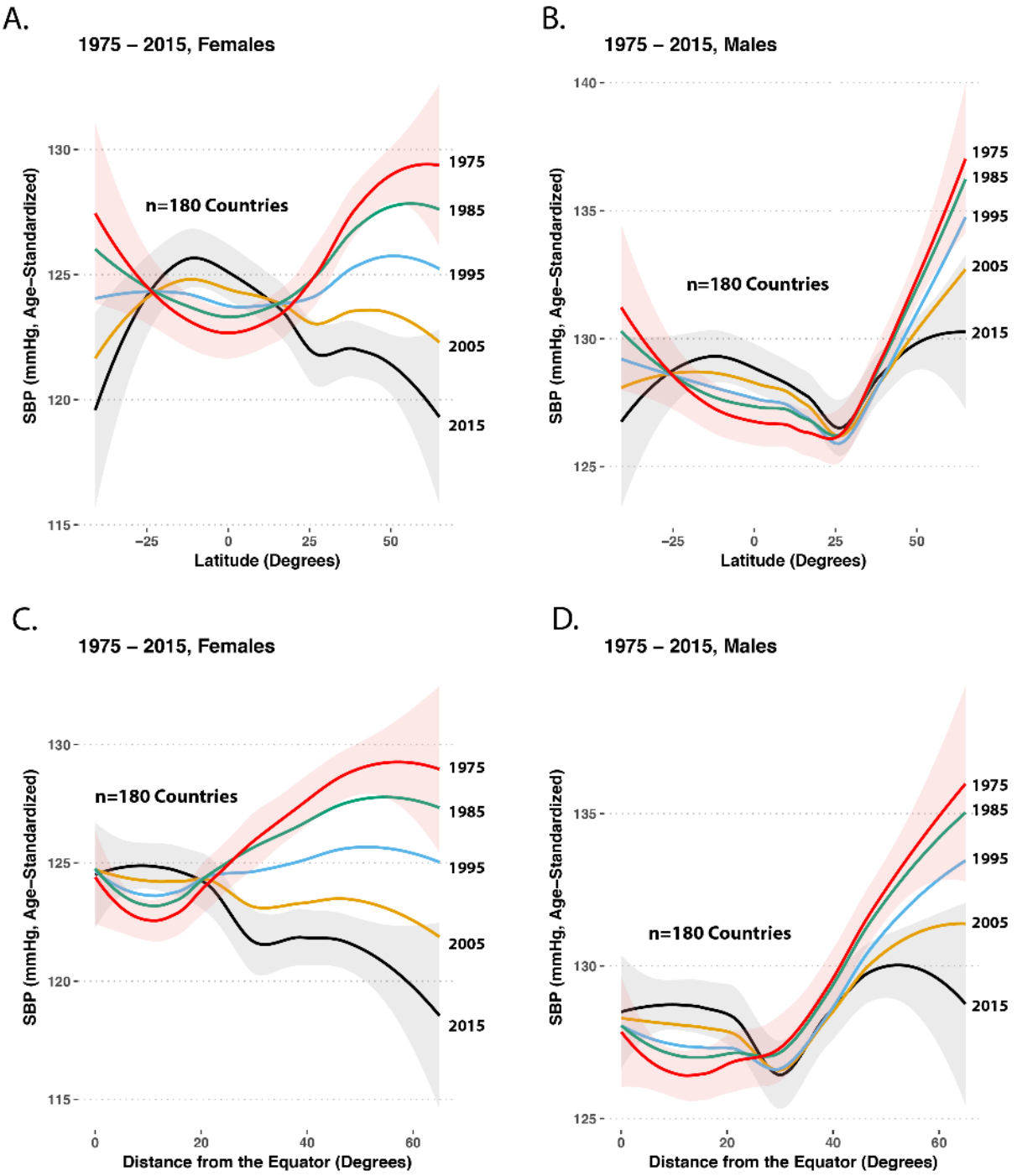
Countries' Geographic Latitude And Their Populations' Cholesterol And Blood Pressure

Authors: Yuhao Liu, Univ of Michigan, Ann Arbor, MI; Robert D. Brook, Univ of Michigan Medical Sch, Ann Arbor, MI; Xuefeng Liu, Univ of Michigan Sch of Nursing, Ann Arbor, MI; **James Brian Byrd**, Univ of Michigan Medical Sch, Ann Arbor, MI

Background: Sunlight has been hypothesized to play a role in variation in cardiovascular disease according to geographic latitude. **Objectives:** To evaluate the plausibility of sunlight as a factor in populations' average cholesterol and BP.

Methods: We analyzed 180 or more countries' age-standardized average cholesterol, age-standardized mean systolic BP, and age-standardized prevalence of raised BP, by latitude, over decades. We also performed analysis by ultraviolet B light (UVB) exposure. **Results:** Mean cholesterol increases with distance from the Equator. This relationship has changed very little since 1980. Similarly, in 1975, mean systolic BP and prevalence of raised BP were higher farther from the Equator. However, the relationship between latitude and BP has changed dramatically (**Figure**); by 2015, the opposite pattern was observed in women. Countries' UVB exposure has a stable relationship with cholesterol over decades, but a changing relationship with BP. **Conclusions:** Since sunlight exposure in a country is relatively fixed and its relationship with BP has changed dramatically in recent decades, countries' average sunlight exposure is an unlikely explanation for contemporary country-level variation in BP. However, our findings are consistent with a putative effect of sunlight on countries' average cholesterol, as well as a no longer detectable effect on BP decades ago. A parsimonious potential explanation for the relationship is that 7-dehydrocholesterol can be converted to cholesterol, or in the presence of ultraviolet light, can instead be converted to vitamin D. This work is archived in unrefereed preprint form: https://doi.org/10.1101/308726.

Figure.



Disclosures: **Y. Liu:** None. **R.D. Brook:** None. **X. Liu:** None. **J.B. Byrd:** None.

Funding:

Funding Component:

P301

Heart Rate Variability is Associated with Future Global Cognitive Performance: the Multi-Ethnic Study of Atherosclerosis

Authors: Chris Schaich, Diego Malaver, Hossam A. Shaltout, Wake Forest Univ Sch Med, Winston-Salem, NC; Adina Zeki Al Hazzouri, Miller Sch of Med, Univ of Miami, Miami, FL; David M. Herrington, Timothy M. Hughes, Wake Forest Univ Sch Med, Winston-Salem, NC

Background: Low heart rate variability (HRV) is associated with major vascular risk factors for cognitive decline, including hypertension and cardiovascular disease (CVD). Therefore, we hypothesized that higher HRV during mid- to late-life is associated with better cognitive performance.
Methods: In a subset of participants from the Multi-Ethnic Study of Atherosclerosis (N = 2,961; aged 45-84 years; 55% female; 40% white, 22% African-American, 25% Hispanic, and 13% Chinese-American), we used multivariate linear regression to study the relationship of short-term HRV to global cognitive performance as measured by the Cognitive Abilities Screening Instrument (CASI; score range 0-100). Two measures of HRV, the standard deviation of normal-to-normal intervals (SDNN) and root mean square of successive differences (RMSSD), were computed at Exam 1 (2000-2002) and Exam 5 (2010-2012). CASI was administered at Exam 5.
Results: In age-, race-, sex- and education-adjusted models, Exam 1 SDNN was significantly associated with performance on the CASI (β = 0.74 ± 0.22; P < 0.001). This association remained significant after adjustment for cardiovascular risk factors, including prevalent CVD, medication use, and *APOE* ε4 allele carriage (β = 0.53 ± 0.23; P = 0.019). Furthermore, participants with highest quartile Exam 1 SDNN scored better than the adjusted mean CASI score (0.61 ± 0.22 points higher; P = 0.022), and 0.81 ± 0.29 points higher than other quartiles (P = 0.006); participants in other Exam 1 SDNN quartiles scored similarly to each other and to the adjusted mean. In contrast, there were no associations between CASI score and Exam 5 SDNN, Exam 1 RMSSD, or Exam 5 RMSSD after adjustment for cardiovascular risk factors, and no interactions between HRV and race or *APOE* were present.
Conclusions: Highest quartile 10-year antecedent SDNN is associated with better global cognitive performance in a multi-ethnic population of middle-aged and elderly adults, independent of sociodemographic factors, traditional cardiovascular risk factors, *APOE* status, and prevalent CVD. These results suggest that mid- to late-life HRV may be an early predictor of future cognitive ability.

Disclosures: C. Schaich: None. **D. Malaver:** None. **H.A. Shaltout:** None. **A. Zeki Al Hazzouri:** None. **D.M. Herrington:** None. **T.M. Hughes:** None.

Funding:

Funding Component:

P302

Prevalence of refractory hypertension in the United States from 1999-2014

Authors: Michael Buhnerkempe, Albert Botchway, Dept of Internal Med, Ctr for Clinical Res, Southern Illinois Univ Sch of Med, Springfield, IL; Carlos E. Nolasco Morales, Vivek Prakash, John M. Flack, Dept of Internal Med, Div of General Internal Med, Hypertension Section, Southern Illinois Univ Sch of Med, Springfield, IL

Background: Refractory hypertension has been defined as uncontrolled blood pressure (BP at or above 140/90 mmHg) when taking five or more classes of antihypertensive medication. Unbiased estimates of the prevalence of refractory hypertension in the United States are lacking. ***Methods:*** Refractory hypertension was assessed in the National Health and Nutrition Examination Survey. Eight cycles of NHANES surveys (1999-2014) representing 41,552 patients were included. Patients younger than 18 and who were pregnant were excluded. Prevalence of refractory hypertension was

estimated in the hypertensive population (systolic BP ≥140 or diastolic BP ≥90 or taking an antihypertensive medication) after adjusting for the complex survey design and standardizing for age. Logistic regression was used to test for temporal trends and to compare prevalence across age classes (18-39, 40-59, 60+), genders, races, and chronic kidney disease status. ***Results:*** Refractory hypertension was found in 0.30% of all hypertensive individuals. Although prevalence of refractory hypertension peaked at 0.54% of hypertensive individuals in the 2005-2006 cycle, there was no significant trend in prevalence through time (p = 0.29). No individuals under 40 years old were observed with refractory hypertension, and refractory hypertension was more common in individuals 60 and older than in individuals 40-60 years old (odds ratio = 4.55, p = 0.004). There was no difference in the prevalence of refractory hypertension between males and females (p = 0.24), but white and black individuals were more likely to have refractory hypertension than individuals of Hispanic or other descent (OR = 4.63, p = 0.002). Finally, individuals with chronic kidney disease were significantly more likely to have refractory hypertension (OR = 9.88, p < 0.001). ***Conclusions:*** We provided the first nationally representative estimate of the prevalence of refractory hypertension in the U.S. Recently lowered BP targets, an aging population, and increased incidence of co-morbidities like chronic kidney disease all create the potential for increases in this hypertensive phenotype in the future.

Disclosures: M. Buhnerkempe: None. **A. Botchway:** None. **C.E. Nolasco Morales:** None. **V. Prakash:** None. **J.M. Flack:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Vascular Dynamics, Bayer, GlaxoSmithKline. G. Consultant/Advisory Board; Modest; Served as a consultant for NuSirt, Served as a consultant for Allergan, Served as a consultant for BackBeat Hypertension, serves on the data safety and monitoring board for Rox Medical.

Funding:

Funding Component:

P303

Inhibition of Group IV Cytosolic Phospholipase $A_2\alpha$ by Cytochrome P450 1B1-Estradiol Derived Metabolite 2-Methoxyestradiol Protects Against Angiotensin II-Induced Hypertension in Female Mice

Authors: Chi Young Song, Purnima Singh, Mustafa Motiwala, Ji Soo Shin, Univ of Tennessee HSC, Memphis, TN; Joseph V. Bonventre, Harvard Medical Sch, Boston, MA; Kafait U. Malik, Univ of Tennessee HSC, Memphis, TN

Previously we showed that the protection against angiotensin (Ang) II-induced hypertension and associated pathophysiological changes in female mice are mediated by cytochrome P450 (CYP) 1B1-estradiol (E2) generated metabolite 2-methoxyestradiol (2-ME). Also, we demonstrated that Ang II-induced hypertension is mediated by group IV cytosolic phospholipase $A_2\alpha$ ($cPLA_2\alpha$) activation resulting in arachidonic acid (AA) release, and generation predominantly of eicosanoids with pro-hypertensive effects in male mice. This study was conducted to determine the contribution of $cPLA_2\alpha$/AA system, and its relationship to CYP1B1 in Ang II-induced hypertension in the female mice. Ang II infusion (700 ng/kg/min, s.c. micro-osmotic pump) for 14 days increased the systolic blood pressure (SBP), measured by tail-cuff in wild-type mice ($cPLA_2\alpha^{+/+}$) but not in $cPLA_2\alpha$ gene-disrupted mice ($cPLA_2\alpha^{-/-}$) (151±8 vs. 126±9 mmHg, P<0.05, n=4-5). Ang II markedly increased SBP in ovariectomized (OVX) $cPLA_2\alpha^{+/+}$ mice but not in OVX $cPLA_2\alpha^{-/-}$ mice (175±3 vs. 121±2 mmHg, P<0.05, n=4-5). AA metabolism inhibitor 5,8,11,14-eicosatetraynoic acid (ETYA, 50 mg/kg, i.p. every 3rd day) attenuated (P<0.05) the Ang II-induced increase in SBP in intact (124±5 mmHg, n=5) and OVX $cPLA_2\alpha^{+/+}$ mice (124±5 mmHg, n=4). E2 attenuated Ang II-induced increase in SBP in OVX *Cyp1b1*$^{+/+}$ mice, but not in OVX *Cyp1b1*$^{-/-}$ mice (125±5 vs. 158±4 mmHg, P<0.05, n=3-4). Ang II-induced increase in SBP was also attenuated by ETYA in the *Cyp1b1*$^{-/-}$ mice (129±7 vs. 185±7 mmHg, P<0.05, n=4). Ang II-induced increase in $cPLA_2\alpha^{+/+}$ activity examined in the kidneys was inhibited in *Cyp1b1*$^{-/-}$ mice treated with 2-ME. Antagonists of prostaglandin (PG) E2 receptors EP1 (SC19220) and EP3 (L-798106) (28 µg/g, s.c. every 2nd day) minimized Ang II-induced increase in SBP in OVX $cPLA_2\alpha^{+/+}$ mice (126±2 and 127±5 vs. 175±3 mmHg, respectively, n=5). These data suggest that CYP1B1-E2 generated metabolite 2-ME protects against Ang II-induced hypertension by inhibiting $cPLA_2\alpha$ activity and production of AA-derived PGE2 that exerts pro-

hypertensive effects by stimulating EP1 and EP3 receptors. Thus, 2-ME and the drugs that selectively block EP1 and EP3 receptors could be useful for the treatment of hypertension and its pathogenesis in females.

Disclosures: C. Song: None. **P. Singh:** None. **M. Motiwala:** None. **J. Shin:** None. **J.V. Bonventre:** None. **K.U. Malik:** None.

Funding: No

Funding Component:

P304

Sex Differences in Mechanisms of Adiposity-Associated Hypertension in Dahl S Rats

Authors: Gregory D Fink, Hannah Garver, Michigan State Univ, East Lansing, MI

In humans, excessive accumulation of visceral adipose tissue is often associated with hypertension, but there is evidence that the *mechanisms* responsible may differ between men and women. Dahl S rats fed a high fat diet (HFD) from weaning gain visceral fat and develop a progressively higher arterial pressure compared to Dahl S rats fed a control diet (CD). We previously showed that the *magnitude* of this effect is similar in males and females. Here we used pharmacological interventions to compare in males and females the contributions of brain prostanoids, the renin-angiotensin system and the sympathetic nervous system to the development of hypertension in this rat model. Dahl S rats were placed on CD or HFD at weaning. All rats received telemetry implants at 15-16 weeks of age to measure arterial pressure, heart rate, and activity. Studies were conducted from 19-23 weeks of age, a time when hypertension is still developing. Drug interventions were separated by at least a week. Mean arterial pressure (MAP) was significantly higher in male HFD (156.0+/- 6.5 mmHg) versus male CD (133.3+/-1.6 mmHg) rats, and in female HFD (140+/-2.6 mmHg) versus female CD (131.4+/-1.2 mmHg) rats during the experimental period. Treatment for one week with the ACE inhibitor enalapril (250 mg/L in the drinking water) lowered MAP significantly more in male HFD (-38.3+/-5.9 mmHg) than in male CD (-23.9+/-1.6 mmHg) rats, but female HFD (-33.2+/-2.2 mmHg) and CD (-28.4+/-1.5 mmHg) rats responded similarly. Treatment for one week with the centrally acting sympatholytic clonidine (2 mg/L in the drinking water) reduced MAP more in females than males, but there were no differences between CD and HFD groups. One week treatment with the prostanglandin D synthase inhibitor AT56 (5.8 nmol/hr sc) caused only a slight fall in MAP (~3 mmHg) that was similar in all groups. Finally, a single injection of the ganglion blocker hexamethonium (30 mg/kg, ip) caused a short-term depressor response that was not significantly different in magnitude among the groups. We conclude that the renin-angiotensin system may have a greater role in adiposity-associated hypertension in Dahl S males versus females, but that sympathetic overactivity or prostaglandin D are not factors in either sex in the age range studied here.

Disclosures: G.D. Fink: A. Employment; Modest; Michigan State University. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH grants as PI and Co-I. E. Honoraria; Modest; Occasional honoraria from Medtronic for meeting attendance. **H. Garver:** None.

Funding: No

Funding Component:

P305

High Fat Diet With Normal Salt Feeding Does Not Change Renal m-TORC Signaling Activities In Male And Female Dahl Salt Sensitive Rats

Authors: Fernandes Roxanne, James J Galligan, Gregory D Fink, **Hui Xu**, MICHIGAN STATE UNIVERSITY, East Lansing, MI

We have previously reported that high fat diet (HFD) with normal salt feeding strongly promotes hypertension in both male and female Dahl salt sensitive (SS) rats. Only males, however, exhibit hypertension-associated renal inflammation/injury. The underlying mechanisms responsible for sex differences in HFD associated renal inflammation/injury are undetermined. It has been reported that increased activity of Mammalian Target of Rapamycin Complex 1/2 (mTORC) contribute to high salt diet associated hypertension and renal inflammation/injury in Dahl SS male rats. In this study, we determined whether HFD increases renal mTORC signaling in Dahl SS rats and if there are sex differences in signaling. Renal cortex and medulla were collected from rats fed a control diet (CD, 10% kcal fat) or a HFD (60% kcal fat) with 0.3% NaCl for 10, 17 or 24 weeks (WKs, starting at weaning, the time points correspond to pre-hypertension, developing hypertension and established hypertension phases of the model. Ratio (%) of expression of phospho-S6 ribosomal protein$^{Ser235/236}$ (pS6)/S6 (a marker for mTORC1), and phospho-AKT^{Ser473} (pAKT)/AKT (a marker for mTORC2) were used to evaluate mTORC activity. The activity of mTORC1 in CD male cortex and female medulla were slightly increased at 17 and 24WKs of feeding compared to 10 WKs (22±4 and 16±7 vs 2±2, P<0.05), but HFD did not change the activity in cortex and medulla significantly in males (cortex,10±5, 14±5, 20±5; medulla 9±2, 18±6, 25±12) or females (cortex, 4±1, 12±3, 13±2; medulla 13±8, 22±8, 24±16) (P>0.5). The overall activity of mTORC2 in CD males and females were very low and mostly unchanged during 24WKs feeding, except for a slight increase in CD male cortex at 17WKs of feeding compared to 10WKs (4±1 vs 0.1±0.02, P<0.05). HFD caused only a slight increase of mTORC2 in medulla from males (4±1 vs 1±0.5) and a decrease in medulla from females (8±2 vs 0.9±0.3) (P<0.05) at 24WKs compared with 10WKs of feeding. Overall HFD did not cause marked changes in renal mTORC1/2 signaling activities. In addition, there were no significant differences in mTORC signaling activities between males and females. Therefore mTORC signaling pathways are not likely to contribute to the sex differences in HFD associated renal inflammation/injury in Dahl SS rats.

Disclosures: F. Roxanne: None. **J.J. Galligan:** None. **G.D. Fink:** None. **H. Xu:** None.

Funding:

Funding Component:

P306

Preterm Birth is Associated with Increased Blood Pressure and Increased Urinary Angiotensinogen in Young Adults

Authors: Andrew Michael South, Patricia A. Nixon, Mark C. Chappell, Debra I. Diz, Elizabeth T. Jensen, Hossam A. Shaltout, Lisa K. Washburn, WAKE FOREST SCHOOL OF MEDICINE, Winston Salem, NC

Introduction: Cardiovascular disease (CVD) is the leading cause of mortality and hypertension (HTN) is a major risk factor for CVD. Preterm birth is an emerging risk factor for both CVD and HTN, but the underlying mechanisms are poorly described. The renin-angiotensin system (RAS) plays a key role in HTN and CVD, and adolescents born preterm have higher blood pressure (BP) with a shift in the balance of the classical and alternative pathways of the RAS exhibited as increased angiotensin II and reduced angiotensin-(1-7) as compared to term-born adolescents. Although numerous factors influence the expression of angiotensins, the precursor protein angiotensinogen in the kidney is implicated in the development of HTN and CVD and may contribute to higher angiotensin II. As the status of renal angiotensinogen in individuals born preterm is unknown, we hypothesized that urinary angiotensinogen is increased in young adults born preterm as compared to their term-born peers. **Methods:** We compared urinary excretion of angiotensinogen corrected for urine creatinine and systolic and diastolic BP in 142 young adults (mean age 19.9 years) born preterm with very low birth weight (<1500 g) to 32 young adults born term using Wilcoxon Rank-Sum test and t-test. We used generalized linear models to compare the ln(x) urinary angiotensinogen between the preterm and term groups adjusted for the potentially confounding factors race, maternal hypertensive pregnancy, and maternal smoking during pregnancy. **Results:** Compared to term, subjects born preterm had higher median urinary angiotensinogen (0.02 μg/g, IQR 0.01 to 0.04 vs. 0.01 μg/g, IQR 0.004 to 0.01, $p < 0.001$) and higher mean systolic BP (111 mmHg, SD 11 vs. 106 mmHg, SD 10, *p*

= 0.03). On crude and adjusted analyses urinary angiotensinogen was associated positively with preterm birth (crude *β*: 0.82, 95% CI 0.47 to 1.16; adjusted *β*: 0.79, 95% CI 0.39 to 1.18). **Discussion:** In addition to higher BP, young adults born preterm demonstrated increased urinary angiotensinogen as compared to their term-born peers. Preterm birth may induce programming of the renal RAS leading to higher angiotensinogen and a higher angiotensin-to-angiotensin-(1-7) ratio, potentially contributing to the increased risk of HTN in individuals born preterm.

Disclosures: A.M. South: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH P01 HD047584 and HD084227; AHA 14GRNT20480131; MCRR/NIH M01-RR07122; NIH UL1 TR001420. **P.A. Nixon:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH P01 HD047584 and HD084227; AHA 14GRNT20480131; MCRR/NIH M01-RR07122; NIH UL1 TR001420. **M.C. Chappell:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH P01 HD047584 and HD084227; AHA 14GRNT20480131; MCRR/NIH M01-RR07122; NIH UL1 TR001420. **D.I. Diz:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH P01 HD047584 and HD084227; AHA 14GRNT20480131; MCRR/NIH M01-RR07122; NIH UL1 TR001420. **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); Significant; NIH P01 HD047584 and HD084227; AHA 14GRNT20480131; MCRR/NIH M01-RR07122; NIH UL1 TR001420. **L.K. Washburn:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH P01 HD047584 and HD084227; AHA 14GRNT20480131; MCRR/NIH M01-RR07122; NIH UL1 TR001420.

Funding:

Funding Component:

P307

Renal Natural Killer Cell Activation and Mitochondrial Oxidative Stress; New Mechanisms in AT1-AA Mediated Hypertensive Pregnancy

Authors: Mark W Cunningham, Venkata Vaka, Kristen McMaster, Tarek Ibrahim, Denise Cornelius, Lorena Amaral, Nathan Campbell, Univ of Mississippi Medical Ctr, Jackson, MS; Gerd Wallukat, Experimental and Clinical Res Ctr, Charité Campus Buch, Max-Delbrück Ctr for Molecular Med, Berlin, Germany; Shyanne McDuffy, Nathan Usry, Univ of Mississippi Medical Ctr, Jackson, MS; Ralph Dechend, Experimental and Clinical Res Ctr, Charité Campus Buch, Max-Delbrück Ctr for Molecular Med, Berlin, Germany; Babbette LaMarca, Univ of Mississippi Medical Ctr, Jackson, MS

Women with preeclampsia (PE) have increased blood pressure (MAP), natural killer (NK) cells, reactive oxygen species (ROS), and agonistic autoantibodies to the angiotensin II type 1 receptor (AT1-AA). AT1-AA's administered to pregnant rodents produces a well-accepted model of PE. However, the role of NK cells and mitochondrial reactive oxygen species (mtROS) in AT1-AA mediated hypertension during pregnancy is unknown. We hypothesize that AT1-AA induced model of PE will exhibit elevated MAP, NK cells, and mtROS; while inhibition of the AT1-AA binding to the AT1R would be preventative. Pregnant rats were divided into 3 groups: normal pregnant (NP) (n=5), NP + AT1-AA infused (NP + AT1-AA) (n= 10), and NP + AT1-AA + AT1-AA inhibitory peptide (NP + AT1-AA + 'n7AAc') (n=8). Day 13, rats were surgically administered mini-pumps with either AT1-AA or AT1-AA+'n7AAc'. Day 19, tissue and blood was collected. MAP was elevated in AT1-AA vs. NP (119±1 vs. 102±2 mmHg, $p<0.05$) and was prevented by 'n7AAc' (108±3). There was a 6 fold increase in renal activated NK cells in AT1-AA vs .NP (1.2±0.4 vs. 0.2±0.1 % Gated, $p=0.05$) and a return to NP levels in AT1-AA +'n7AAc' (0.1±0.1 % Gated). Renal mtROS (317±49 vs. 101±13 % Fold, $p<0.05$) was elevated with AT1-AA vs NP and was decreased in AT1-AA +'n7AAc' (128±16, $p<0.05$). AT1-AA's infused into pregnant rats increased MAP, NK cells, and mtROS which were prevented by AT1-AA inhibition, thus highlighting new mechanisms of AT1-AA and the importance of drug therapy targeted to AT1-AAs in hypertensive pregnancies. **Research Supported by NIH grants**

HL78147, HL51971, and HD067541 awarded to BL. DC is supported by NIH grant HL130456 and the American Heart Association 16SDG27520000.

Disclosures: M.W. Cunningham: None. **V. Vaka:** None. **K. McMaster:** None. **T. Ibrahim:** None. **D. Cornelius:** None. **L. Amaral:** None. **N. Campbell:** None. **G. Wallukat:** None. **S. McDuffy:** None. **N. Usry:** None. **R. Dechend:** None. **B. LaMarca:** None.

Funding: No

Funding Component:

P308

Differential VEGF-Dependent Induction of sFlt-1 From Vascular Endothelial and Placental Cells

Authors: Heather Chapman, Adrian C Eddy, Gene L Bidwell III, **Eric M George**, Univ Mississippi Medical Ctr, Jackson, MS

Preeclampsia is a disorder of pregnancy characterized by new-onset hypertension and maternal endothelial dysfunction. Although the origins of the disease are unclear, it is believed that placental hypoxia/ischemia leads to the release of soluble factors which cause the maternal syndrome. The best characterized factor elevated in PE is soluble fms-like Tyrosine Kinase-1 (sFlt-1), a soluble splice variant of the VEGFR1 receptor which acts as a decoy receptor; and thus a VEGFA antagonist. Overproduction of this protein is believed to be a major contributor to the maternal endothelial dysfunction, causing much speculation that VEGF supplementation could be an effective supplementation could be an effective therapy. Recently we described a VEGFA chimera therapy (ELP-VEGFA) which alleviated the hypertension associated with placental ischemia in a rodent model, but also led to a dramatic increase in maternal circulating sFlt-1. Here we tested the hypothesis that chimeric VEGF would significantly increase sFlt-1 production from placental tissue in vitro. Surprisingly, there was no effect of ELP-VEGF on sFlt-1 production in either BeWo placental trophoblasts or in rodent placental tissue explants at either the mRNA or protein level. Instead, we found that vascular endothelial cells (HUVECs) responded robustly to ELP-VEGFA at the protein (6593 ± 199 vs 14547 ± 364 pg/ml, $p<0.05$) and mRNA (100 ± 2 vs 401 ± 3.6%, $p<0.05$) levels. This effect was unique to ELP-VEGFA as ELP-based proteins based on the VEGF family members VEGFB or PlGF had no effect on sFlt-1 production from either placental or endothelial cells. Finally, we found that 24hr post-administration of ELP-VEGF, pregnant rats demonstrated a trend for increased sFlt-1 mRNA in aorta (83 ± 7 vs 225 ± 63 AU, $p<0.05$) but no increase in placental tissue. These data suggest that therapeutic peptides based on either VEGFB or PlGF could have significantly improved safety profiles over those based on the VEGFA sequence. Furthermore, it raises questions about the origin of much sFlt-1 protein in preeclamptic patients.

Disclosures: H. Chapman: None. **A.C. Eddy:** None. **G.L. Bidwell:** None. **E.M. George:** None.

Funding: Yes

Funding Component: Greater Southeast Affiliate (Alabama, Florida, Georgia, Louisiana, Mississippi, Puerto Rico & Tennessee)

P309

Aberrant Corin and PCSK6 in the Placenta of Hyperinsulinemic Dams

Authors: Zaid Abassi, Safa Kinaneh, Rappoport Faculty of Med, Technion, Haifa, Israel; Galina Skarzinski, Einat Cinnamon, Hadassah Univ Hosp Ctr Mt Scopus, Jerusalem, Israel; Yoav Smith, Hebrew Univ-Hadassah Sch of Med, Jerusalem, Israel; Ilana Ariel, **Michael Bursztyn**, Hadassah Univ Hosp Ctr Mt Scopus, Jerusalem, Israel

Introduction: Corin, a serine protease converts pro-atrial natriuretic peptide (pro-ANP) and pro-brain natriuretic peptide (pro-BNP) to the mature respective peptides. There is evidence that the pregnant uterus produces ANP, where it promotes spiral artery remodelling, essential for adequate blood supply to the developing conceptus. **Aim**: Here we examine corin and PCSK6, a key enzyme in the conversion of pro-corin to corin, in the placenta of hyperinsulinemic dams (HD) in comparison to normal pregnant dams (NPD). **Materials and Methods**: Female Wistar rats were rendered hyperinsulinemic by subcutaneous insulin pellet, mated and followed to day 21 of pregnancy. Both groups were then sacrificed and their placentas were dissected together with the implantation site designated mesometrial triangle (MT). Placentas and MT were sectioned from the maternal through the fetal surface to the base of the MT. For western blot (WB) the placentas and MT were separated, snap frozen in liquid nitrogen and stored at −80°C. RNA expression of corin was carried out by *in-situ* hybridization in 5 NPD and 6 HD with *corin* probe (RNAscope® Assay 2.5 Detection Reagent, Cat No. 507231) and image analysis was performed. **Results:** As previously described, HD (n=20) developed hypertension as compared with in NPD (n=16). Moreover, HD exhibited lower placental and foetal weights. Corin and PCSK6 mRNA and immunoreactive peptide were detected in the mesometrial tissues. Corin abundance as determined by western blot was significantly decreased in the placenta/ mesometrial triangle by ~40% ($P<0.02$), as was PCSK6 immunoreactivity. Corin and PCSK6 mRNAs displayed similar pattern of alterations as protein abundance, where *in-situ* hybridization for corin mRNA revealed a 50% ($P<0.001$) expression reduction. Corin mRNA was specifically expressed by cytotrophoblasts in the labyrinth. Concomitant with the downregulation of corin/PCSK6, ANP levels decreased in the MT of HD. **Summary and Conclusion**: Corin, PCSK6 and ANP are all expressed in the placenta of NPD but declined in dams with chronic hyperinsulinemia, suggesting a role of corin/PCSK6 pathway in the prevention of pregnancy-induced hypertension and intrauterine growth restriction, in this setting.

Disclosures: Z. Abassi: None. **S. Kinaneh:** None. **G. Skarzinski:** None. **E. Cinnamon:** None. **Y. Smith:** None. **I. Ariel:** None. **M. Bursztyn:** None.

Funding: No

Funding Component:

P310

Hypermethylation of Dlk1-meg3 Loci in Human Umbilical Veins: Insights Into Offspring Vasculardysfunction Born After Pre-Eclampsia

Authors: Ying Jiang, women's hospital, school of medicine, zhejiang university, Hangzhou, China; Yi-Chen Yu, Dept of General Surgery, Sir Run Run Shaw Hosptial, Sch of Med, Zhejiang Univ, Hangzhou, China; **Qiong Luo**, Women's hospital, Sch of Med, Zhejiang Univ, Hangzhou, China

Increasing epidemiological studies have confirmed the association between maternal pre-eclampsia and elevated blood pressure in their offspring. In our study, we explored the potential epigenetic regulation of Dlk1-Meg3 region in human umbilical vein endothelial cells, and its connection with endothelium-derived factors. We recruited 58 singletons born spontaneously and 67 singletons born with pre-eclampsia during 2016 to detect blood pressure and growth development index, and found that diastolic blood pressure was significantly lower in pre-eclampsia offspring who born over 34 weeks compared to normal offspring (53.59±1.38mmHg VS 59.9±1.40mmHg, $P<0.01$), which leads to higher pulse pressure difference. RT-qPCR showed that imprinted gene Dlk1 level significantly increased and Meg3 level decreased in HUVECs than that in control group, accompanying with lower expression of endothelial nitric oxide synthase(eNOS) and vascular endothelial growth factor (VEGF) , higher expression of endothelin-1 (ET1), which are close related with vascular endothelial function. Meanwhile, ELISA assay of ET1, Nitrite, VEGF were consistent with Real-time results. Besides, we used pyrosequencing to find that abnormal expression of Dlk1-Meg3 expression were caused by hyper-methylation status of IG-DMR. And methylation status of IG-DMR highly correlated with ET1 concentration and Nitrate concentration, these might be one of the mechanisms for impaired endothelial function (Coeffient=0.5806,

P=0.0115; Coeffient=-0.4883, P=0.0398). Our results demonstrated that altered expression of imprinted gene Dlk1 and Meg3 were caused by hypermethylation of IG-DMR in pre-eclampsia HUVECs, accompanied by lower secretion of nitrite, VEGF, and higher secretion of ET1. It might be one potential mechanism for higher risk of cardiovascular disease in pre-eclampsia offspring later in life.

Disclosures: Y. Jiang: None. **Y. Yu:** None. **Q. Luo:** None.

Funding: No

Funding Component:

P311

Static Urine Osmolality With Elevated First Trimester Urine Copeptin in Human Preeclampsia

Authors: Donna A Santillan, Sabrina M Scroggins, Alyssa T Ray, Shao Y Zhang, Curt D Sigmund, Gary L Pierce, Justin L Grobe, Mark K Santillan, Univ Iowa Carver Coll Med, Iowa City, IA

We have previously shown that maternal plasma copeptin (CPP), as a marker of vasopressin, is highly predictive of preeclampsia (PE) in the first trimester and remains elevated throughout pregnancy. Furthermore, in maternal urine samples we demonstrated that CPP was also significantly elevated in the first trimester in women who later developed PE. Because a urine dipstick test could be easily used in the clinic, we sought to validate this finding in a new and expanded cohort of samples and to determine whether these changes persist throughout pregnancy. In addition, to begin to address the mechanism for this difference, we also assessed urine osmolality to further probe renal function. In a case-control study (IRB# 2015038355), banked maternal urine samples and clinical data from each trimester from women who developed PE (N=117) and controls (N=593) were obtained from the University of Iowa Maternal Fetal Tissue Bank (MFTB) (IRB# 200910784). CPP concentrations were measured by ELISA. Osmolality was determined by freezing point depression. Differences between groups were detected by Chi square, Student's T Test, or ANOVA as appropriate. Using a validation cohort and a different ELISA (USCN vs Phoenix Pharmaceuticals), we validated our earlier findings. We again find a significant increase in the concentration of urine CPP in the first trimester in women who developed preeclampsia compared to women who did not (0.304 ± 0.03 vs 0.223 ± 0.01 pg/ml, *P*=0.03). No significant differences in copeptin were observed in urine CPP in the 2nd and 3rd trimesters from PE and control women (2^{nd} tri: 0.205 ± 0.04 vs 0.213 ± 0.02; 3^{rd} tri: 0.290 ± 0.03 vs 0.336 ± 0.02 pg/ml *P*>0.05 in 2^{nd} and 3^{rd} trimesters). In this cohort, we did not detect differences in urine osmolality between preeclamptics and controls in any trimester (1^{st} tri: 606.09 ± 50.16 vs 604.28 ± 16.72; 2^{nd} tri 582.10 ± 36.30 vs 596.96 ± 16.98; 3^{rd} tri: 479.63 ± 58.81 vs 583.75 ± 19.61 mOsm/kg H_2O). We conclude that (i) PE is associated with increased maternal urinary CPP in the 1st trimester, (ii) this increase does not correlate with expected increases in urine osmolality. Future work will focus on understanding the mechanisms involved in the elevation in urinary CPP such as early immunologic changes or renal concentrating responses.

Disclosures: D.A. Santillan: None. **S.M. Scroggins:** None. **A.T. Ray:** None. **S.Y. Zhang:** None. **C.D. Sigmund:** None. **G.L. Pierce:** None. **J.L. Grobe:** None. **M.K. Santillan:** None.

Funding: Yes

Funding Component: National Center

P312

The Impact of Arterial Hypertension in Maternal and Perinatal Outcomes in Pregnancies With Takayasu Arteritis

Authors: Luiz Bortolotto, Heart Inst (InCor), São Paulo, Brazil; Maria R Bortolotto, Rodrigo R Codarin, Tiago J Gomes, Carolina B Testa, Rossana P Francisco, Marcelo Zugaib, Departament of Obstetrics, Hosp das Clinicas da FMUSP, São Paulo, Brazil

OBJECTIVES: To describe the clinical features, obstetric and perinatal outcomes of pregnancies in patients diagnosed Takayasu arteritis associated to arterial hypertension. METHODS: A retrospective, observational and descriptive study evaluating pregnant patients at a Brazilian tertiary hospital between the years 2002 and 2016 that had been diagnosed with Takayasu arteritis (TA) based on the modified Ishikawa criteria. Arterial hypertension (AH) previous to pregnancies was considered as BP≥ 140/90 mmHg or under treatment. The variables considered for analysis were clinical characteristics, diagnostic criteria, obstetrics and perinatal outcomes of these pregnancies. RESULTS: Twenty-nine pregnancies in 24 patients were followed in the period. Hypertension was detected in 20 patients (83.3%). The most prevalent angiographic criteria were injury in the abdominal aorta, found in 15 women (62.5%). Angiographic classification type 5 was the most common feature. Aside from two drop-outs that were not followed up, the pregnancies resulted in 25 live births. Five (20%) of the newborns were classified as small for gestational age and eight (32%) were premature, most of them in patients with AH. Eighteen deliveries (69.2%) were caesarean sections and the main anesthetic method was the combined spinal-epidural. Preeclampsia was the main maternal complication, present in five cases, all of them with previous diagnosis of AH. There were no acute cardiovascular complications during pregnancy related to underlying disease. The only fetal death in this study was an abortion after judicial authorization by lethal fetal malformation, CONCLUSION: In general, patients with TA had a good perinatal outcomes despite of severity of disease. Hypertension is highly prevalent and is related to major reported obstetric and perinatal complications, such as preeclampsia, prematurity and newborns that are small for gestational age.

Disclosures: L. Bortolotto: None. **M.R.F.L. Bortolotto:** None. **R.R. Codarin:** None. **T.J.O. Gomes:** None. **C.B. Testa:** None. **R.P.V. Francisco:** None. **M. Zugaib:** None.

Funding: No

Funding Component:

P313

Isolated Central Hypertension and Features of the Pregnancy Courseisolated Central Hypertension and Features of the Pregnancy Course

Authors: Maria Evsevyeva, Sergeeva Oksana, Evgeniy Shchetinin, Victoria Frantseva, Tatiana Bondar, Stavropol State Medical Univ, Stavropol, Russian Federation

Background. Physiological features of hemodynamic changes in pregnant Women seem to be important for the implementation of personalized prevention and effective prediction of the upcoming vascular events of Women itself and its offspring too. The problem of hypertension in pregnant women (PW) is a subject of many studies. However, to question of isolated increasing of aortic pressure during pregnancy was given much less attention. We aimed to evaluate the indices of vascular stiffness in pregnant women in the presence of isolated central hypertension (ICH) in correlation with some complications of pregnancy. Methods. The study included 89 women in the first trimester of pregnancy. Evaluated the signs of pre-eclampsia (PE) and syndrome of delayed fetal growth (SDFG). Determining of central pressure (CP) was performed using diagnostic system BPLab (Petr Telegin, Russia) on software Vasotens Office. Inclusion criteria - level of systolic pressure on shoulder in the range of 110-129 mm Hg. Among these women were identified bearers of CP on the level above 90th percentile for the relevant age group in accordance with established reference indicators by

Herbert A. e.a. (2014). These women were included in first group with ICH (27 persons). The rest ones with normal pressure on shoulder and in the aorta constituted the second control group (62 persons). The groups did not differ by age. Data were processed using software package "Statistica 10.0" (StatSoft Inc, USA). Results. It turned out that carriers of ICH had higher parameters of vascular stiffness compared with women in the control group. Particularly significant difference is revealed from the side of the aortic augmentation index - 5,4±1,1 vs 2,1±0,8. All cases of complications of pregnancy were identified only in the first group. So the frequency of PE and SDFG amounted to 11,1%.The average weight of newborns of the first group also differed from the control in direction of its decrease - 3338±99,6g vs 3487±125,3g . Conclusions. The presented results reflect the approach to the formation of new and more effective system of health care of pregnant women that is focused on predictive diagnostics of personalized threat profile for implementation targeted preventive measures in subsequent.

Disclosures: M. Evsevyeva: None. **S. Oksana:** None. **E. Shchetinin:** None. **V. Frantseva:** None. **T. Bondar:** None.

Funding: No

Funding Component:

P314

Regulatory Dendritic Cell Treatment Prevents the Development of Vasopressin-Induced Preeclampsia

Authors: Sabrina M Scroggins, Donna A Santillan, Jeremy A Sandgren, Gary L Pierce, Curt D Sigmund, Justin L Grobe, Mark K Santillan, Univ of Iowa, Iowa City, IA

The concept that persistent feto-placental intolerance is important in the pathogenesis of preeclampsia (PE) has been demonstrated by our lab and others. Arginine vasopressin (AVP) infusion during pregnancy induces cardiovascular, renal, and immune alterations in mice consistent with human PE. These findings identify AVP as a potential contributor to poor fetal tolerance and the development of PE. In addition to their conventional immuno-stimulatory role, dendritic cells (DCs) also play a vital role in immune tolerance. In contrast to conventional DCs, regulatory DCs (DCregs) express low levels of co-stimulatory markers, produce anti-inflammatory cytokines, induce T regulatory cells, and promote tolerance. In mice, DCregs are able to prevent pro-inflammatory responses and induce antigen-specific tolerance. Given these known functions of DCregs, we hypothesize that DCregs will prevent the development of AVP-induced PE. C57BL/6J dams were infused with AVP (24 ng/hour) or saline throughout gestation via osmotic minipump. To generate DCregs, bone marrow derived cells from C57BL/6J mice were cultured with human TGF-b1, and murine GM-CSF and IL-10 and phenotype confirmed via flow cytometry. At the time of pump implantation or early post-placentation on gestational day (GD) 7, AVP dams received a single intravenous injection of DCregs. Blood pressure was taken throughout pregnancy and total urine protein was measured on GD 17. Maternal tissues were collected on GD 18. Cytokine concentrations were determined via commercially available ELISAs and normalized to total protein. Treatment of AVP-infused dams with DCregs before mating (GD -3) and on GD 7 prevented AVP-induced hypertension (AVP: 120 ±1.8, n=27 vs GD -3: 108 ±3.3, n=7 vs GD 7: 110 ±4.4, n=5 $p<0.05$) and elevations in urine protein (AVP: 37.4 ±2.3, n=24 vs GD -3: 25.6 ±2.9, n=7 vs GD 7: 24.1 ±3.1, n=5 $p<0.05$). Treatment with DCregs also reversed AVP-induced suppression of anti-inflammatory TGFb (AVP: 1.3, n=9 vs GD -3: 3.2, n=7 vs GD 7: 2.9, n=5 ug/g, $p<0.05$) in the plasma. These data support the hypothesis that DCreg treatment prevents AVP-induced PE. It further provides evidence for the use of personalized, cellular therapy in the prevention of cardiovascular, renal, and immune alterations induced in PE.

Disclosures: S.M. Scroggins: None. **D.A. Santillan:** H. Other; Modest; Patent on Therapeutics and Diagnostics for Preeclampsia. **J.A. Sandgren:** None. **G.L. Pierce:** None. **C.D. Sigmund:** None. **J.L. Grobe:** H. Other; Modest; Patent on Therapeutics and Diagnostics for Preeclampsia. **M.K. Santillan:** H. Other; Modest; Patent on Therapeutics and Diagnostics for Preeclampsia.

Funding: Yes

Funding Component: Midwest Affiliate (Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota & Wisconsin)

P315

Progesterone Supplementation Abolishes Sflt-1 Induced Hypertension In Pregnant Rats

Authors: Lorena M Amaral, Mark W. Cunningham Jr., Venkata Ramana Vaka, Tarek Ibrahim, Babbette LaMarca, Medical Ctr- UMMC, Jackson, MS

Preeclampsia (PE) affects 7% of pregnancies in the United States and is characterized by new onset hypertension in association with elevated soluble fms-like tyrosine kinase-1 (sFlt-1). We and others have shown that infusion of sFlt-1 into pregnant rodents causes hypertension, indicating an important role for sFlt-1 in mediating the pathophysiology of the disease. Currently, there is no effective treatment for PE except for early delivery of the fetal placental unit, making PE the leading cause for premature births worldwide. Administration of 17-hydroxyprogesterone caproate (17-OHPC, Makena) is used for prevention of recurrent preterm birth, but not for treatment of PE. This study was designed to test the hypothesis that 17-OHPC improves clinical characteristics of PE such as hypertension in response to sFlt-1 in pregnant rats. sFlt-1 was infused into normal pregnant (NP) Sprague-Dawley rats (3.7 $\mu g \cdot kg^{-1} \cdot day^{-1}$ for 6 days, gestation day *13-19*) in the presence or absence of 17-OHPC (3.32mg/kg) administered via intraperitoneal injection on gestational days 15 and 18. Infusion of sFlt-1 into NP rats elevated mean arterial pressure (MAP) compared with control NP rats: 112 $\pm$2 (n=11) vs. 98$\pm$2 mmHg (n=15, $p<0.05$). Administration of 17-OHPC attenuated this hypertension reducing MAP to 99 $\pm$4 mmHg in sFlt-1 treated pregnant rats (n=8). Neither pup nor placental weight was affected by sFlt-1 or 17-OHPC. In conclusion, administration of 17-OHPC improves hypertension in response to elevated sFlt-1 during pregnancy and should be considered for merit further study to the management of PE

Disclosures: L.M. Amaral: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; LUMARA- AMAG Pharmaceutical Grant. C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Significant; Receipt of drug: 17-OHPC (MAKENA). **M.W. Cunningham Jr.:** None. **V.R. Vaka:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; AHA-Predoctoral Grant. **T. Ibrahim:** None. **B. LaMarca:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; NIH: RO1HD067541. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; LUMARA-AMAG Pharmaceutical Grant.

Funding:

Funding Component:

P316

Effects of Paternal Contribution of a Null Allele of Regulator of G Protein Signaling-2 (*Rgs2*) upon the Placental Transcriptome of C57BL/6J Mice

Authors: Katherine J Perschbacher, Guorui Deng, John W Walsh, Donna A Santillan, Eric J Devor, Gary L Pierce, Curt D Sigmund, Rory A Fisher, Katherine N Gibson-Corley, Mark K Santillan, Justin L Grobe, UNIVERSITY OF IOWA, Iowa City, IA

Preeclampsia (PreE), a cardiovascular disorder of pregnancy, remains a major cause of maternal and fetal mortality worldwide. The early etiology of the disorder is unclear, though increased G protein-coupled receptor signaling has been implicated. Regulator of G protein Signaling-2 (RGS2) is a negative regulator of G protein signaling, and mutations of *RGS2* have been associated with increased risk for PreE in humans. Our ongoing work has demonstrated that breeding wildtype C57BL/6J dams with *Rgs2*-deficient (*Rgs2*-KO; B6.129P2-*Rgs2*^{tm1Dgen}/Mmnc) sires is sufficient to cause ~50%

reduced placental expression of *Rgs2* mRNA, and to induce hypertension, renal and placental morphological phenotypes typical of PreE in the wildtype dams. To test the hypothesis that reduced RGS2 (via paternal contribution of an *Rgs2*-null allele) is sufficient to cause molecular changes within the placenta that are typical of PreE, we performed RNA sequencing on placentas collected on gestational day 12.5 from C57BL/6J dams mated with either *Rgs2*-KO sires or wildtype littermates of these sires. RNA was isolated and sequenced using an Illumina HiSeq 4000. Transcript counts were quantified and aligned to the mouse genome (UCSC genome browser) using Kallisto, followed by differential gene expression analysis using DEseq2 (FDR = 0.1). 726 genes were differentially expressed (479 up, 247 down), including some (*Hsd11b2* (up), *Adm* (down)), that are similarly changed in human PreE placenta (PMID: 28618048 & 26268791). Ingenuity Pathway Analysis indicated that reduced placental *Rgs2* expression was associated with mitochondrial dysfunction, unfolded protein response, and oxidative stress (all $p < 1e\text{-}3$), which also parallels findings in human PreE placenta. Collectively these data support the conclusion that reduced *Rgs2* expression in the feto-placental unit is sufficient to induce transcriptomic changes within the placenta that are typical of PreE.

Disclosures: **K.J. Perschbacher:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA. **G. Deng:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA. **J.W. Walsh:** None. **D.A. Santillan:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH, AHA. 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; Patents. **E.J. Devor:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH. **G.L. Pierce:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH. **C.D. Sigmund:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH. **R.A. Fisher:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH. **K.N. Gibson-Corley:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH. **M.K. Santillan:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH. 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; Patents. **J.L. Grobe:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH. 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; Patents.

Funding:

Funding Component:

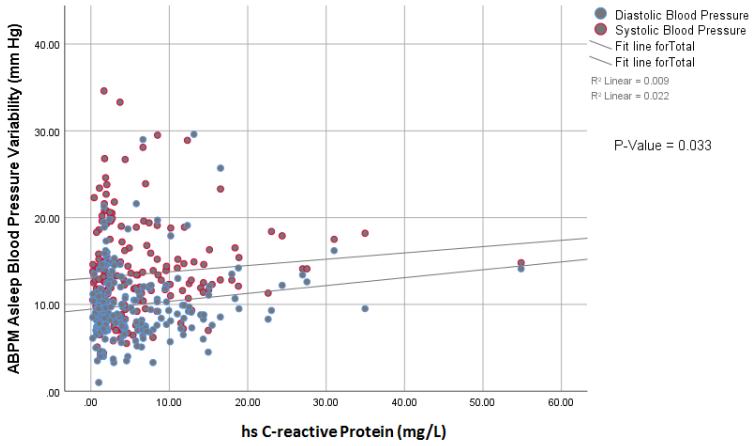
P317

Nocturnal Blood Pressure Variability is associated with hs-CRP in Hypertensive Patients

Authors: Badhma Valaiyapathi, Mohammed Siddiqui, Maria El Hachem, Eric Judd, Tanja Dudenbostel, Suzanne Oparil, David A Calhoun, Univ of Alabama at Birmingham, Birmingham, AL

Background: 24-hr Blood pressure (BP) variability and high sensitivity C-reactive protein (hs-CRP) have been found to be independently associated with increased cardiovascular diseases (CVD). Studies have shown a positive association between hs-CRP and BP variability in hypertensive population. In particular, nocturnal BP variability is associated with increased risk for CVD. However, the relationship between inflammation and nocturnal BP variability has not been examined in hypertensive patients. **Methods**: In this prospective study, 237 hypertensive patients were recruited from

University of Alabama at Birmingham Hypertension clinic. As part of the study protocol, hs-CRP and 24-hr ambulatory blood pressure monitoring (ABPM) was performed at same visit. Patients with chronic kidney disease and those who did not have complete ABPM and hs-CRP values were excluded. Nocturnal BP variability was assessed using standard deviation. **Results**: Out of 202 patients who were included, 49.0% were women and 55.9% were African American. The mean age was 58.2 ± 10.9 years, mean nighttime BP (Systolic BP 132.4 ± 22.9 mmHg and diastolic BP 71.9 ± 14.0 mmHg), mean nighttime BP variability (systolic BP 13.4 ± 5.3 and diastolic BP 9.9 ± 4.2) and mean hs-CRP was 5.9 ± 7.0 mg/L. Regression analysis showed significant positive association (p-value = 0.033) between nocturnal BP variability and hs-CRP. **Conclusion**: In this study, we demonstrated that hs-CRP is strongly correlated with nocturnal BP variability in hypertensive patients, suggesting a likely mechanistic role of inflammation in the pathogenesis of nocturnal BP variability and a possible additive role in adverse cardiovascular outcomes.



Disclosures: B. Valaiyapathi: None. **M. Siddiqui:** None. **M. El Hachem:** None. **E. Judd:** None. **T. Dudenbostel:** None. **S. Oparil:** None. **D.A. Calhoun:** None.

Funding:

Funding Component:

P318

Cardiac and Vascular Changes in Patients With Morbid Obesity at 12 Months After Bariatric Surgery

Authors: Anna Oliveras, Ana M Granados, Lluís Molina, Albert Goday, Susana Vazquez, Laia Sans, Maria Vera, Berta Xargay, Julio Pascual, Hosp del Mar, Barcelona, Spain

Aim: To analyze changes in echocardiographic parameters and vascular stiffness in patients with morbid obesity, with either normal or high blood pressure, at 12 months after BS.
Methods: We prospectively studied 62 patients (61% normotensive) with morbid obesity (BMI 42.7 ± 5.6 Kg/m^2) before and 12 months post-BS. Pulse wave velocity (PWV) was determined, as well as different morphological and functional echocardiographic parameters.
Results: generalized estimation equations showed a decrease in PWV at 6 months [mean (95% CI): -0.13 (-0.25 to -0.02), p=0.021], losing statistical significance at 12 months. Regarding cardiac involvement, the Table shows the changes in the different parameters at 12 months post-BS. Significant decreases were

observed in the left ventricular mass (LVM), thickness of the interventricular septum (IST) and posterior wall (PWT), and relative wall thickness (RWT), as well as in the index E/e 'of ventricular filling, but not in the left ventricular mass index (LVMI). These changes were especially significant in normotensive patients (p=0.005 for RWT and IST, p=0.001 for the PWT and p = 0.004 for the LVM). In the adjusted analyzes, it was found that the changes observed in the LVM were determined by changes in weight (p <0.001) and changes in 24h-systolic BP(p=0.011).
Conclusion: patients with morbid obesity experience 12 months after BS a
significant reduction of the structural parameters of the left ventricle, especially when they are assessed with the RWT but not by LVMI. These changes are also significant in normotensive people, suggesting that factors other than obesity *per se* determine structural cardiac changes in them.

	Change at 12 months Median (95% CI)	p
Left ventricular mass (g)	-24.4 (-37.9 a -10.9)	0.001
Interventricular septum thickness (mm)	-0.96 (-1.39 a -0.53)	<0.001
Posterior wall thickness (mm)	-0.87 (-1.29 a -0.45)	<0.001
Diastolic left ventricular diameter (mm)	-0.67 (-2.25 a 0.84)	0.376
Left ventricular mass index (g/m²)	0.99 (-5.33 a 7.31)	0.754
Relative wall thickness	-0.03 (-0.05 a -0.01)	0.004
Ejection fraction	1.57 (-0.41 a 3.55)	0.117
E' lateral	2.29 (1.66 a 2.91)	<0.001
E' septal	0.71 (-0.27 a 1.69)	0.150
E/e'	-0.83 (-1.28 a -3.76)	0.001

Disclosures: A. Oliveras: None. **A.M. Granados:** None. **L. Molina:** None. **A. Goday:** None. **S. Vazquez:** None. **L. Sans:** None. **M. Vera:** None. **B. Xargay:** None. **J. Pascual:** None.

Funding: No

Funding Component:

P319

Effects on Cardiac Output, Arterial Stiffness and Blood Pressure of Sacubyrtil + Valsartan in Hypertensive Patients With Heart Failure Whose Previously Received Ace Inhibitors or Arb + Diuretics. Prospective Study and Follow-up to 24 Weeks

Authors: Ricardo Cabrera-Sole, Caridad Turpin Lucas, Liliana Urrego Rivera, Ana Nuñez Galdamez, Santiago Garcia Ruiz, Erik Stephan Luekpe, Manuel Aguilera Saldaña, Univ General Hosp, Albacete, Spain

INTRODUCTION: Hypertension (HTA), is behind a high percentage of patients with left ventricle dysfunction (LVHF) and the recent use of sacubitril + valsartan (ARNi), has revolutionized the treatment of heart failure. In hypertensive patients with LVHF, the use of this combination has been especially useful for clinical improvement and better control of blood pressure figures, however, we have little information about the effect of them on arterial stiffness (AS) and cardiac output (CO), for what we present the following work.
OBJECTIVES: to evaluate the effect of ARNi on AS,CO and antihypertensive response in hypertensive patients with heart failure, who previously received ACE inhibitors or ARB.
MATERIAL AND METHODS: we studied 48 patients (P) with LVHF from those we selected 38 P (68 ± 8 years old, 30 men) who had hypertension and were receiving ACEI or ARB alone or with diuretics for treatment. After discontinuing the previous treatment, ARNi was started until the tolerated dose and in all P had been done before introducing this drug and at 24 ± 2 weeks, a 24-hour ambulatory blood pressure study , and we measured the augmentation index (AI), pulse wave velocity (PWV), and CO non-invasively by means a MobilOgraph © device.The results were compared and exposed in the following table:
RESULTS:

DATA	Sistolic BP	Diastolic BP	AI	PWV	CO
PRE ARNi	136±3*	85±2*	27±3*	8±2	3.9±1*
24 weeks after ARNi	111±5	79±3	17±2	6±2	5.1±1

*means p value less than 0.05.
CONCLUSIONS: use of ARNi, significantly improves the control of HTA as well as the AS, and CO of hypertensive patients with LVHF. There was also a tendency towards improvement, of the PWV, without reaching significant level, probably due to the number of patients studied, which provides more data of better prognosis to use ARNi in this group of patients.

Disclosures: R. Cabrera-Sole: None. **C. Turpin Lucas:** None. **L. Urrego Rivera:** None. **A. Nuñez Galdamez:** None. **S. Garcia Ruiz:** None. **E. Stephan Luekpe:** None. **M. Aguilera Saldaña:** None.

Funding: No

Funding Component:

P320

Influence of Diabetes Mellitus on the Prevalence of Atrial Fibrillation in Patients With Diastolic Dysfunction in a Large Community Practice in Greenville, South Carolina

Authors: Naveen Raj Saxena, Vinita Srivastava, Peace Medical Ctr, Greenville, SC; Juhi Saxena, Anju Saxena, Furman Univ, Greenville, SC

Atrial Fibrillation and diastolic dysfunction causes adverse cardiovascular events. Review of medical literature shows prevalence of atrial fibrillation in diastolic dysfunction in the range of 8% to 24%. The aim of this study is to examine the influence of diabetes on the prevalence of atrial fibrillation in patients with diastolic dysfunction in a large community practice. This is a retrospective chart review of patients with diastolic heart disease and atrial fibrillation from 2013 to 2018. 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 systolic heart disease and valvular heart disease were excluded from the study. Atrial Fibrillation was diagnosed using EKG and Holter Recording. The age distribution of the patients included in the study ranged from 50 to 75 years of age with a mean of 65. There were 1,047 patients with diastolic dysfunction and 93 patients had atrial fibrillation. There were 472 patients with diastolic dysfunction with diabetes and 575 patients with diastolic dysfunction without Diabetes. There were 569 females and 478 males in the study. The prevalence of atrial fibrillation in diastolic dysfunction with diabetes was 11%. The prevalence of atrial fibrillation in diastolic dysfunction without Diabetes was 7.1%.
Given the data, we conclude that the presence of Diabetes increases the prevalence of atrial fibrillation in diastolic dysfunction.

Table 1. Prevalence of Atrial Fibrillation in Diabetic vs Non-Diabetic Patients with Diastolic Dysfunction.			
Population of Diastolic Dysfunction	**Total Patients**	**Number of Patients With Atrial Fibrillation**	**Proportion**
Non-diabetic	575	41	0.07130435
Diabetic	472	52	0.11016949

Table 1. The results show a 0.03886514 (3.89%) higher prevalence of atrial fibrillation in diabetic patient populations compared to the non-diabetic population. The results of a two population proportion t-test validate this difference as statistically significant at a p-value of 0.0278.

Disclosures: N.R. Saxena: None. **V. Srivastava:** None. **J. Saxena:** None. **A. Saxena:** None.

Funding: No

Funding Component:

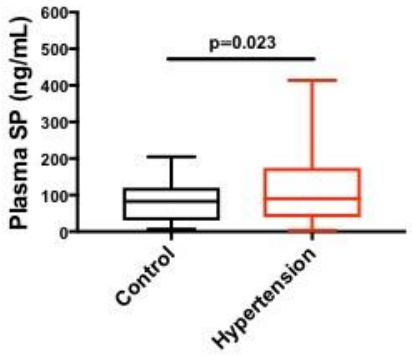
P321

Substance P is Associated with Hypertension-induced Cardiac Fibrosis

Authors: Alexander Widiapradja, Christine Yu, Katharine Kott, Gemma A Figtree, Scott P Levick, Kolling Inst of Medical Res, St Leonards, Australia

Myocardial remodelling such as fibrosis is a major contributor to heart failure with preserved ejection fraction. We sought to determine the relationship between substance P (SP), a sensory neuropeptide, and this adverse remodelling. Using the angiotensin II mouse model of pressure overload we determined that *Tac1*$^{-/-}$ mice that are genetically deficient in SP were protected from developing cardiac fibrosis (WT+Ang II = 1.452±0.1523% (n=9) vs *Tac1*$^{-/-}$+Ang II = 0.5678±0.078% (n=10), $p<0.0001$). We then investigated the association between SP and fibrosis in hypertensive individuals. Plasma from consented individuals showed significantly higher levels of SP in the hypertensive group in comparison to the control group (control = 78.3 ±11.54 ng/mL (n=22), hypertensive = 127±17.5 ng/mL (n=43), p=0.023) (Figure). Interestingly, hypertensive males showed significantly higher levels of plasma SP than the male control group (control = 74.94±14.54 ng/mL (n=17), hypertensive = 143.2±23.96 ng/mL (n=29), $p<0.002$), while no significant difference existed between these groups in females. Plasma levels of procollagen type 1 C-terminal pro-peptide (PICP), a marker of fibrosis, indicated that hypertensive individuals with SP levels greater than one standard deviation from the mean, had significantly higher levels of PICP (below = 5.539±0.688 ng/mL (n=38), above = 10.52±3.75 ng/mL (n=5), p=0.035). These findings indicate that high levels of plasma SP are associated with increased levels of a marker of fibrosis in hypertensive individuals. The human studies combined with the genetically modified mouse studies indicate an important role for SP in hypertension-induced cardiac fibrosis.

Figure



Disclosures: **A. Widiapradja:** None. **C. Yu:** None. **K. Kott:** None. **G.A. Figtree:** None. **S.P. Levick:** None.

Funding:

Funding Component:

P322

Different Responses To Intrarenomedullary Infusion of Anandamidevs. PF-3845 Reveal Additional Mechanisms Of Pressure-lowering And Diuresis-stimulation By Renomedullary FAAH Inhibition

Authors: Ashfaq Ahmad, Sara K Dempsey, Zdravka Daneva, Ningjun Li, Pin-Lan Li, **Joseph K Ritter**, Virginia Commonwealth Univ, Richmond, VA

The fatty acyl ethanolamides comprise a group of lipids having biological activities consistent with multiple mechanisms of blood pressure-lowering, including vasodepressor, diuretic and natriuretic, and sympatholytic activities. One of these, N-arachidonoylethanolamide (anandamide, AEA), is an agonist at cannabinoid type 1 receptors (CB1) and is enriched in the renal medulla together with two of its metabolizing enzymes, fatty acyl amide hydrolase (FAAH) and cyclooxygenase-2 (COX-2). The purpose of the present study was to characterize and compare the effects of AEA with those of a selective FAAH inhibitor, PF-3845, on blood pressure and urine excretion parameters after intramedullary infusion into the outer medulla of anesthetized C57BL/6 (WT) or FAAH knockout (KO) mice. Intramedullary infusion of PF-3845 into WT mice stimulated UV but had a delayed MAP-lowering effect. Homozygous FAAH KO mice were refractory to intramedullary PF-3845- induced changes in MAP but UV was still increased. Both the MAP and UV responses to intramedullary PF-3845 in C57BL/6J mice were diminished by pretreatment with the cannabinoid type 1 receptor (CB1)-selective antagonist but not COX2-selective inhibitor. In contrast, intramedullary AEA treatment stimulated UV to similar extents in WT and FAAH KO mice but had no effect on MAP. The increase in UV by AEA was blocked by pretreatment with CB1 antagonist and COX-2 inhibitor. These data indicate fundamental differences in mechanisms of blood pressure-lowering by a FAAH inhibitor vs. AEA.

Table 1
Effect of intramedullary infusion of PF-3845 and AEA on urine formation rate (in μL/min/g kidney weight) in C57 BL and FAAH knock out mice

Groups	C1	C2	LD	MD	HD	P1	P2
C57BL+Sham	11±2	12±2	11±2	11±3	11±3	11±3	11±1
C57BL+PF-3845	11±1	12±1	17±3	22±5*	28±5*	25±4*	21±5*
C57BL+AEA	12±1	13±1	15±2	25±3*	26±2*	20±2*	21±3*
FAAHKO+PF-3845	4±1	3±1	18±3*	20±2*	24±3*	22±3*	22±3*
FAAH KO+AEA	11±1	11±1	17±2	23±3*	28±2*	24±3*	23±3*

* represents P<0.05 vs. C1 phase of experiment; C1 and C2 is measurement after 10th and 20th min of experiment after acclimation time of 1 hr; LD: Low dose for AEA is 15 nmol/kg/min and that for PF-3845 is 7.5 nmol/kg/min; MD: Medium dose for AEA is 30 nmol/kg/min and that for PF-3845 is 15 nmol/kg/min; HD: High dose for AEA is 60 nmol/kg/min and that for PF-3845 is 30 nmol/kg/min; values represent the mean +/- SEM of 5-7 mice per group.

Disclosures: A. Ahmad: None. **S.K. Dempsey:** None. **Z. Daneva:** None. **N. Li:** None. **P. Li:** None. **J.K. Ritter:** None.

Funding:

Funding Component:

P323

TMEM16A and TMEM16B Contribute to Mice Cholecystokinin Response in Sex-Specific Manner

Authors: Runping Wang, Michael Z Cicha, Mark W Chapleau, Francois M Abboud, The Univ of Iowa, Iowa City, IA

High fat diet (HFD) leads to obesity and the metabolic syndrome. However, the effect are less pronounced in females than in males. We suggest that a gender difference is in their response to the satiety peptide Cholecystokinin (CCK). We previously reported that the Ca^{2+}-activated Cl^- channel (CaCC) subunit *Ano2*/TMEM16B is essential for the CCK-induced current in intestinal nodose neurons in male mice, and is down regulated in obese mice fed on HFD. We hypothesize that the CCK-induced current in female mice is mediated predominantly by *Ano1*/TMEM16A, the alternative subunit of TMEM16B. We studied the CCK-induced current in nodose neurons from female C57BL/6 mice using whole cell patch-clamp. We found that 10nM of CCK induces an inward current in these neurons with a current density averaging 13.4±2.5 pA/pF (n=7), and this current is inhibited to 3.1±1.2 pA/pF (n=7, p<0.01) by TMEM16A specific inhibitor $T16A_{inhi-A01}$. We further tested whether HFD which effectively suppressed the expression of TMEM16B in male mice, would also decrease TMEM16A in female mice. Our results show that the CCK-induced TMEM16A current in nodose neurons is not decreased in female, but tend to increase from 12.5±1.6 (n=10) in control to 17.4±4.1 pA/pF (n=11, p=0.298) in female mice fed on HFD. Concurrently the mRNA level of *Ano1* in mice fed on HFD also show a trend to increase by 1.33±0.41 fold (n=2 pairs of mice, p=0.37) compared to mice on regular chow. Our findings indicate that activation of CCK-sensitive *Ano1*/TMEM16A may partially protect female mice from HFD induced obesity and metabolic syndrome.

Disclosures: R. Wang: None. **M.Z. Cicha:** None. **M.W. Chapleau:** None. **F.M. Abboud:** None.

Funding: No

Funding Component:

P324

Role of GABAergic Signaling in AgRP Neurons in the Dipsogenic and Metabolic Responses to Brain Angiotensin

Authors: Lisa L Morselli, Guorui Deng, Nicole A Pearson, Justin L Grobe, Univ of Iowa, Iowa City, IA

Obesity is a major risk factor for development of hypertension, and evidence supports a role for the suppression of resting metabolic rate (RMR) in the development and maintenance of obesity. Angiotensin II (ANG) AT1A receptors, localized to cells expressing the leptin receptor and Agouti-related peptide (AgRP) within the arcuate nucleus of the hypothalamus (ARC), are involved in transcriptional control of *AgRP* and γ-aminobutyric acid (GABA) synthetic genes (*Gad65*, *Gad67*, *Slc32a1*) within the ARC, as well as the integrative control of thermogenic sympathetic nerve activity (SNA) and RMR responses in vivo to various stimuli including leptin and high fat diet. These data led us to hypothesize that AT1A functions to suppress GABA signaling in AgRP neurons, ultimately to disinhibit SNA and RMR. Treatment of cultured mouse hypothalamic N43/5 cells (which express endogenous *Agtr1a*, *AgRP* and *Gad67*) with 100 nM ANG for 1 hour (n=6) resulted in reduced *AgRP* (0.57-fold of control, p=0.03) and *Gad67* (0.72-fold of control, p=0.09) mRNA. Mice with targeted disruption of *Slc32a1* (vesicular GABA transporter; VGAT) in AgRP neurons ($VGAT^{AgRP\text{-}KO}$ mice) were generated on a mixed background. Compared to littermates (male n=9-17, female n=7-8), $VGAT^{AgRP\text{-}KO}$ mice (male n=8-13, female n=2-3) at 8 weeks of age exhibited normal body mass (genotype p=ns, sex $p<0.05$, interaction p=ns), no changes in food intake (genotype p=ns, sex p=ns, interaction p=ns), but a sex-dependent genotype effect on fluid intake was observed (male con 4.5±0.3 vs KO 5.1±0.4; fem con 5.1±0.4 vs KO 3.7±0.6 mL/d; interaction $p<0.05$). A preliminary study of male mice instrumented for chronic infusion of ANG (5 ng/hr, 21 days icv) was also performed. This caused modest increases in drinking (con+aCSF n=7, 3.3±0.8, con+ANG n=4, 5.2±1.0, KO+ANG n=3, 7.9±1.1 mL/d, $p<0.05$) but no change in energy efficiency (11.2±2.9, 11.3±3.5, 10.1±4.1 mg/kcal), indicating no change in energy expenditure, possibly due to the mixed background of this strain. We conclude that ANG acts at AgRP cells of the ARC to suppress GABA and AgRP signaling, and this contributes to energy and fluid homeostasis in a complex, strain- and sex-dependent manner.

Disclosures: L.L. Morselli: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH. **G. Deng:** None. **N.A. Pearson:** None. **J.L. Grobe:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA, NIH.

Funding: Yes

Funding Component: National Center

P325

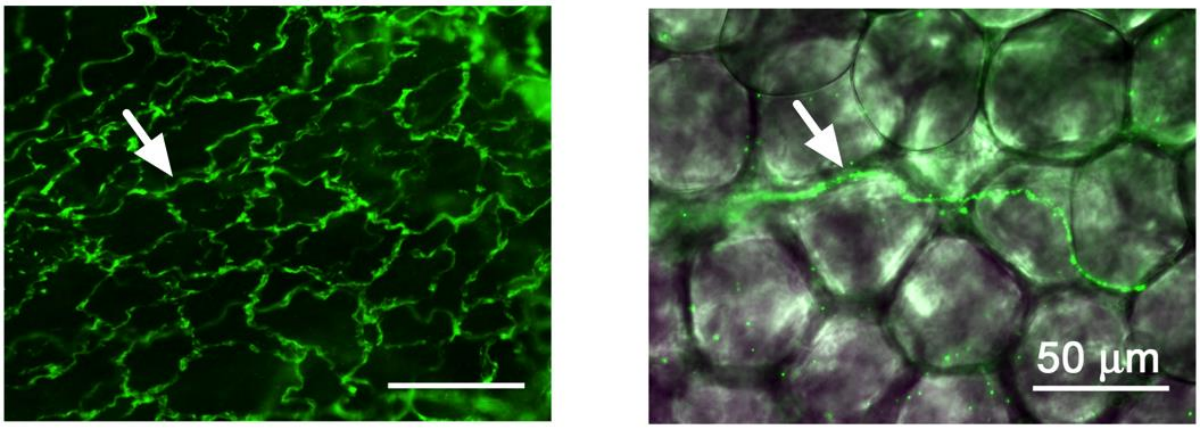
High-Fat Feeding Does not Alter the Sparse Sympathetic Nerve Density in Mesenteric Resistance Artery Perivascular Adipose Tissue in Male Sprague-Dawley Rats

Authors: William F. Jackson, Gregory D. Fink, Stephanie W. Watts, Michigan State Univ, East Lansing, MI

Chemical signals from perivascular adipose tissue (PVAT) modulate the structure and function of blood vessels. This may be partially under sympathetic neural control, however, little is known about the density of sympathetic innervation of PVAT. We hypothesized sympathetic nerve (SN) density in PVAT surrounding mesenteric resistance arteries (MRAs) is

similar to that in the adventitia of the MRAs, and that high-fat feeding (HFF, 16 weeks) would decrease the density of SNs on MRAs and in PVAT. To test these hypotheses, we labeled SNs on MRAs and in PVAT with rabbit anti-tyrosine hydroxylase (TH) antibodies/AlexaFluor488-conjugated donkey-anti-rabbit antibodies, ex vivo. TH staining density (fraction occupied by TH-labeled structures) was computed from thresholded, background subtracted, maximum intensity z-projections of image stacks. TH staining density was higher at the surface of MRAs (0.21±0.03, n = 14) than within PVAT (0.013±0.003, n = 42, p<0.0001, Fig. 1 - arrows point to TH-positive nerves). HFF increased PVAT adipocyte diameter from 39±0.5µm (n=672) to 72±0.6µm (n = 365, p<0.0001). However, HFF had no effect on TH staining density on MRAs or within PVAT (p=0.83). Thus, while MRA's are robustly innervated by SNs, only the first layer of adipocytes adjacent to MRA adventitia is exposed to a similar SN density; SN density in PVAT more than one adipocyte away from MRAs is >16 fold lower. HFF does not affect SN density on MRAs or in PVAT. However, given the larger size of adipocytes in HFF rats, this may mean an increase in SN density/adipocyte. Nonetheless, the low density of SNs in mesenteric PVAT suggests limited direct SN control of PVAT function in the bulk of PVAT adipocytes.

Fig. 1 - TH immunoreactivity



Disclosures: **W.F. Jackson:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; P01 HL70687. **G.D. Fink:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; P01 HL70687. **S.W. Watts:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; P01 HL70687.

Funding: No

Funding Component:

P326

Enhancement of Endogenous Epoxyeicoastrienoic Acids Production Ameliorates Heart Function via Increased Pgc1 Mitochondrial Functions in Metabolic Syndrome

Authors: Jian A Cao, Chinese PLA General Hosp, Beijing, China

Metabolic syndrome (MS) is a constellation of ailment which increases blood pressure, high blood sugar, excess visceral fat, and abnormal triglyceride levels that together instigates menace of heart disease, stroke and diabetes. We

hypothesized that an EET-agonist (AUDA) would increase expression of PGC 1α and improve mitochondrial and endothelial functions which recuperate heart function in a rat model of MS. Rats were randomly divided into four groups: 1) Control; 2) MS+ABCT; 3) MS+AUDA; and 4) MS+AUDA+SnMP. MS rats were fed a high-fructose diet for 16 weeks. Rats developed MS with elevated inflammatory mediators, oxidative stress, and significant decreases in fractional shortening and hemodynamic parameters indicated cardiac dysfunction ($p<0.05$). Histology revealed myocardial fibrosis and myocyte hypertrophy. AUDA improves mitochondrial function proven by increase in mt copy no and ATP production and significantly increased expression of PGC-1α and HO-1 in the rats and normalization of inflammatory cytokines, oxidative stress, and improves in cardiac function and myocardial fibrosis ($p<0.05$). These benefits of EETs were reversed by SnMP. Furthermore AUDA increases eNOS but decreases iNOS expression which improved endothelial function ($p<0.05$). Therefore, it suggests that increase of endogenous EET has novel role in protecting the heart from MS by regulating mitochondrial and endothelial function. **Keywords:** epoxyeicoastrienoic acids, PGC-1α, Heme oxygenase-1, mitochondrial function, metabolic syndrome

Disclosures: J.A. Cao: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; 16BJZ15.

Funding: No

Funding Component:

P327

Real-Time Analysis of Blood Glucose Dynamics by a Glucose Telemetry System in Canagliflozin-Treated Diabetic Mice

Authors: Ryousuke Satou, T Cooper Woods, Kayoko Miyata, Akemi Katsurada, Courtney M Dugas, Natasha C Klingenberg, L. Gabriel Navar, Dept of Physiology and Hypertension and Renal Ctr of Excellence, Tulane Univ Sch of Med, New Orleans, LA

SGLT2 inhibitors lower blood glucose (BG) levels and have the potential to reduce cardiovascular and renal complications of type 2 diabetes mellitus (T2DM). Since BG levels can vary significantly during a treatment regime, hemoglobin A1c is often utilized as a stable indicator of BG status but dynamic measurements of BG may provide greater insights. A blood glucose telemetry systems was used to investigate BG dynamics in canagliflozin (CANA, an SGLT inhibitor)-treated T2DM mice. Male New Zealand Obese mice were fed a high fat diet to induce diabetes. When the mice exhibited a BG of ~350 mg/dl, the mice were treated with vehicle for 5 days followed by 10 mg/kg/day CANA for 5 days by daily oral gavage (single dosing/day). BG levels were monitored continuously via implanted telemetry devices. Chronic treatment (6 weeks) was also performed to investigate blood pressure monitored by a telemetry system and to assess kidney injury. During vehicle treatment for 5 days, BG levels in the mice averaged 336.7 mg/dl. Lower BG levels during early morning compared to night time (active time) were observed during vehicle treatment. BG levels demonstrated a large variation over 24 hours during the vehicle treatment (maximum minus minimum BG: 368.5 mg/dl on day 5). CANA rapidly reduced BG levels within 3 hours following treatment (214.8±25.4 mg/dl), and the lowered BG was sustained until the next dosing. The average 24-hour BG was gradually suppressed during the CANA treatment reaching 170.1 mg/dl on the last day of CANA treatment. Moreover, CANA reduced the variation of BG (maximum minus minimum BG: 214.8 mg/dl on day 5 of CANA treatment). In mice receiving chronic treatment, CANA started lowering systolic blood pressure on week 2 and significant suppression was observed on week 6 (vehicle: 157.9±2.2 vs. CANA: 124.7±7.6 mmHg). The development of kidney injury, especially renal tubular fibrosis and inflammation, was attenuated by CANA. These findings demonstrate that CANA treatment results in rapid and sustained reductions of both BG levels and BG variability which precede the reduction of blood pressure. The temporal dissociation between the lowering of BG and of arterial pressure levels by CANA suggests hyperglycemia-induced factors mediate the development of hypertension in T2DM.

Disclosures: **R. Satou:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; IIS Janssen Research & Development. C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; Drug from Janssen Research & Development. **T. Woods:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; IIS Janssen Research & Development. C. Other Research Support (includes receipt of drugs, supplies, equipment or other in-kind support); Modest; Drug from Janssen Research & Development. **K. Miyata:** None. **A. Katsurada:** None. **C.M. Dugas:** None. **N.C. Klingenberg:** None. **L. Navar:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; IIS Janssen Research & Development, Drug from Janssen Research & Development.

Funding: No

Funding Component:

P328

Effect of Sitagliptin on the Hemodynamic and Metabolic Response to a Mixed Meal in Individuals With Type 2 Diabetes and Hypertension

Authors: Jessica R Wilson, Univ of Pennsylvania, Philadelphia, PA; Scott J Kerman, Hui Nian, Scott Hubers, Nancy J Brown, Vanderbilt Univ, Nashville, TN

Dipeptidyl peptidase IV (DPP4) inhibitors such as sitagliptin are anti-diabetes drugs that may also have cardiovascular effects. We tested the hypothesis that there is an interactive effect between sitagliptin and angiotensin-converting enzyme (ACE) inhibition on blood pressure (SBP, DBP, MAP) and heart rate (HR) after a meal. We enrolled 52 type 2 diabetics (T2DM) with hypertension. The mean age was 56.1±10.2 years. 58.5% were men, 28.3% were black and 67.9% were white. Individuals were randomized to one of three BP agents: ramipril 10mg/d, valsartan 320mg/d [a renin-angiotensin-aldosterone system (RAAS) inhibiting drug without enzyme activity], and amlodipine 10mg/d (a RAAS-independent control) for 15 weeks. On the 5^{th}, 10^{th}, and 15^{th} week of anti-hypertensive therapy each participant underwent one-week crossover treatment with sitagliptin 100 mg/d + placebo, placebo + placebo, and sitagliptin + aprepitant 80 mg/d (a substance P receptor blocker, as DPP4 and ACE degrade substance P). On the last day of each treatment, participants were given a mixed meal (712 calories: 45% from fat, 33% carbohydrate, 22% protein).
There was no difference in pre-prandial BP among the three crossover treatments. Following the meal, SBP ($p=0.002$) and MAP ($p<0.001$) were significantly decreased during either DPP4 inhibitor crossover treatment compared to placebo. In particular, in patients treated with an ACE inhibitor, post-prandial SBP (0.015) and MAP ($p=0.021$) were lower during DPP4 inhibition. This was true after controlling for significant effects of race ($p=0.002$) and gender ($p=0.0007$). Post-prandial HR was not affected by DPP4 inhibition during ACE inhibition. The effect of DPP4 inhibition on fasting glucose but not on postprandial glucose differed among anti-hypertensive treatment groups. DPP4 inhibition decreased the formation of PYY3-36 from PYY following the meal.
There is an interactive effect between DPP4 inhibition and anti-hypertensive therapy on post-prandial BP and metabolic parameters.

Disclosures: **J.R. Wilson:** None. **S.J. Kerman:** None. **H. Nian:** None. **S. Hubers:** None. **N.J. Brown:** None.

Funding: No

Funding Component:

P329

Ankle Brachial Index Profile in a Diabetic Population With High Risk of Cardiovascular Disease

Authors: Bernardo Pires de Freitas, FTESM, Rio de Janeiro, Brazil; Beatriz Glielmo Saraiva, Laura Martins Peçanha, Andressa Regina da Fonseca Wolff, UNESA, Rio de Janeiro, Brazil; Bruno Vasconcelos Coimbra, FTESM, Rio de Janeiro, Brazil; Fernando Ribas Brustolin, Nicole Ceccon de Castro, Odara da Costa, Renan Alves Garcia, Maria Clara Dal Pai, UNESA, Rio de Janeiro, Brazil; Caroline Campos Garcia, FTESM, Rio de Janeiro, Brazil; **Lilian Soares da Costa**, FTESM and UNESA, Rio de Janeiro, Brazil

Introduction: The Ankle Brachial Index (ABI) is a noninvasive and effective measure in the evaluation of peripheral vascular disease, also being indicative of atherosclerosis. This measure is based on the ratio of ankle systolic pressure values to ipsilateral arm systolic pressure. ABI values below 0.90 are considered risk factors for coronary and cerebrovascular diseases and for the presence of high cardiovascular risk. Objective: To describe the ABI and risk factors (RF) of participants in a cross-sectional sample of diabetic and hypertensive patients at high cardiovascular risk. Method: After analyzing a group of 147 high-risk cardiovascular patients, 25.9% of hypertensive diabetic patients (n = 38) were selected for ABI and the demographic profile analysis, related to the presence of brain and/or cardiovascular events (CVD). To evaluate the project, a cross-sectional descriptive analysis of data collected through questionnaires and anthropometric evaluation was used. Results: Of the 38 subjects (57.9% women), with a mean age of 63 ± 9 years, other RF history were found: physical inactivity (73.7%); dyslipidemia (63.2%); family history of cardiovascular diseases (60.5%); reports of previous cardiovascular events, myocardial infarction and / or stroke (71.1%); overweight (34.2%); obesity (36.8%) and smoking (7.9%). When comparing the ITB values found in the subgroup with CVD (n = 27) and without CVD (n = 11), we observed values of 1.00 (0.73-1.25) x 1.02 (0.68- 1.13), respectively, with significant difference between the groups by the chi-square Monte Carlo analysis (p 0.13), although with a small analysis sample. Conclusion: In a population with a high cardiovascular profile, with multiple risk factors, the assessment of ABI was a risk marker in the presence of previous CVD, and should be a method to be encouraged in daily clinical practice as a possible marker of cardiovascular risk in the diabetic population.

Disclosures: B.P. de Freitas: None. **B.G. Saraiva:** None. **L.M. Peçanha:** None. **A.D. Wolff:** None. **B.V. Coimbra:** None. **F.R. Brustolin:** None. **N.C.C. de Castro:** None. **O. da Costa:** None. **R.A. Garcia:** None. **M.C.D. Dal Pai:** None. **C.C. Garcia:** None. **L.S. da Costa:** None.

Funding: No

Funding Component:

P330

Alterations in Maternal Heart during Diabetic Rat Pregnancy

Authors: Till Schuette, Charité – Univsmedizin Berlin, Berlin Inst of Health, German Ctr for Cardiovascular Res, Berlin, Germany; Michaela Golic, Experimental and Clinical Res Ctr, Max Delbrück Ctr for Molecular Med, Charité – Univsmedizin Berlin, Berlin Inst of Health, Berlin, Germany; Kristin Kraeker, Nadine Haase, Experimental and Clinical Res Ctr, Max Delbrück Ctr for Molecular Med, Charité – Univsmedizin Berlin, Berlin Inst of Health, German Ctr for Cardiovascular Res, Berlin, Germany; Florian Herse, Experimental and Clinical Res Ctr, Max Delbrück Ctr for Molecular Med, Charité – Univsmedizin Berlin, Berlin Inst of Health, Berlin, Germany; Natalia Alenina, Max Delbrück Ctr for Molecular Med, Berlin Inst of Health, German Ctr for Cardiovascular Res, Berlin, Germany; Dominik N Mueller, Experimental and Clinical Res Ctr, Max Delbrück Ctr for Molecular Med, Charité – Univsmedizin Berlin, Berlin Inst of Health, German Ctr for Cardiovascular Res, Berlin, Germany; Michael Bader, Max Delbrück Ctr for Molecular Med, Charité – Univsmedizin Berlin, Berlin Inst of Health, German Ctr for Cardiovascular Res, Berlin, Germany; Michael Schupp, Charité – Univsmedizin Berlin, Berlin Inst of Health, Berlin, Germany; Ralf Dechend, Experimental and Clinical Res Ctr, Charité – Univsmedizin Berlin, Berlin Inst of Health, German Ctr for Cardiovascular Res, Helios Clinic, Berlin, Germany

Introduction: Pregnancy is an enormous challenge for the maternal cardiovascular system. Women with pre-gestational cardiovascular impairments face an increased risk for developing pathological pregnancies. Diabetes affects cardiovascular function already in the non-pregnant state. We aimed at evaluating the influence of pre-gestational diabetes on heart morphology and gene expression using a transgenic rat. **Methods:** We generated a pre-gestational hyperglycemic condition in transgenic rats (Tet29) by applying doxycycline, which induces a knock down of the insulin receptor via RNA interference in this strain. Wildtype Sprague-Dawley (SD) rats receiving doxycycline served as a control. Heart analysis was performed on day 21 of pregnancy (with appearance of a vaginal plug being pregnancy day 1). Gene expression levels were measured by qRT-PCR. Heart and body weight were obtained and analyzed. **Results:** Tet29 rats were hyperglycemic throughout pregnancy (blood glucose of 487±24 mg/dl vs. 95±5 md/dl in SD rats) and had a lower body weight in comparison to SD rats at the end of pregnancy (402.3±9.0 g vs. 471.3±12.0 g). Hearts of diabetic Tet29 rats were smaller (absolutely and relatively, regarding body weight), in comparison to SD rats at the end of pregnancy (0.60±0.02 g vs. 0.87±0.02 g; 0.150±0.005 vs. 0.186±0.004, respectively). Expression of inflammation markers MCP1 (1.34±0.20 vs. 0.47±0.13) and TNFα (1.76±0.30 vs. 0.48±0.15) was higher in diabetic hearts. Additionally, the expression of the fibrosis markers CTGF (1.83±0.98 vs. 0.18±0.04) and NGAL (1.30±0.55 vs. 0.41±0.22) was elevated in diabetic hearts, whereas the markers fibronectin (0.74±0.07 vs. 1.23±0.29) and collagen 1 (0.45±0.08 vs. 2.04±0.55) were decreased. **Discussion:** Diabetic pregnant Tet29 rats reveal an altered morphology and gene expression profile in the heart. This phenotype does not fit in a classical category of heart diseases. Further studies such as echocardiography and follow-up analysis in postpartum life are necessary to elucidate possible functional changes.

Disclosures: T. Schuette: None. **M. Golic:** None. **K. Kraeker:** None. **N. Haase:** None. **F. Herse:** None. **N. Alenina:** None. **D.N. Mueller:** None. **M. Bader:** None. **M. Schupp:** None. **R. Dechend:** None.

Funding:

Funding Component:

P331

Transition From Pre-Diabetes To Diabetes Is Associated With Worsening Of Cardiac Autonomic Neuropathy: Reversal By Anti-Diabetic Drugs

Authors: Nour Bakkar, Nahed Mougharbil, Souha Fares, **Fouad A Zouein**, Ahmed F El-Yazbi, AUBMC, Beirut, Lebanon

Cardiac autonomic neuropathy (CAN) occurs early in the course of diabetes leading to significant cardiovascular mortality and morbidity. Manifested as impaired heart rate variability (HRV) and baroreflex sensitivity (BRS), CAN 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. Here, we assessed the incidence and manifestation of CAN in a rat model of metabolic challenge prior to and after the development of diabetes. 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. The pre-diabetic stage was associated with signs of perivascular and epicardiac adipose inflammation (4- and 40- fold increase in IL-1β and TGF-β and 2-fold increase in NK-κB expression; and macrophage infiltration) and increased brainstem oxidative stress accompanied with impairment of HRV (total power decrease from 3098 ± 233 to 89 ± 88μs^2) and the parasympathetic arm of the baroreflex (slope decrease from -1.02 ± 0.12 to -0.34 ± 0.04). These abnormalities were reversed upon treatment with non-hypoglycemic doses of either metformin or pioglitazone or upon lowering the rat caloric intake. On the other hand, after progression to diabetes, the autonomic deficit progressed to the sympathetic arm of the baroreflex (Slope reduction from -0.35 ± 0.02 to -0.09 ± 0.03). The same hypoglycemic doses of metformin and pioglitazone failed to reverse the impaired HRV and BRS. Whereas, reduction of hyperglycemia to control levels by insulin was only capable of reversing the sympathetic impairment (BRS slope of -0.28 ± 0.04). Our present results outline a framework for sequential involvement of different

detrimental mechanisms in the course of metabolic disease leading to differential impact on cardiac autonomic control. Furthermore, our findings emphasize the value of early intervention with antidiabetic drugs with potential protective pleiotropic effects.

Disclosures: N. Bakkar: None. **N. Mougharbil:** None. **S. Fares:** None. **F.A. Zouein:** None. **A.F. El-Yazbi:** None.

Funding:

Funding Component:

P332

Hesperidin in Citrus Fruit Juice Plays a Role in Preventing Cognitive Impairment Induced by Ischemic Brain Damage

Authors: Moe Kawakami, Jun Iwanami, Kana Tsukuda, Akinori Higaki, Li-Juan Min, Masaki Mogi, Masatsugu Horiuchi, Ehime Univ, Graduate Sch of Med, Tohon, Ehime, Japan

Recently we reported that drinking *Citrus iyo* juice (CI) inhibited more effectively vascular remodeling in the inflammation-induced vascular injury mouse model than *Citrus unshiu* juice (CU). These results led us to explore the possibility that citrus fruits juice drinking could attenuate cognitive decline in transient cerebral ischemia mouse model, focusing on the effects of flavanone, hesperidin, which is more abundantly contained in CI compared with CU and has antioxidant activity. Eight-week-old male 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. The Y maze task was performed 2 weeks after BCCAO operation. After cognitive task, cerebral blood flow (CBF) was measured by a laser speckle flowmetry. Morphological changes in the hippocampus were examined. Administration of CI, CU or hesperidin did not influence systolic blood pressure, body weight and brain weight. Cognitive function was significantly impaired (sham, 71% (16 of 23) vs BCCAO, 55% (14 of 27) in Y maze) with the increase in superoxide anion production after BCCAO. The cognitive impairment was more effectively attenuated by the administration of CI than CU (CI, 66% (13 of 23); CU, 61% (17 of 27)) with the significant increase in CBF. Interestingly, we also observed that the treatment with hesperidin significantly prevented cognitive decline (67% (13 of 21)) after BCCAO. The increase in superoxide anion production 24 hours after BCCAO (expressed as fold-increase compared to sham) was attenuated by CI or hesperidin, not by CU (BCCAO, 4.0; CI, 1.2; hesperidin, 1.1; CU, 3.0). The fold-increase in TNF-α mRNA in the hippocampus 24 hours after BCCAO was prevented by hesperidin (BCCAO 5.0; hesperidin, 2.8). Cell number in CA1 region of hippocampus decreased in BCCAO-operated mice. Hesperidin treatment attenuated this decrease (sham, 163±5; BCCAO, 136±4; hesperidin, 154±4). These results suggest that the intake of hesperidin in citrus fruits juice should prevent cognitive decline after brain ischemia at least in part due to reduction of oxidative stress, inflammatory cytokine and an increase in CBF.

Disclosures: M. Kawakami: 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 lngelheim Co. Ltd.. **K. Tsukuda:** None. **A. Higaki:** None. **L. Min:** None. **M. Mogi:** 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 lngelheim Co. Ltd., Novartis Pharma K. K..

Funding: No

Funding Component:

P333

Effect of Autonomous Cortisol Secretion on Cerebrovasuclular Events in Patients With Primary Aldosteronism

Authors: Masataka Kudo, Tohoku Univ Hosp, Div of Nephrology, Endocrinology and Vascular Med, Sendai, Japan; Shunji Mugikura, Tohoku Univ Hosp, Dept of Diagnostic Radiology, Sendai, Japan; Ryo Morimoto, Tohoku Univ Hosp, Div of Nephrology, Endocrinology and Vascular Med,, Sendai, Japan; Yuta Tezuka, Kei Omata, Beata Shiratori, Yasuhiro Igarashi, Tohoku Univ Hosp, Div of Nephrology, Endocrinology and Vascular Med, Sendai, Japan; Kazumasa Seiji, Tohoku Univ Hosp,Dept of Diagnostic Radiology, Sendai, Japan; Kei Takase, Tohoku Univ Hosp, Dept of Diagnostic Radiology, Sendai, Japan; Sadayoshi Ito, Tohoku Univ Hosp, Div of Nephrology, Endocrinology and Vascular Med, Sendai, Japan; Fumitoshi Satoh, Tohoku Univ Hosp, Div of Clinical Hypertension, Endocrinology & Metabolism, Sendai, Japan

Background: Primary aldosteronism (PA) display a higher risk of cerebrovascular events (CVE) compared with essential hypertension (EH).On the one hand, it was also reported that the PA patients associated with autonomous cortisol secretion had high incidence of CVE.**Objective:** Our objective was to investigate the effect of autonomous cortisol secretion on the prevalence of CVE in the patients with PA and/or Cushing syndrome (CS).**Design and Setting:** This was a retrospective cross-sectional study from Tohoku University Hospital between 2008 and 2017.**Patient:** 291 patients were analyzed. In the patients with PA, the recent continuous cases performed adrenal venous sampling (AVS) were extracted. They were divided into four groups. Group A: 32 CS patients, Group B: 34 PA patients with autonomous cortisol secretion, Group C:121 PA patients with aldosterone producing adenoma (APA) and Group D: 104 PA patients with idiopathic hyperaldosteronism (IHA). They were also performed and analyzed MRI brain scans during a hospitalization and examined 1-mg dexamethasone suppression test . The patients in Group A, B and C were underwent adrenalectomy. **Results**: In Group A and Group B, the female-to-male ratio was significantly high (about 4:1). The prevalence of symptomatic stroke prevalence of symptomatic stroke before the confirmed diagnosis in each group were as follows: Group A:2/32, Group B: 0/34, Group C:13/121 and Group D: 3/104. The unruptured cerebral aneurysms were detected in Group A:1/32, Group B: 0/34,Group C:2/121 and Group D: 3/104. Aside from this results, 3 patients underwent surgical clipping for unruptured cerebral aneurysms. During this follow-up period, 5 patients had new onset of CVE(cerebral infarction: 1, cerebellar hemorrhage: 4) in Group C albeit carried out adrenalectomy. In comparison with MRI findings between Group A and B, we found no significant difference in the prevalence of Cerebral microbleeds (CMBs:5.8-9.3%) and High grade white matter hyperintensities (HWHs:43-50%). **Conclusion**: In this study, we could not demonstrate that the patients with PA associated with autonomous cortisol secretion had high incidence of CVE. Nevertheless, we demonstrate that the patients with APA had relatively high incidence of CVE.

Disclosures: M. Kudo: None. **S. Mugikura:** None. **R. Morimoto:** None. **Y. Tezuka:** None. **K. Omata:** None. **B. Shiratori:** None. **Y. Igarashi:** None. **K. Seiji:** None. **K. Takase:** None. **S. Ito:** None. **F. Satoh:** None.

Funding: No

Funding Component:

P334

Demographic, Risk Factors and Knowledge Profile of the Participants From a Vascular Disease Prevention Campaign - Red September

Authors: Maurício Mello Mendes Junior, Abraão Iuri Medeiros Angelim, Ana Carolina Lessa Neno, **Lais Felix da Cunha**, Lívia Pinto Almeida, Lílian Soares da Costa, UNESA, Rio de Janeiro, Brazil

Introduction: Studies have suggested beneficial effect of the continuous educational work in the prevention of chronic degenerative diseases. **Objective**: To describe the demographic, risk factors (RF) profile and knowledge of the participants of a Vascular Disease (VD) Prevention Campaign - Red September. **Method**: A cross-sectional descriptive

analysis of data collected through a form with questions about RF associated with VD and the subject's knowledgement about them. **Results**: In a total of 572 subjects (55% female), with a mean age of 43.7±16.4 years, the prevalence of RF for women and men respectively, was 29.1% x 28.3% for arterial hypertension (AH); 11.2% x 8.3% for diabetes mellitus; 15.5% x 13.3% for dyslipidemia; 55% x 65.1% for self-reports of anxiety and/or depression (A/D); 26.8% x 33.1% for family history of early VD and 7.6% x 6.7% of previous cardiovascular (CV) event. Dividing women by age group, the prevalence of AH, spontaneous report of A/D and insufficient physical activity found was, respectively, 5%, 36% and 63% for the group between 18-50 y/o; 50%, 52% and 65.5% for 51-70 y/o group, and 82.4%, 76.5% and 88.2% for women above 71 y/o. The participants' knowledge results reveal that 72% had a satisfactory answer on "what is an artery?", only 25% knew the doctor specialized in the care of VD, and 70.5% of the women and 84.4% of the men are unaware that fatigue may be a symptom. When questioned about which RFs are most related to VD, the first, second and third responses were, respectively: fatty diet (36.9%), inadequate diet (18.1 %) and sedentarism (19.3%). Despite of the knowledge of the causality of VD and CV presence, these RFs are significantly present in this population. **Conclusion**: The need for better strategies to reduce the discrepancy found in this sample is notorious, aimed at reducing the high percentage of modifiable RF that were evidenced in the analyzed population and have been already seen and published in previous studies.

Disclosures: M.M. Mendes Junior: None. **A.M. Angelim:** None. **A.L.B. Neno:** None. **L.F.T. da Cunha:** None. **L.P. Almeida:** None. **L.S. da Costa:** None.

Funding: No

Funding Component:

P335

Effect of Aerobic Walking Exercise on Cardiovascular Health in Non-Ambulatory Stroke Survivors, a Pilot Study

Authors: Abdulfattah S Alqahtani, Ramzi A Alajam, Sarah Eickmeyer, Wen Liu, Univ of Kansas Medical Ctr, Kansas City, KS

Background: Most of rehabilitation interventions in stroke rehabilitation are designed with a focus on improving the impaired sensorimotor function. However, around 75% of stroke survivors are prone to having cardiovascular diseases, the main cause of death in stroke survivors. Much less efforts have been made on how to control risks of cardiovascular diseases. Furthermore, past studies of aerobic exercise have involved only stroke survivors who could walk independently. Stroke survivors who were unable to walk were greatly ignored in past studies that measured risk of cardiovascular diseases. In this project, we examined the effect of aerobic walking exercise on cardiovascular risk factors in non-ambulatory stroke survivors using a treadmill and body weight support system. **Methods**: So far, we have completed a low intensity walking exercise program with body weight support system (30 minutes/session; three sessions/week for eight weeks) in three ischemic stroke survivors (two males, mean age 60.3±17.6 years). Blood pressure (BP) and resting heart rate (RHR) were measured pre- and post-intervention. **Results**: Pre- and post-intervention systolic BP decreased from 151.3±17.2 mmHg to 137.3±27.6 mmHg; diastolic BP decreased from 95±14.4 mmHg to 86±8.6 mmHg; RHR decreased from 74±19.3 beats/minute to 68±20.2 beats/minute, respectively. **Conclusion**: This is an ongoing study, and we are still recruiting study participants. The current results are promising and suggest that the aerobic walking exercise may decrease BP and RHR in non-ambulatory stroke survivors.

Disclosures: A.S. Alqahtani: None. **R.A. Alajam:** None. **S. Eickmeyer:** None. **W. Liu:** None.

Funding:

Funding Component:

P336

Efficacy and Safety of Vasodilator Beta-Blocker in Elderly Hypertensive Patients: Result From BEFIT-KOERA Study

Authors: Eun Joo Cho, Catholic Univ of Korea, Seoul, Korea, Republic of; Geu-Ru Hong, Yonsei Univ Severance hospital, Seoul, Korea, Republic of; YoungYoup Koh, Chosun Univ, Gwangju, Korea, Republic of; Dong Woon Jeon, Natl Health Insurance Service Ilsan Hosp, Ilsan, Korea, Republic of; In Hyun Jung, Inje Univ, Sanggye Paik Hosp, Seoul, Korea, Republic of; Min Kyoung Kang, Hallym Univ Kangnam Sacred Heart hospital, Seoul, Korea, Republic of; Young Mo Yang, Cheongju St. Mary's Hosp, Cheongju, Korea, Republic of; SangWon Park, A.Menarini Korea Ltd,, Seoul, Korea, Republic of; Jae Hyung Kim, Catholic Univ of Korea, Seoul, Korea, Republic of

Purpose: Beta-blockers are efficacious in young hypertensive patients with sympathetic activation. Elderly patients have been known to be at higher risk of hypotension, bradycardia, and other side-effects of beta-blockers when compared with younger patients. This study was to evaluate efficacy and safety of vasodilator beta-blocker in elderly hypertensive patients.
Methods: BENEFIT-KOREA study (BEnefits after 24 weeks of NEbivolol administration For essential hypertensIon patients wiTh various co-morbidities and treatment environments in KOREA) was a open labeled non-controlled, prospective, multicenter observational study enrolled total 3,140 subjects [1,871 male(59.6%), mean age=63.5±12.9 years old]. We evaluated efficacy and safety of vasodilator beta-blocker in elderly patients.
Results: Subjects of more than 65 years old [n=1,655, 839 male (49.3%), mean age=73.6±5.8] were analyzed. Table shows that vasodilator beta-blocker is effective in reduction of blood pressure, mean arterial pressure and pulse pressure in elderly patients regardless of add-on or switched to beta-blocker. Adverse drug reaction rate was not different between age group (n=19, 1.15% vs. n=17, 1.15%). Table shows lipid profile and body mass index were not aggravated after 24-week treatment of vasodilator beta-blocker.
Conclusion: Vasodilator beta-blocker is efficacious and safe without metabolic derangement in elderly patients regardless of prescription patterns. These drug effects in elderly are comparable to in younger patients.

	≥ 65 years old			< 65 years old		
	N=1,655			N=1,485		
	Baseline	24-week treatment	p	Baseline	24-week treatment	p
Systolic blood pressure (mmHg)	140.3±18.0	130.5±15.6	<0.0001	142.7±18.8	130.4±14.3	<0.0001
Diastolic blood pressure (mmHg)	79.2±11.9	73.5±10.7	<0.0001	86.9±13.6	79.0±10.7	<0.0001
Mean arterial pressure (mmHg)	99.5±12.5	92.5±10.9	<0.0001	105.5±14.4	96.1±11.1	<0.0001
Pulse pressure (mmHg)	61.2±14.6	57.1±13.2	<0.0001	55.8±12.5	51.4±10.3	<0.0001
Heart rate (BPM)	75.4±14.7	68.7±13.6	0.0002	77.0±14.4	69.5±11.6	<0.0001
Body mass index (kg/m^2)	25.1±3.4	25.4±3.5	0.7220	26.2±4.0	26.2±4.2	0.9342
Total cholesteol (mg/dl)	162.3±38.8	149.9±33.5	0.0057	176.4±47.4	153.9±34.0	<0.0001
Triglyceride (mg/dl)	136.1±76.1	143.4±84.0	0.6039	181.3±143.8	156.4±86	0.2185
LDL-cholesterol (mg/dl)	93.0±32.8	81.4±28.6	0.0020	110.3±38.3	87.6±29.6	<0.0001
HDL-cholesterol (mg/dl)	47.4±11.6	46.4±12.9	0.8964	45.2±11.1	46.8±11.9	0.6301

Disclosures: E. Cho: None. **G. Hong:** None. **Y. Koh:** None. **D. Jeon:** None. **I. Jung:** None. **M. Kang:** None. **Y. Yang:** None. **S. Park:** A. Employment; Modest; director. **J. Kim:** None.

Funding: No

Funding Component:

P338

Aging and Inhibition of Nox1/4 Modulate Perivascular Inflammation in Spontaneous Hypertension

Authors: Ryszard Nosalski, Tomasz Mikolajczyk, Univ of Glasgow, Glasgow, United Kingdom; Mateusz Siedlinski, Joanna Maciag, Jagiellonian Univ Collegium Medicum, Krakow, Poland; Tomasz Guzik, Univ of Glasgow, Glasgow, United Kingdom

INTRODUCTION: Hypertension is associated with enhanced oxidative stress and perivascular inflammation. Although, that aging and oxidative stress are major factors in the development of hypertension their effect on perivascular inflammation remains unclear. **METHODS:** Using flow cytometry we studied leukocytes infiltrating perivascular adipose tissue (PVAT) in 1-, 3-, 6- and 12-month-old SHR (Spontaneously Hypertensive Rats) and normotensive WKY (Wistar-Kyoto) rats. Additionally, 1-month-old rats were treated with GKT137831 (60mg/kg) or ML171 (NOX1/4 and NOX1 inhibitor, respectively) for 4 weeks. Blood pressure (PB) was measured by the tail cuff. **RESULTS:** Aging in SHRs was associated with elevation of BP (139±4 vs 180±4 vs 202±2 vs 208±2 mmHg; 1 vs 3 vs 6 vs 12-month-old, respectively) when this effect was not seen in WKY rats. While the total number of leukocytes infiltrating PVAT were comparable between 1-month-old WKY and SHR (p=0.8) aging escalated their numbers only in SHRs (2096±164 vs 1994±296 vs 2311±470 vs 3255±408 cell/mg; p^{int}<0.001). Similar effect was observed among NK cells (p^{int}<0.001) and macrophages (p^{int}<0.001). Moreover, spontaneous hypertension was associated with 2-fold elevation of T cells residing in PVAT in

comparison to WKY, however, aging did not affect their number in both groups. While the age-related increase of Nox4 mRNA was observed in both groups, this increase was more dynamic in SHRs, (p^{int}<0.05). Furthermore, 5-, 6- and 9-fold induction of Nox1 mRNA was observed in the vessels of 3-, 6- and 12-months-old SHRs, respectively (p<0.01). GKT137831 treatment significantly increased BP (p<0.01, 2way ANOVA) in both WKY and SHR (150±2 vs 164±3 mmHg, 198±4 vs 209±3 mmHg, respectively). This was accompanied by elevation of the total number of leukocytes (988±180 vs 1471±88 cell/mg, 1487±945 vs 1878±164 cell/mg) and macrophages (107±14 vs. 153±14 cell/mg, 228±26 vs. 298±42) in PVAT of WKYs and SHRs treated with GKT137831. On the contrary, ML171 treatment protected against increased accumulation of CD45+ cells in PVAT, without affecting BP.
CONCLUSIONS: Aging in spontaneous hypertension is associated with elevation of BP and aggravation of perivascular inflammation which are hastened after NOX1/4 inhibitor treatment.

Disclosures: R. Nosalski: None. **T. Mikolajczyk:** None. **M. Siedlinski:** None. **J. Maciag:** None. **T. Guzik:** None.

Funding:

Funding Component:

P340

Variable Pressor and Sympathetic Responsiveness Reflects Neurotransmitter Imbalance in the Paraventricular Nucleus that is Prevented by Loss of Renal Sensory Nerves

Authors: Mark M Knuepfer, St. Louis Univ Sch of Med, Saint Louis, MO; Hong Zheng, Xuefei Liu, Basic Biomedical Sciences, Vermillion, SD; Neeru M. Sharma, Kaushik P. Patel, Univ of Nebraska Medical Ctr, Omaha, NE

The paraventricular nucleus of the hypothalamus (PVN), a key regulator of sympathetic activity, is dependent on a balance of excitatory (NMDA) and inhibitory (nNOS) neurotransmission. Ischemic heart failure (HF), hypertension and renal disease are associated with excessive NMDA and reduced nNOS in the PVN. Individuals with essential hypertension have greater sympathetic tone and exaggerated pressor responsiveness to acute behavioral stress. We observed variable pressor responsiveness in Sprague-Dawley and Borderline Hypertensive rats (SDR and BHR). We hypothesized that those SDR or BHR with greater pressor responses to stress have greater NMDA and/or reduced nNOS activity in PVN. We proposed that renal denervation (RDN) or deafferentation resolves the difference in stress responses and the PVN imbalance. We recorded arterial pressure (AP), and, in some rats, cardiac output and sympathetic nerve activity. After recovery, SDR and BHR were exposed to acute stress (1 cm deep cold water) repeatedly. Average AP responses were greater in male BHR compared to SDR ($\dot{x}$=60±2 vs 49±1 mmHg). Responses were divided between those with AP responses greater or lesser than these means. SDR with larger pressor responses (LP), had elevated NMDA NR1 mRNA and protein levels in the PVN and reduced nNOS mRNA (p<0.05). Likewise, in female BHR, larger AP responses ($\dot{x}$=57±3 mmHg) LP had greater NMDA NR1 mRNA and protein in the PVN and reduced nNOS protein. Therefore, the imbalance in PVN exists in normotensive rats and correlates with greater AP and sympathetic responses predisposing an individual to cardiovascular disease. In related studies in male SDR and BHR, we examined the effects of RDN on acute stress responses to cold water and to air jet. In both rat strains, RDN or selective renal deafferentation with capsaicin or resiniferatoxin selectively prevented the exaggerated AP responses only in LP rats. Finally, RDN or deafferentation reversed the HF-induced increase in sympathetic nerve activity and the increase NADPH-diaphorase and nNOS staining and protein levels in the PVN. These data suggest that renal sensory nerves are responsible for variable excitatory and inhibitory influences in the PVN that contribute to the predisposition to cardiovascular disease.

Disclosures: M.M. Knuepfer: None. **H. Zheng:** None. **X. Liu:** None. **N.M. Sharma:** None. **K.P. Patel:** None.

Funding: No

Funding Component:

P341

Melanocortin Receptor 4 Activation Mediates Leptin-Induced Sensitization of the Angiotensin II-Elicited Hypertensive Response in Mice

Authors: Baojian Xue, Terry G. Beltz, Fang Guo, Alan Kim Johnson, Univ Iowa, Iowa City, IA

Obesity has been shown to promote the renin-angiotensin system (RAS) activity and inflammation in the hypothalamus that increase BP and sympathetic activity. Our previous studies using an **Induction-Delay-Expression** (IND-DEL-EXP) experimental design demonstrated that in the rats high-fat diet (HFD) or central leptin pretreatment resulted in an enhanced hypertensive response to subsequent treatment with angiotensin (ANG) II. The present study tested whether the leptin sensitization of ANG II-induced hypertension is mediated by activation of brain melanocortin receptor 4 (MC4R) in mice. The animals pretreated with either 3-week HFD or systemic leptin during IND responded with enhanced hypertension to ANG II (1000 ng/kg/min, sc.; HFD, Δ44.0±2.1 mmHg vs. Δ29.4±3.2 mmHg; leptin, Δ43.0±1.7 mmHg vs. Δ29.4±2.0 mmHg). This sensitized ANG II hypertensive response produced by systemic leptin given during IND was blocked by co-administration of icv SHU9119, a MCR4 antagonist (Δ24.0±2.9 mmHg). Moreover, a similar sensitization of the ANG II-induced hypertensive response was produced by icv pretreatment with MT II, a MC4R agonist (Δ39.6±2.1 mmHg). The results indicate that leptin-induced sensitization of ANG II-elicited hypertension is mediated by activation of brain MC4R.

Disclosures: B. Xue: None. **T.G. Beltz:** None. **F. Guo:** None. **A.K. Johnson:** None.

Funding: No

Funding Component:

P342

Management of Coexistent Neurogenic Orthostatic Hypotension and Supine Hypertension: A Case Study

Authors: David S Cannom, Good Samaritan Hosp, Los Angeles, CA

A 65-year-old woman presented for evaluation of syncopal spells, up to 5 per week, which typically occurred with assuming the upright posture and on one occasion led to a femoral fracture. Routine orthostatic blood pressure determinations revealed coexistent supine hypertension (SH) with a BP of 150/70 mmHg and neurogenic orthostatic hypotension (nOH), with BP that dropped to 70-80/50 mmHg upon standing associated with a 10-20 beat per minute increase in pulse rate. She was not on diuretics, was euvolemic, and had no evidence of systemic neurological disease or diabetes. A pacemaker was implanted, but it did not resolve her hemodynamic issues and she was admitted to the hospital for medical management of her condition. Losartan was initiated at 50 mg hs and titrated to 100 mg hs to manage the nocturnal SH. Midodrine was started at 2.5 mg TID and titrated to 20 mg TID to manage her nOH. This regimen reduced her orthostatic BP drop by 50 mmHg and relieved her symptoms of syncope, although occasional lightheadedness persisted. As an outpatient, her SH became more severe, reaching levels of 200 mmHg. The dose of midodrine was lowered to 10 mg TID, support stockings were recommended, and clonidine 0.1 to 0.2 mg hs was added to the nighttime regimen. However, this regimen resulted in a recurrence of syncope. She subsequently had a small hemorrhagic stroke, presumably due to the drug-induced hypertension. The patient was readmitted and midodrine was discontinued and replaced with droxidopa, which was titrated up to 300 mg TID. Droxidopa was well tolerated and reduced her orthostatic BP drops by 40 mmHg. She has now been stable with no reported syncopal spells or falls for 3 years. It appeared clinically that droxidopa had a longer half-life, with fewer alarming spikes in her SH than were noted with midodrine. Interestingly, although her presentation suggests OH associated with autonomic dysfunction, she has not demonstrated any primary neurological disease. Midodrine and droxidopa are approved to treat symptoms of orthostatic hypotension and nOH, respectively, and both carry the risk of SH. In this patient with coexisting nOH and

severe SH, improvement in nOH symptoms and alleviation of SH were achieved after treatment with droxidopa and a combination of losartan and clonidine hs.

Disclosures: D.S. Cannom: D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Medtronic. G. Consultant/Advisory Board; Modest; Medtronic, Boston Scientific, Lundbeck.

Funding: No

Funding Component:

P343

Utility of Autonomic Testing for the Diagnosis of Neurogenic Orthostatic Hypotension and Subsequent Management With Droxidopa

Authors: Sami B Alam, Waiel Almardini, Amer Suleman, The Heartbeat Clinic, McKinney, TX

A 62-year-old man with a history of Hodgkin lymphoma, lupus, type 2 diabetes mellitus, neuropathy, hypercholesterolemia, hypothyroidism, and orthostatic hypotension who was taking gabapentin, saxagliptin, pravastatin, levothyroxine sodium, omeprazole, prednisone, midodrine, and fludrocortisone was evaluated for syncope, collapse, and fluctuating blood pressure (BP). He reported a 2-year history of syncope, with progressive worsening over a 3-month period. Initially, his episodes of syncope were preceded by dizziness and nausea, but began to occur without warning. He also reported a 5-year history of orthostatic hypotension, with supine BP of 100/60 mmHg and standing BP of 50-60/40 mmHg. Treatment with midodrine (10 mg three times daily) and fludrocortisone (0.1 mg twice daily [BID]) did not relieve his symptoms. Initial cardiac evaluation revealed a systolic murmur, but was otherwise unremarkable. Autonomic function tests, including continuous electrocardiography, BP and heart rate monitoring at 2-min intervals, and transcranial Doppler, were performed in the supine and 80° head-up tilt positions. A BP drop from 112/68 mmHg to 76/60 mmHg occurred within 2 min of tilt, accompanied by dizziness and presyncope. Heart rate with deep breathing revealed a depressed baseline autonomic tone, and a Valsalva maneuver test showed a depressed Valsalva response (Valsalva ratio: 1.15). Taken together, these results indicated a diagnosis of neurogenic orthostatic hypotension (nOH). Treatment with droxidopa (100 mg BID) was initiated; this dose was titrated to 100 mg once daily due to hypertension, for which, a nitroglycerin patch (0.2 mg nightly) was prescribed. All other medications were continued. Treatment with droxidopa significantly improved his symptoms; he no longer loses consciousness upon positional change, but still experiences some symptoms of presyncope (eg, slight lightheadedness, dizziness). This has allowed greater integration into activities of daily living and a less strenuous routine for the patient and his caretakers. In conclusion, autonomic function testing in this patient effectively led to a timely diagnosis of nOH which, in turn, allowed for implementation of appropriate pharmacologic intervention to manage symptoms.

Disclosures: S.B. Alam: None. **W. Almardini:** None. **A. Suleman:** None.

Funding: No

Funding Component:

P344

Vascular Purinergic Neurotransmission is Not Altered in High Fat-Fed Dahl S Hypertension

Authors: Sutheera Sangsiri, Faculty of Med, Thammasat Univ, Pathumthani, Thailand; James J. Galligan, Neuroscience Program, Michigan State Univ, East-Lansing, MI

Mesenteric arteries (MAs) are supplied densely by sympathetic nerves releasing NE and ATP to increase vascular tone. Increased neuronal ATP/NE storage and release and impaired clearance cause hypertension. Our previous studies of DOCA-salt hypertensive rats showed that 1) impairment of the prejunctional autoreceptors regulating ATP and NE release contributes to elevated sympathetic neurotransmission, and 2) purinergic neurotransmission was impaired due to decreased ATP bioavailability in sympathetic nerves. As hyperactivity of sympathetic nerves occurs in obesity-related hypertension, we investigated alterations in purinergic transmission in high fat-fed (HF) Dahl S rats. MAs maintained *in vitro* were stimulated focally. Intracellular recording of excitatory junction potential (EJP) from smooth muscle cells was obtained from male and female, control and HF rats. EJP amplitude, facilitation and rundown were assessed. EJPs from all groups were abolished by tetrodotoxin (300 nM), a Na^+ channel blocker, and P2X receptor antagonist, PPADS (10 μM), indicating EJPs were neurogenic and purinergic. At 17 weeks, we found that 1) frequency (0.5-10Hz) response curves from control and HF were similar in male and female arteries. 2) When using short trains of stimulation (0.5Hz; 5 pulses), UK14304 (0.0001-1μM)-, an α2-adrenergic receptor agonist, blocked EJPs response equally well in arteries from control and HF rats. Moreover, the α-adrenergic receptor antagonist, yohimbine (1 μM), potentiated EJPs similarly in control and HF groups. 3) There were no differences in EJP rundown caused by trains of stimulation (10Hz, 50 pulses) in MA from control and HF fed rats. Differential facilitation of purinergic neurotransmission was detected in male *vs* female. In the presence of yohimbine, degree of EJP facilitation was higher in female arteries compared to male control arteries (2.69±0.26 *vs* 1.96±0.09 N=4, $p<0.05$) indicating higher prejunctional ATP storage in female nerves. However, HF did not affect the degree of facilitation. These data suggested that sympathetic neurovascular function is not affected in HF Dahl S rats. Elevated blood pressure in this animal model is not dependent on altered sympathetic neurovascular function.

Disclosures: S. Sangsiri: None. **J.J. Galligan:** None.

Funding:

Funding Component:

P345

The Cost-Effectiveness of Home BP Telemonitoring in Patients With a Cerebrovascular Event

Authors: Raj Padwal, Peter Wood, Helen So, Scott Klarenbach, Univ of Alberta, Edmonton, AB, Canada

Background: Home BP telemonitoring, with pharmacist case management, leads to clinically important BP reductions. Our objective was to determine the incremental cost-effectiveness of this intervention compared with usual care BP control in patients with cerebrovascular disease in Alberta, Canada. **Methods:** A cost-utility analysis using a Markov decision model was created, examining a cohort of high-risk patients with a recent cerebrovascular event residing in their own residence. A lifetime time horizon and health care payer perspective was used. Achieved BP and risk of future cardiovascular events (recurrent stroke, myocardial infarction, unstable angina, or death) were modelled, with attendant consequences on quality adjusted life years and costs. BP telemonitoring was assumed to occur monthly until BP was controlled, then quarterly. Canadian life tables were used to determine overall mortality, adjusted by CVD mortality. Relative efficacy on intervention-associated BP lowering were obtained from published data. Reduction in BP of 9.7/5.1 mmHg at 12-months was used in the base case. Resource use and costs were obtained from Canadian published literature. **Results**: Telemonitoring with case management led to net health care savings of $2326, and an additional 0.83 QALYs (see Table). Results were robust in sensitivity analysis (see Table). **Conclusion:** Home BP telemonitoring and pharmacist case management was a dominant strategy, as it lowered costs and improved QALYs, and should be implemented.

Parameters	Incremental Cost ($ Canadian)	Incremental Effectiveness (QALY)	Cost per QALY ($/QALY)
Base case	-1,676	0.826	Dominant
SBP reduction of 4.89 mmHg (base case 9.7 mmHg)	37	0.424	86
SBP reduction of 15 mmHg (base case 9.7 mmHg)	-3,424	1.218	Dominant
Double telemonitoring cost	950	0.826	1,151
Triple telemonitoring cost	3,577	0.826	4,331
Baseline risk decreased by 50%	-650	0.623	Dominant
Baseline risk increased by 50%	-1,714	0.861	Dominant

Disclosures: **R. Padwal:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Alberta Innovates. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; mmHg Inc. **P. Wood:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; mmHg Inc.. **H. So:** None. **S. Klarenbach:** None.

Funding: No

Funding Component:

P346

Impact of the Changing Guidelines in the Classification of Patients: Insights from a Real Life mHealth Digital Registry

Authors: Gaia Del Mauro, Tommaso Bordignon, Martina Fabi, Eros Colombo, Amicomed Inc, San Francisco, CA; **Domenico Cianflone**, Univ Vita-Salute San Raffaele, Milano, Italy

Background:The recent updated AHA/ACC guidelines have spiked academic discussions on the changing prevalence of hypertensive subjects with the new thresholds. **Aim**:To determine the changes in classification (hypertensive or not) on a real life and spontaneous mHealth digital registry, via mobile App and website. **Population and Methods**:7956 consecutive subjects (71% males; 53±11 years old) subscribed, spontaneously, a real-time BP -recording, - interpretation and -trend evaluation service (via smartphone-Health App and website platforms). Thru the digital platforms, each BP value, after consistency-checks, feeds a proprietary algorithm providing both individual BP value interpretation in comparison to the previous BP history, and BP trend and fluctuations assessments. These latter are fed back to users on their smartphone and computer screens instantly. The service complies with the most strict data privacy, safety and security requirements. **Results**:We collected 176432 BP measurements, overall. BP were optimal according to the AHA-ACC guidelines values (<=120/80mmHg) were in 29% of instances, while either only the systolic or the diastolic BP values were optimal in 53% of instances. Conversely, according to the most commonly used normal thresholds of BP <=130/85mmHg 63% of both systolic and diastolic BP values were normal, while either only the systolic or the diastolic BP values were optimal in 39% of instances. **Conclusion**: In a population of subjects, who subscribed a digital remote BP monitoring and evaluation service because they are so aware of the need of controlling their BP, the change of guidelines threshold for optimal and event reducing BP doubles the impact of non-controlled BP. This registry highlights the wide gaps still existing in achieving target blood pressure levels in real-life subjects, even in the most aware hypertensive subjects.

Disclosures: **G. Del Mauro:** A. Employment; Modest; AMICOMED. **T. Bordignon:** A. Employment; Modest; AMICOMED EUROPE SA. **M. Fabi:** A. Employment; Modest; AMICOMED EUROPE SA. **E. Colombo:** A. Employment; Modest; AMICOMED EUROPE SA. **D. Cianflone:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; Co-Founder, Chief Scientific Officer Amicomed.

Funding: No

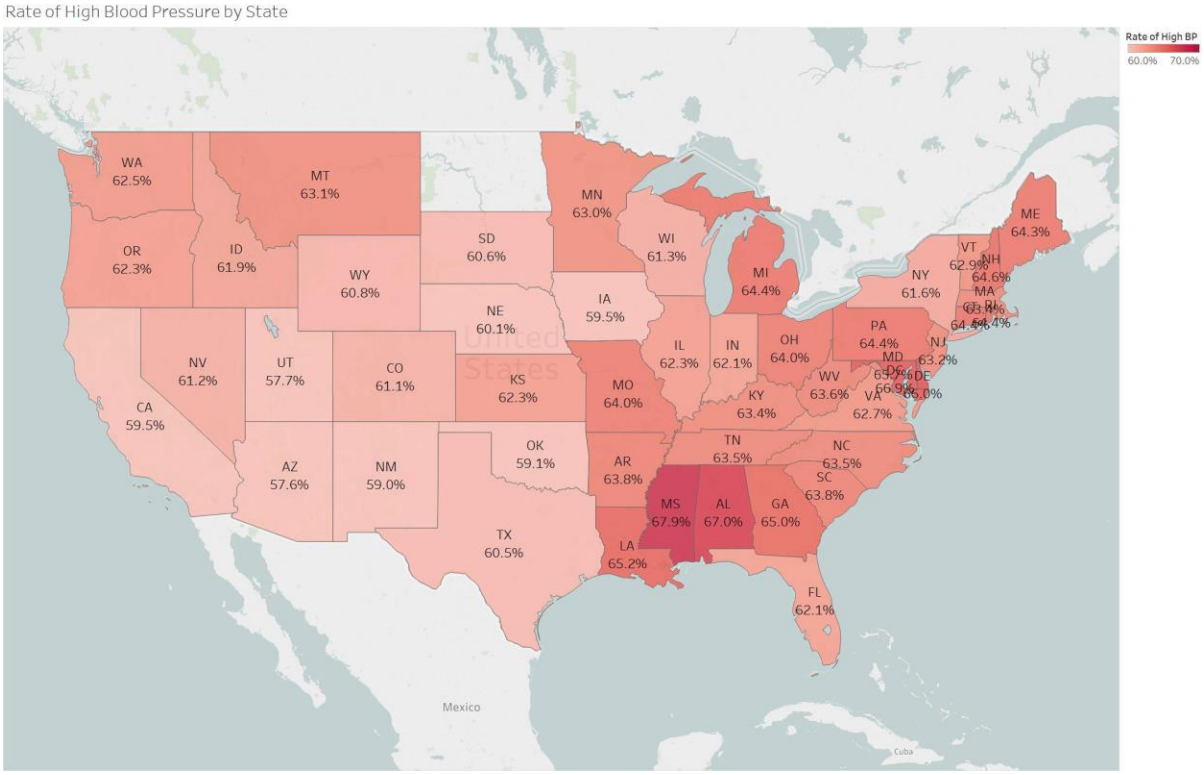
Funding Component:

P347

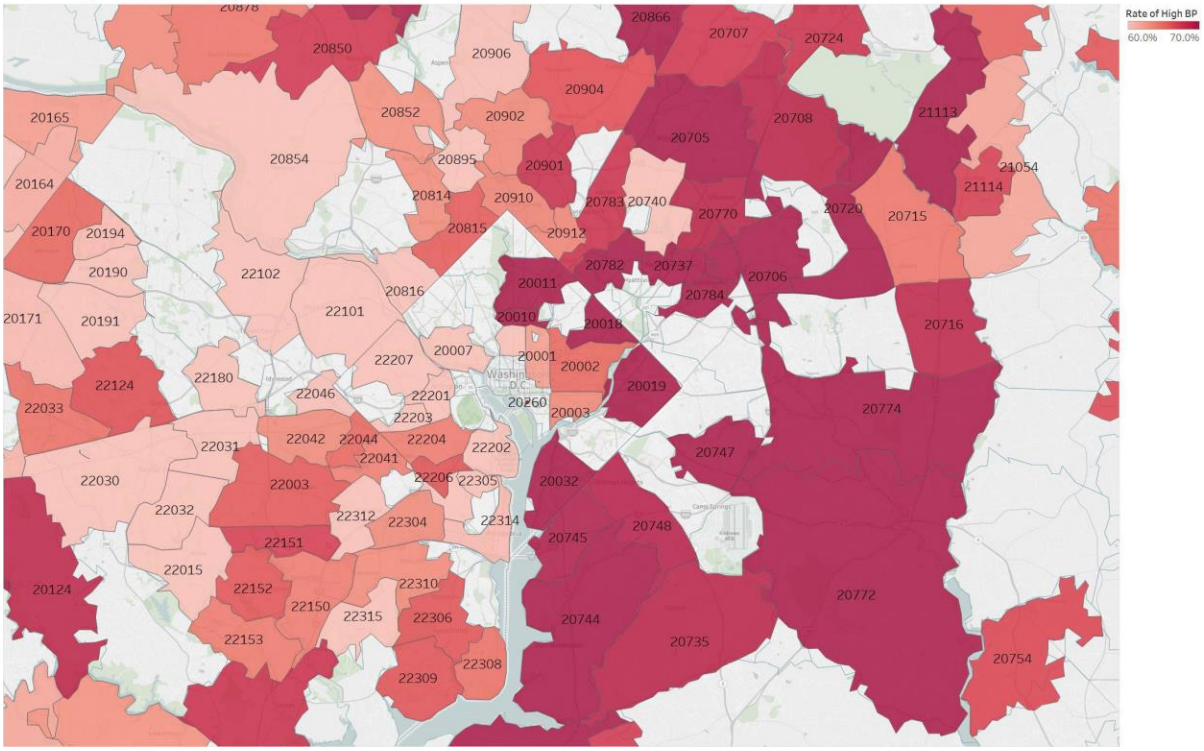
How Did Another 30% of Americans Develop High Blood Pressure Overnight? An Analysis of the New AHA/ACC Blood Pressure Guidelines and Their Impact in Communities Across the Country

Authors: Khan Siddiqui, Johns Hopkins Univ Sch of Med; Higi, Chicago, IL; **Ross Goglia**, Nikole Wiley, Daniel Neems, Higi, Chicago, IL

According to data from retail blood pressure kiosks, almost one-third of Americans just moved into the hypertensive category, roughly doubling its size. National costs of hypertension treatment were already estimated at $40-50B, so understanding the detailed impact of this development is important for healthcare providers, payors, and policymakers alike. We leverage the 42,614,330 blood pressure readings that took place across the national network of 11K+ higi health kiosks in 2017 to study the effect of the new guidelines on both macro and micro (i.e. zip code) levels, and within sub-populations of interest. We find that new blood pressure guidelines do not impact all states, or all communities within a given metro area, equally. (It's also not the case that size of impact positively correlates with rate of high blood pressure under the old guidelines - i.e. healthy populations often see greater impact.) Furthermore, the guidelines affect certain cohorts of patients differently than others. This study identifies the communities and cohorts that pose the blood pressure greatest risk post-2017.



Rate of High Blood Pressure by Zip Code



Disclosures: **K. Siddiqui:** None. **R. Goglia:** None. **N. Wiley:** None. **D. Neems:** None.

Funding: No

Funding Component:

P348

Evaluation of Check. Change. Control. in the Asian American & Pacific Islander Community

Authors: Cevadne Lee, Sang-Mi Oh, American Heart Association, Los Angeles, CA

Background: Heart Disease is the #1 cause of death and stroke is #3 for Asian Americans and Pacific Islanders (AAPI) 65 years and older. Approx. 21% of deaths among certain AAPIs are due to high blood pressure (HBP). In response, the Western States Affiliate (WSA)-American Heart Association (AHA) adapted our evidence-based self-monitoring community program to reduce and control HBP with AAPI senior citizens. Blood pressure (BP) awareness, treatment and control remains suboptimal among US adults with hypertension. Objectives: We evaluated the efficacy, acceptability, and cultural relevancy among Chinese, Korean, Vietnamese, Pacific Islander older adults in California and Washington. Methods: We collaborated with 4 community-based AAPI organizations and recruited 800+ AAPI senior citizens. We used a multi-pronged evaluation approach including pre and post surveys to determine existing lifestyle partners and readiness to change, health assessments to identify risk factors, monthly self-monitoring blood readings, and aggregated biometric data to demonstrate progress towards control and HBP reduction. Results: At baseline, more than 30% of participants experienced high blood pressure. There were significant reductions in both SBP and DBP overall and especially among those with elevated BP at baseline. SBP drop changes varied by site and by ethnic group from baseline to final visit. Each site implemented adapted self- monitoring program for cultural relevancy and readiness level. Conclusion: Adapting our self-monitoring program had a significant impact in reducing BP for AAPI participants, especially among those with elevated BP at baseline. Further study is needed across multiple sites and AAPI ethnicities.

Disclosures: **C. Lee:** A. Employment; Significant; American Heart Association. **S. Oh:** A. Employment; Significant; American Heart Association.

Funding: Yes

Funding Component: Western States Affiliate (California, Nevada & Utah)

P349

Establishing Correction Factors for Oscar and SpaceLabs 90207 Oscillometric Ambulatory Blood Pressure Monitors

Authors: Vincent Patteson Lombardi, Patrick C Reichhold, Nicholas R. Dietz, Natalie J. DeBell, Donald L. Pate, Univ of Oregon, Eugene, OR

PURPOSE & BACKGROUND: To obtain a more accurate picture of diurnal variations in blood pressure (BP), we establish correction factors based on differences between mercury (Hg) column & 24-hr ambulatory blood pressure (ABPM) measurements in the lab. In normotensives, hypertensives & alcoholics, an *auscultatory* ABPM underestimated diastolic BP (DBP) & misclassified DBPs in > 75% of cases. Our goal was to explore *oscillometric* ABPMs accuracy & reliability. **METHODS**: In the lab, simultaneous opposite arm BPs by the Oscar 2 and SpaceLabs 90207 ABPMs & simultaneous same arm BPs by observers (O1, O2) using a ThinkLabs digital electronic stethoscope with ambient noise reduction, were obtained in 5 subjects seated with arms at the phlebostatic axis. Postural tests included simultaneous, same arm Hg BPs by two observers & 45 sequential recordings by each ABPMs (SBP range 92-183; DBP range 30-124 mm Hg). **RESULTS**: Baseline SBPs for O1, O2, Oscar & SpaceLabs were 112.8 ± 22.3, 114.0 ± 23.4 (O1 - O2 Δ = - 1.2), 123.7 ± 23.4 & 119.0 ± 19.4 (Oscar - SpaceLabs Δ 4.7) mm Hg, respectively. The Oscar overestimated O1O2 SBPs by 10.3 mm Hg ($P < 0.05$). DBPs for O1, O2, Oscar & SpaceLabs were 61.7 ± 22.2, 62.4 ± 22.4 (O1 - O2 Δ = -0.7), 66.5 ± 16.6 & 67.4 ± 15.7 (Oscar - SpaceLabs Δ -0.9) mm Hg, respectively. From postural tests, average SBP/DBP correction factors were -4.5/-3.2 mm Hg for the Oscar and -1.3/-2.4 mm Hg for the SpaceLabs, but with extreme variation from -25 to +16 mm Hg based on supine, seated, standing and normotensive vs. hypertensive conditions. The Oscar significantly ($P <0.01$) overestimated SpaceLabs 24-hr SBP (154 ± 11 vs 146 ± 10, Δ = 8 mm Hg) in a hypertensive, but overestimations were less pronounced for 24-hr DBP (101 ± 9 vs 98 ± 8, Δ 3 mm Hg). In a normotensive, SBP & DBP corrections for 24-hr ABPMs were less clinically significant, except during sleep. **CONCLUSIONS**: Our results though limited, indicate that recordings obtained may depend entirely upon the ABPM selected and the BP range and postures assumed by the subject over 24 hr, demanding further investigation and assessment of postural BPs. Unless correction factors are developed on an individual basis and subjects happen to match the population from which oscillometric nomograms were developed, the accuracy of 24-hr ABPMs should be questioned.

Disclosures: **V.P. Lombardi:** None. **P.C. Reichhold:** None. **N.R. Dietz:** None. **N.J. DeBell:** None. **D.L. Pate:** None.

Funding: No

Funding Component:

P350

Early Signs of Stress Induced Arterial Hypertension During Cardiopulmonary Testing and Advantages in Cardiac Risk Lowering

Authors: Elena A Kolesnikova, Gregory P Arutyunov, Anna Rylova, Pirogov Russian Natl Res Medical Univ, Moscow, Russian Federation

Goal: to study early signs and hypertension reaction features during cardiopulmonary exercise testing (CPET) in intellectually working men with rare episodes of stress induced blood pressure (BP) increase.Methods: 47 patients (pats) with periodic episodes of high BP due to high level of stress were included (all men, 42 ± 4.6 years old). Risk factors: smoking had 83% of pats, high cholesterol level 87%, glucose intolerance 39%, family history of high BP 14%. All pats had normal BMI and no signs of visceral fat and target organ damage, 54% used hypotensive therapy non-systemically, 98% complained for sleep disorders. CPET was done for optimal training regime appointment. Baseline BP was 134± 2,8 mm Hg. Most of the pats demonstrated low physical tolerance level (6,7±0.4 MET). All patients demonstrated hypertensive reaction during test, and that was the reason for test stop. 68% of pats demonstrated systolic BP 220 mm Hg at respiratory compensation point (RCP) and RER 1,17 ± 0.01, 32% of pats demonstrated it earlier than RCP and at RER 0.85± 0.06. All pats got recommendations with optimal physical and pulse regime for training. All the patients had 3 ± 1 moderate intensive aerobic training per week for 34 ± 10.7 min .Results: in 6 months all the patients demonstrated increase in physical tolerance (ΔVO2 5,3 ±1,3 ml/kg/min). Patients with hypertension reaction at RCP showed significantly lower baseline BP (124.6± 3.5mm Hg vs 131.2 ± 4.1 mm Hg in other patients, p <0.05), only 14% demonstrated hypertensive reaction during CPET vs 89% in other patients, p <0.05 and mentioned less hypertension episodes during 6 months (7 ± 3 episodes vs 24 ± 2, p <0.05). 96% pats of this group demonstrated higher level of physical tolerance (load 9,8 ± 0.3 MET vs 7.6± 1.1 MET, p <0,05). Also, they didn't show abnormalities in glucose intolerance test. Conclusion: hypertension reaction at RCP during CPET in intellectually working man with episodic stress induced increase of blood pressure can be a predictor of great benefit in life style modification, cardiac risk lowering and arterial hypertension control

Disclosures: E.A. Kolesnikova: None. **G.P. Arutyunov:** None. **A. Rylova:** None.

Funding: No

Funding Component:

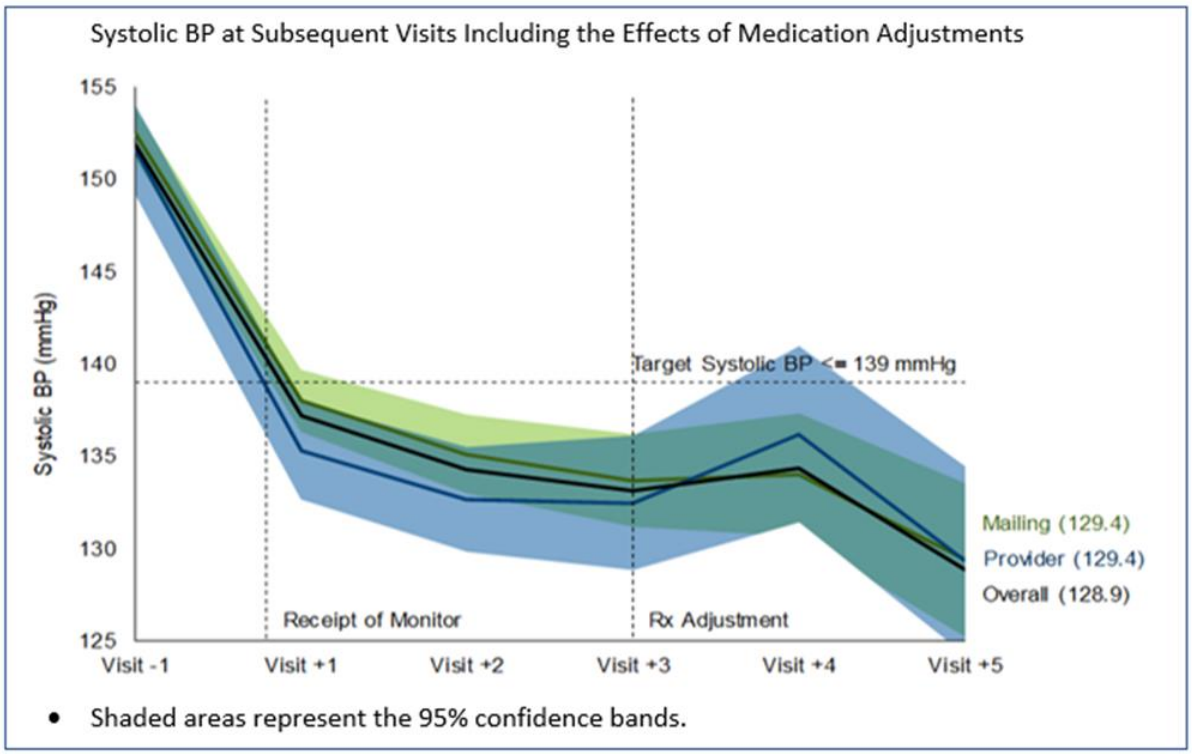
P351

Improving the Management of Hypertension for Adults: The Impact of Home Blood Pressure Monitoring and Lessons Learned from a System-Level Hypertension Control Initiative

Authors: Roy R Champion Jr., Zachary Rice, Emran Rouf, Scott & White Health Plan, Temple, TX

Purpose: To examine the effects of a pragmatic HBPM program on improving HTN control.
Methods: SWHP commercial and Medicare members 18-85 y/o with persistently uncontrolled HTN were given a free home BP monitor and related educational tools to effective engage in and document HBPM via 3 separate cohorts:
- Provider distribution during an office visit
- Based on medical record reviews, mailed to the patient with PCP's permission
- Patient's request at various health fairs

Using the office-based BP readings in the EHR, we calculated the change in BP control for each subsequent visit and end of year.
Results: From Sept 2016 through May 2017, with no undue burden placed on providers or their staff, 2550 members received the tools necessary to engage in HBPM. By the 3rd office visit, 66.79% [±3.7%; 95% CI] achieved office-based BP readings of less than 140/90 mmHg. It was also noted that by the 3rd office visit many providers were able to reduce the patient's medications and still maintain control.



The retrospective review, like that of previous studies, found HTN control rates continued to climb with 79.03% (78.18%-88.57%) [± 3.7%; 95%CI] achieving HTN control per HEDIS 2018 standards. The average sustained change in BP control was an 18.3 mmHg (11.9-19.0 mmHg) reduction in SBP and a 7.0 mmHg (4.2-9.5 mmHg) reduction in DBP. It is also worth noting 72.6% (71.1-77.14%) [± 3.7%; 95%CI] achieved HTN control per the new AHA/ACC guidelines.
Conclusions: Clinical guidelines recommend HBPM to evaluate and treat HTN. Our HTN Control Initiative supports a multi-modality approach as being cost-effective and sets the groundwork for further QI efforts to better discern each process regarding the use of HBPM.

Disclosures: R.R. Champion: None. **Z. Rice:** None. **E. Rouf:** None.

Funding:

Funding Component:

P352

The Quality of Blood Pressure Measurement in the Hemodialysis Unit

Authors: Jesse Bittman V6R 1W7, Univ of British Columbia, Vancouver, BC, Canada; Thuy Pham, Mari Sarian, Sheldon Tobe, Sunnybrook Health Sciences Ctr, Toronto, ON, Canada

Objectives: The association of intradialytic blood pressure with interdialytic blood pressure and clinic outcomes is inconsistent. Poor technique of routine blood pressure measurement in the hemodialysis unit may contribute to this inconsistency. We aim to assess adherence to current blood pressure measurement standards in the hemodialysis unit.
Methods: We assessed a convenience sample of 79 hemodialysis unit patient visits for adherence to five blood pressure measurements standards, per Hypertension Canada guidelines. The frequency of errors in blood pressure measurement was assessed by descriptive statistics.
Results: In 79 blood pressure measurements only 6% had no errors in measurement technique across the five standards we assessed. The most common errors were cuff position (29% correct) and patient position (29% correct), while the

upper arm was appropriately chosen in 94% of patients.
Conclusion: Errors in blood pressure measurement technique were common and may contribute to inaccuracy in blood pressure measurement.

Disclosures: J. Bittman: None. **T. Pham:** None. **M. Sarian:** None. **S. Tobe:** None.

Funding:

Funding Component:

P353

Comparability of Oscillometric to Simultaneous Auscultatory Blood Pressure Measurement in Children

Authors: Jennifer S Ringrose, Abdullah Alabbas, Afrooz Jalali, Harsimran Khinda, Catherine Morgan, Verna Yiu, R. Todd Alexander, Raj Padwal, Univ of Alberta, Edmonton, AB, Canada

Objective: Uncertainty exists regarding the accuracy of automated blood pressure (BP) measurement in children. We recorded oscillometric waveforms in children, derived oscillometric BPs using two standard algorithms, and compared the results to simultaneous auscultation.**Methods:** Twenty children aged 2-12 years were recruited from a tertiary-care Pediatric Nephrology Clinic. Sex, height, weight, arm circumference, history of hypertension and clinic BP were recorded. Two, simultaneously measured, oscillometric and auscultatory BP readings were obtained 30-60 seconds apart. The first reading was discarded and, the second, used for analyses. Fixed-ratio and slope-based algorithms were used for BP derivation.**Results:** Mean age was 7.95 ± 2.82 years, 40% were female, mean arm circumference was 21.86 ± 4.06 cm, and 50% had hypertension or a history of hypertension. Mean auscultatory BP for all participants (systolic ± SD/diastolic ± SD) was 93.40 ± 11.80/50.50 ± 9.04 mmHg, oscillometric fixed-ratio BP was 99.20 ± 11.90/57.35 ± 7.15 mmHg and oscillometric slope-based algorithm was 91.60 ± 13.94/60.65 ± 7.71 mmHg. Compared to auscultation, the fixed-ratio method differed by 5.80 ± 12.72/6.85 ± 7.51 mmHg ($p=.06$ and $p<.01$) and the slope-based method differed by -1.80 ± 13.59/10.15 ± 8.07 mmHg ($p=0.56$ and $p<.01$). Differences from auscultation were statistically significant for diastolic BP with both fixed-ratio and slope-based methods for all age categories but of greatest magnitude in the youngest children. **Conclusions:** Oscillometric BP derived using two commonly used algorithms differed by more than 5 mmHg in either SBP or DBP from simultaneous auscultatory BP in children aged 2-11. These findings emphasize the need for greater understanding of the functionality and accuracy of oscillometry in children.

Disclosures: J.S. Ringrose: F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; Start up company that will manufacture BP monitors. **A. Alabbas:** None. **A. Jalali:** None. **H. Khinda:** None. **C. Morgan:** None. **V. Yiu:** None. **R.T. Alexander:** None. **R. Padwal:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; Owner of start up company that will manufacture BP monitors.

Funding:

Funding Component:

P354

Performance of Different Oscillometric Blood Pressure Algorithms in Patients with Chronic Kidney Disease

Authors: Jennifer S. Ringrose, Kevan Smith, Afrooz Jalali, Harsimran Khinda, Isaac Wirzba, Hannah Eche-Ameh, Aminu K Bello, Branko Braam, Raj Padwal, Univ of Alberta, Edmonton, AB, Canada

Objective: The extent to which different oscillometric blood pressure (BP) algorithms differ in how they derive BP in patients with chronic kidney disease (CKD) is unknown. We compared the performance of three different oscillometric algorithms against a known oscillometric reference standard in this patient population. **Methods:** Thirty intermittent hemodialysis (HD) patients and 30 stage 3 CKD (CKD) patients were recruited from a quaternary care, academic hospital in Edmonton, Canada. In random order, three sequential readings with an Omron HEM 907XL device and three sequential readings with a laptop-driven oscillometric device capable of detecting and recording oscillometric waveforms were obtained 30 seconds apart. The mean of each three reading set was used for analyses. Oscillometric algorithms (two fixed-ratio and one slope- based) were applied to the raw oscillometric data to derive BP. Paired t-tests were used to assess for statistical significance at the 0.05 level. **Results:** Mean age was 63.4 ± 16.3 y (HD group) and 65.9 ± 10.7 y (CKD group); percent female was 37% (HD) and 47% (CKD); mean BMI was 28.3 kg/m^2(HD) and 30.3 kg/m^2(CKD). Over 80% of participants in each group had hypertension. BP comparisons are summarized in Table 1. **Conclusions:** Varying the type of oscillometric algorithm results in markedly different systolic BP estimates in patients with CKD. The fixed-ratio algorithm produced results most comparable to the Omron device. These findings help clarify why different devices using different algorithms produce different results in patients with CKD.

Table 1. Blood Pressure Comparisons

	Hemodialysis (n = 30)		Stage 3 Chronic Kidney Disease (n= 30)	
Systolic Blood Pressure	**Mean ± SD**	**Difference versus Omron Device (p-value)**	**Mean ± SD**	**Difference versus Omron Device (p-value)**
Omron Device	136.9 ± 25.4	-	136.7 ± 21.1	-
Fixed-ratio Gaussian envelope	134.6 ± 23.5	-2.3 ± 8.8 (0.16)	135.7 ± 17.4	-1.0 ± 10.8 (0.63)
Fixed-ratio smoothened envelope	135.0 ± 23.4	-1.9 ± 7.8 (0.19)	137.4 ± 17.7	0.7 ± 10.8 (0.73)
Slope-based	124.2 ± 23.0	-12.8 ± 10.2 (< 0.01)	127.0 ± 17.4	-9.7 ± 12.1 (< 0.01)
Diastolic Blood Pressure	**Mean ± SD**	**Difference versus Omron Device (p-value)**	**Mean ± SD**	**Difference versus Omron Device (p-value)**
Omron Device Mean ± SD	72.7 ± 15.8	-	73.1 ± 10.0	-
Fixed-ratio Gaussian envelope Mean ± SD	75.0 ± 16.7	2.3 ± 10.2 (0.22)	76.4 ± 11.0	3.3 ± 5.7 (0.01)
Fixed-ratio smoothened envelope Mean ± SD	72.6 ± 14.7	-0.1 ± 6.9 (0.95)	75.1 ± 10.4	2.0 ± 5.2 (0.04)
Slope-based Mean ± SD	74.4 ± 17.4	1.7 ± 9.8 (0.36)	75.3 ± 13.7	2.2 ± 8.2 (0.15)

Disclosures: **J.S. Ringrose:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; Ownership in start up company that will manufacture BP devices. **K. Smith:** None. **A. Jalali:** None. **H. Khinda:** None. **I. Wirzba:** None. **H. Eche-Ameh:** None. **A.K. Bello:** None. **B. Braam:** None. **R. Padwal:** F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; Owner of start-up company that will manufacture BP devices.

Funding:

Funding Component:

P355

Is Knowledge of Proper Technique a Barrier to Accurate Blood Pressure Measurement in the Hemodialysis Unit?

Authors: Jesse Bittman V6R 1W7, Univ of British Columbia, Vancouver, BC, Canada; Thuy Pham, Mari Sarian, Sheldon Tobe, Sunnybrook Health Sciences Ctr, Toronto, ON, Canada

Objectives: Hypertension and hypotension are associated with adverse outcomes in hemodialysis patients. However, routine blood pressure measurement in the hemodialysis unit has poor correlation with interdialytic blood pressure and clinical outcomes. Poor measurement technique in routine practice may contribute to this poor correlation. We aim to assess hemodialysis unit nurses' knowledge of proper blood pressure measurement technique.
Methods: All hemodialysis unit nurses were asked to anonymously answer 12 multiple-choice questions on proper blood pressure measurement technique, based on Hypertension Canada guidelines. Frequency of incorrect responses were used to gauge gaps in knowledge.
Results: 73 hemodialysis nurses completed the survey with an average score of 9.5/12 (SD 1.4). Only four respondents answered all questions correctly. The most frequent errors were regarding patient position during measurement. 96% of respondents graded their confidence in their blood pressure measurement technique as ≥4/5.
Conclusion: Despite routine use and high confidence, there are gaps in knowledge of proper blood pressure measurement technique.

Disclosures: J. Bittman: None. **T. Pham:** None. **M. Sarian:** None. **S. Tobe:** None.

Funding:

Funding Component:

P356

Community Hypertensive Control is Improved by Pharmacists in a Closed-Loop Digital Healthcare Ecosystem: A Real World Study of Large Samples

Authors: Yingjie Li, Ying Chen, Dongxing Li, Zhen Wang, Hongguang Zhang, **Kai Liu**, CareLinker Co., Ltd., Shanghai, China

Objective: To evaluate the effects of pharmacist-led digital care for hypertension management at retail pharmacies in China. Methods: For the past 3.5 years we have been developing and operating a digital healthcare ecosystem that formed a closed-loop of healthcare delivery by pharmacists to hypertensive patients at drug stores. The system included a set of BP tele-monitor, an App and a cloud engine that ran 5 proprietary algorithms to support personalized lifestyle coaching and medication guidance. Between Jan 1st 2015 and Apr 30th 2018, we served 2,683,865 walk-in patients in 8,730 pharmacies in 199 cities, and 54,032 of them were selected in this report 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. Results: The study population demonstrated an average reduction of SBP by 12.2 mm Hg (baseline: 152.0 ± 14.4 mm Hg, last time: 139.8 ± 19.2 mm Hg, P< 0.001) and of DBP by 6.8 mm Hg (baseline: 89.7 ± 11.7 mm Hg, last time: 82.9 ± 12.7 mm Hg, P<0.001). Among them, 31,493 (58.3%) patients had improved hypertension stages with 24,326 (45.0%) of them returning to the normal BP range. The first time health intervention had the strongest BP control effect, accounting for 81.6% reduction of SBP and 78.0% reduction of DBP over time. Linear simulation revealed 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, $y=141.222+9.613x^{-1}-0.025x$ ($R^2=0.835$); DBP, $y = 83.450+5.419x^{-1/2}-0.015x$ ($R^2=0.911$). Conclusion: Pharmacists are valuable resources in a closed-loop digital healthcare setup to improve community hypertensive control.

Disclosures: **Y. Li:** None. **Y. Chen:** None. **D. Li:** None. **Z. Wang:** None. **H. Zhang:** None. **K. Liu:** None.

Funding: No

Funding Component:

P357

A Pharmacy-Based Software as a Service (SaaS) Approach Improved Medication Adherence of Hypertensive Patients

Authors: Yingjie Li, Ying Chen, Dongxing Li, Zhen Wang, Hongguang Zhang, **Kai Liu**, CareLinker Co., Ltd., Shanghai, China

Objective: To enhance medication adherence of hypertensive patients by connecting them to a cloud healthcare knowledge platform which was synchronized to the pharmacy Enterprise Resource Planning (ERP) system. Methods: A cloud engine was developed to house a continuously growing knowledge database of 6,000 drugs. This database consisted of 5 different categories of text messages to provide educational materials for each drug's 1) indication and dosage, 2) precautions and side effects, 3) relevant disease information, 4) relevant lab test interpretation, and 5) medication reminders. This cloud solution was offered as SaaS by connecting to pharmacies' ERP, thus to identify the patients each time when they filled prescriptions within the drug store, triggering auto-sent text messages at dosage-adjusted intervals to the patients. During the past 12 months, 18,362 patients at 937 pharmacies in 13 cities in China received this intervention and medication adherence was measured by Proportion of Days Covered (PDC). Results: The study population demonstrated an improved overall PDC from 51.4% at baseline to 61.7% at last time, P<0.001. We further analyzed PDC changes by medications of use and intervention durations (Table 1 A&B), and found a cross-board enhancement of PDC even though a gradual reducing trend of PDC seemed to appear over time. Conclusion: The pharmacy SaaS offered an efficient way to auto-deliver text knowledge tailored to each prescription, leading to increased medication adherence of hypertensive patients.

Table 1A: Changes of PDC by medications of use

Medication	# Patients	PDC (%, SE), Baseline	PDC (%, SE), Last Time	Diff. (Mean, SE)	Paired-Samples t test
Calcium Channel Blockers	12,704	56.1%±0.3%	67.3%±0.3%	11.1%±0.3%	P<0.001
Beta Blockers	3,265	30.2%±0.4%	36.2%±0.5%	6.0%±0.4%	P<0.001
ARB and ACEI	1,645	56.1%±0.8%	69.8%±0.8%	13.7%±0.8%	P<0.001
Combination (> 1 drug)	748	53.2%±1.0%	60.9%±1.0%	7.7%±0.9%	P<0.001

Table 1B: Changes of PDC by intervention durations

Intervention Duration Month	# Patients	PDC (%, SE), Baseline	PDC (%, SE), Last Time	Diff. (Mean, SE)	Paired-Samples t test
1	613	72.2%±1.4%	91.5%±0.8%	19.4%±1.3%	P<0.001
2	2,520	65.7%±0.6%	82.9%±0.5%	17.3%±0.6%	P<0.001
3	2,619	55.6%±0.6%	70.8%±0.6%	15.2%±0.6%	P<0.001
4	2,649	46.4%±0.6%	58.3%±0.6%	11.9%±0.6%	P<0.001
5	2,253	48.3%±0.7%	57.1%±0.7%	8.8%±0.6%	P<0.001
6	2,247	46.1%±0.7%	52.6%±0.7%	6.5%±0.6%	P<0.001
7	2,120	53.6%±0.7%	58.8%±0.7%	5.2%±0.6%	P<0.001
8	776	37.4%±1.1%	47.3%±1.2%	9.8%±1.0%	P<0.001
9	904	37.4%±0.9%	45.1%±1.0%	7.6%±0.9%	P<0.001
10	825	41.6%±1.0%	46.1%±1.1%	4.5%±0.9%	P<0.001
11	720	49.8%±1.1%	48.8%±1.2%	-0.9%±1.0%	p=0.360
12	116	56.1%±2.7%	52.9%±2.9%	-3.2%±2.6%	p=0.222

Disclosures: **Y. Li:** None. **Y. Chen:** None. **D. Li:** None. **Z. Wang:** None. **H. Zhang:** None. **K. Liu:** None.

Funding: No

Funding Component:

P358

Improvement in Antihypertensive Medication Adherence and Persistence Using a Mobile Technology Application

Authors: Catherine McGuiness, IQVIA, Plymouth meeting, PA; **Jon Michaeli**, Medisafe, Boston, MA; Jennifer Millard, Xin Wang, Rolin L. Wade, IQVIA, Plymouth meeting, PA

Background: The high costs and negative outcomes associated with poor medication adherence are well established. This study evaluated antihypertensive adherence and persistence in patients using the Medisafe personalized medication management application (app) compared to a matched control group. **Methods:** Patients installing the app entering an antihypertensive medication (users) between 1/1/2014-12/31/2017 (index date) were anonymously linked to IQVIA's longitudinal prescription claims (LRx) database and matched 1:3 to non-app users (controls) with prescription claims for antihypertensive therapy in the same month as the app user; further matched on age (+/- 3 years), gender, geographic region, and categorical number of pre-index antihypertensive therapy claims (0-5, 6-11, ≥ 12). All patients had ≥ 2 prescription claims pre-index and ≥ 3 claims post-index, while pre- and post-index periods were equalized for app users and controls. Adherence was measured during the persistent period in LRx via medication possession ratio (MPR) during the pre-index and post-index periods established for both groups. Persistence was defined as no gaps in therapy >60 days. Differences in MPR change between app users and controls were tested using difference in differences (DID) models. Persistence was evaluated using Kaplan-Meyer analysis. **Results:** There were a total of 4,019 cases and 12,057 controls with mean (SD) pre-index periods of 14.2 (8.8) months. The mean (SD) age was 55 (12) years; 55% were male. The net increase in MPR was significantly higher in app-users compared to the controls (+0.083 vs. +0.036, p<.0001) as was the change in the proportion of patients with MPR ≥ 0.80 (+15.40% vs. +7.81%, p<.0001). Persistence on therapy was significantly higher for app users (log-rank test p<0.001) and at 12 months was 81.4% for app users compared to 34.5% for controls. **Conclusions:** Patients using the Medisafe app were significantly more persistent on therapy compared to matched controls and had a higher adherence to therapy during the persistent period. Broad use of this mobile app technology could play an important role in improving clinical and economic outcomes in patients with hypertension.

Disclosures: C. McGuiness: A. Employment; Significant; Employment. **J. Michaeli:** A. Employment; Significant; Employment. **J. Millard:** A. Employment; Significant; Employment. **X. Wang:** A. Employment; Significant; Employment. **R.L. Wade:** A. Employment; Significant; Employment.

Funding: No

Funding Component:

P359

Management of Hypertension and Diabetes Comorbidity by Pharmacists in a Digital Setup

Authors: Wei Wang, Shanghai RuiJin Hosp, JiaoTong Univ Sch of Med, Shanghai, China; Zhen Wang, Hongguang Zhang, Ying Chen, Yingjie Li, Dongxing Li, Kai Liu, CareLinker Co., Ltd., Shanghai, China; **Jiumei Cao**, Shanghai RuiJin Hosp, JiaoTong Univ Sch of Med, Shanghai, China

Objective: To identify the health outcomes of using a digital approach to manage patients of hypertension and diabetes comorbidity in China. Methods: We developed and promoted a digital solution that consisted of BP and blood glucose (BG) tele-monitors, an App, and a cloud engine with 5 proprietary algorithms for personalized care in 1,150 drug stores in 101 cities. During Jun 1st 2015 and Apr 30th 2018, a total of 4,775 patients of both uncontrolled BP and BG received monthly pharmacy services of: 1) BP and BG tele-monitoring, 2) App-assisted lifestyle coaching for meal plans and

exercises, and 3) App-guided medication consultation. Results: The study population had an average age of 65.7 ± 10.4, BMI of 24.4 ± 3.3, and male of 47.5%. SBP and DBP were reduced on average by 9.3 mm Hg (baseline: 154.4 ± 14.7 mm Hg, last time: 145.1 ± 18.5 mm Hg, P< 0.001) and 5.0 mm Hg (baseline: 86.7 ± 11.2 mm Hg, last time: 81.7 ± 11.7 mm Hg, P<0.001). Their fasting blood glucose (FBG) and random blood glucose (RBG) were reduced by 0.8 mM (baseline: 9.1 ± 2.2 mM, last time: 8.3 ± 2.3 mM, P<0.001) and 3.1 mM (baseline: 14.5 ± 3.3 mM, last time: 11.4 ± 4.0 mM, P<0.001). The percentage of patients who had controlled BP or BG or both measurements were 36.0% (n=1,717, P<0.001), 28.8% (n=1,376, P<0.001) and 11.4% (n=542, P<0.001) by the time of this analysis. Patients in general felt comfortable about data sharing with pharmacists and were satisfied with this interventional approach that provided easy data access to them and their close relatives who in many cases played an important role in helping the patients achieve better compliance. The health outcomes were not associated with geographic differences except for RBG (r=0.1, P<0.001) or economic status of the site. Conclusion: Patients of hypertension and diabetes comorbidity can be effectively managed by pharmacists when digital tools were integrated into the interventional approach.

Disclosures: W. Wang: None. **Z. Wang:** None. **H. Zhang:** None. **Y. Chen:** None. **Y. Li:** None. **D. Li:** None. **K. Liu:** None. **J. Cao:** None.

Funding: No

Funding Component:

P360

Potential Need for Expanded Clinical- and Community-based Pharmacologic Treatment and Lifestyle Modification Services Under the 2017 Hypertension Guideline

Authors: Gregory Wozniak, American Medical Association, Chicago, IL; Matthew Ritchey, Cathleen Gillespie, Ctrs for Disease Control and Prevention, Atlanta, GA; Christina Shay, American Heart Association, Dallas, TX; Angela Thompson-Paul, Fleetwood Loustalot, Yuling Hong, Ctrs for Disease Control and Prevention, Atlanta, GA

Objective: Describe the effects of the 2017 Hypertension Guideline (HTN GL) compared to JNC-7 guideline on recommended blood pressure (BP) treatment status among US adults and identify the potential need for expanded clinical- and community-based BP management services. Methods: Analyze data from 2011-2014 National Health and Nutrition Examination Survey, with analytic sample of 10,031 aged ≥18 years.
Results: The new HTN GL reclassifies 32.3 million US adults as newly hypertensive and recommends BP-related treatment for 133.7 million adults, including 4.9 million newly recommended pharmacologic therapy and 50.5 million newly recommended lifestyle modifications alone. An estimated 1.1 million adults newly recommended to initiate pharmacologic treatment and 20.6 million adults newly recommended lifestyle modification alone report not having established healthcare linkages. Application of the new HTN GL affects some groups significantly more than others in being reclassified as having hypertension or being newly recommended to initiate BP treatment. This includes adults aged 18-64 years and males, two groups who historically have limited access to or low utilization of healthcare services for hypertension management. In addition, 3.6 million of the 4.9 million US adults who are newly recommended pharmacologic treatment and 36.4 million of the 50.5 million of those newly recommended lifestyle modification alone, are overweight or obese. Conclusions: The new HTN GL results in millions of additional US adults being recommended for lifestyle modification to manage their BP and a smaller proportion for pharmaceutical treatment. With many of those individuals not having established linkages to healthcare, these results can aid in translating the new HTN GL into clinical practice and public health programs necessary to meet the increased demand for services. Expanded clinical and public health resources are likely to be required to manage the additional millions of US adults recommended to newly initiate pharmacologic treatment and/or lifestyle modification, including many who previously had only limited healthcare system interaction. Community-based prevention strategies can aid in addressing this added burden on the health care system.

Disclosures: G. Wozniak: None. **M. Ritchey:** None. **C. Gillespie:** None. **C. Shay:** None. **A. Thompson-Paul:** None. **F. Loustalot:** None. **Y. Hong:** None.

Funding: No

Funding Component:

P361

Single Pill Combination Therapy is Not Superior to Free Combination Therapy for Controlling Hypertension

Authors: Wasseem Rock, Shereen Dabbah, Sofia Rimbrot, Emek Medical Ctr, Afula, Israel; Shiraz Vered, Statistics Dept, Haifa University, Israel; Ehud Grossman, Yehonatan Sharabi, Sheba Medical Ctr, Tel Hashomer, Israel

Introduction:
The 2017 AHA guidelines advocate the use of ambulatory blood pressure monitoring (ABPM) for assessing the response to anti-hypertensive therapy and favor the use of single pill combination therapy for patients on multiple drugs. We aimed to investigate whether single pill combination therapy was superior to free pill combination therapy for attainment of goal blood pressure.
Methods:
We used data from 1074 consecutive ABPM studies performed between February 2017 and March 2018 to identify patients who received the following drug combinations as either single pill or free pills: ACEI/HCTZ, ARB/HCTZ, ACEI/CCB, ARB/CCB. Goal blood pressure was defined as an average awake blood pressure below 130/80 mmHg.
Results
There were 193 patients in the single pill combination group and 136 patients in the free pill combination group. There was no difference between the groups regarding age, gender, number of antihypertensive drugs, and previous cardiovascular disease except for previous stroke which was more common in the free pill combination group. Compared to single pill combination, free pill combination showed a trend to superiority in the rate of blood pressure control in all drug combinations, with statistical significance reached only when comparing pooled groups (see table).
Conclusions
Single pill combination therapy was not superior to free pill combination therapy for attainment of goal blood pressure.

Table: Single pill combination therapy vs. free combination therapy for the control of hypertension

		Number treated	Controlled (%)	P-value
ACE/HCTZ	Separate pills	25	20 (80)	0.39
	Single pill	10	6 (60)	
ARB/HCTZ	Separate pills	38	26 (68.4)	0.67
	Single pill	37	27 (72.9)	
ACEI/CCB	Separate pills	60	47 (78.3)	0.34
	Single pill	29	20 (69)	
ARB/CCB	Separate pills	82	64 (78.1)	0.09
	Single pill	122	82 (67.2)	
Any combination	Separate pills	136	110 (80.9)	0.02
	Single pill	193	133 (68.9)	

Disclosures: W. Rock: None. **S. Dabbah:** None. **S. Rimbrot:** None. **S. Vered:** None. **E. Grossman:** None. **Y. Sharabi:** None.

Funding: No

Funding Component:

P362

Change of P Wave Parameters After Switching Anti-Hypertensive Agent From Free Drug Combination Into Fixed Dose Combination

Authors: Young Jae Ki, Joong Wha Chung, Dept of Internal Med, Chosun Univ Sch of Med, Gwangju, Korea, Republic of; Young Uk Seo, Dept of Internal Med Cheomdan Hosp, Gwangju, Korea, Republic of

Background: In hypertensive patients, it is well-known that PWD (P wave dispersion = maximal P wave duration - minimal P wave duration) is associated with the level of BP and the level of PWD correlates with the prevalence of arrhythmias and left atrial enlargement in previous studies. Fixed-dose combination(FDC) of anti-hypertensive agents effectively lower BP compared with free-drug combination. There have been some studies comparing the effect of anti-hypertensive agents on P-wave parameters. However, there have been no studies assessing the change of PWD after switching anti-hypertensive agents from free drug combination into fixed-dose combination Objective: The aim of this study was to assess the change of PWD after switching anti-hypertensive agents from free drug combination of angiotensin II receptor blocker(ARB)/Calcium channel blocker(CCB) into fixed-dose combination Design and method: This study included 20 patients who changed anti-hypertensive agents from free drug combination of ARB/CCB into same dosage fixed-dose combinations (FDCs) of ARB/CCB during 2011 to 2014. The maximal P wave duration(maxPWD), minimal P wave duration(minPWD), P wave dispersion(PWD), cPWD(corrected by heart rate, i.e. corrected P wave dispersion) measured by 12-lead electrocardiogram(ECG) at a paper speed of 50mm/s and 20mm/mV. Results: The PWD was reduced non-statically after changed into fixed dose combination (54.45±13.14ms) compared with free drug combination (56.73±11.90ms, p=0.070). The cPWD was reduced statically after changed into fixed dose combination (56.72±11.90ms) compared with free drug combination (60.02±12.28ms, p=0.003). Conclusions: The cPWD significantly reduced after switching anti-hypertensive agents from free drug combination into fixed dose combination

Disclosures: Y. Ki: None. **J. Chung:** None. **Y. Seo:** None.

Funding: No

Funding Component:

P363

Risk Factors Associated With Uncontrolled Hypertension in Low Income Jordanians

Authors: Mohd Shara, Jordan Univ of Science & Technology, Irbid, Jordan; Samar Zayadeen, Yarmouk Univ, Irbid, Jordan

Background: Hypertension (HTN) is one of the most important modifiable and contributing factors to stroke, cardiovascular and renal disease. Globally, HTN control remains inadequate, with about half attaining BP goal. This study evaluated potential risk factors associated with uncontrolled HTN in a low income population managed by the ministry of health (MOH) which provides coverage for the vast majority of Jordan's population. **Methods**: All subjects (400) in this multi-center, cross-sectional study, were recruited at MOH primary care clinics, were using the same HTN medication regimen for a minimum of 6 months and were all initiated on HTN therapy under JNC-7 guidelines. BP control was assessed based on JNC 7 and 8 guidelines. In addition, obesity, diabetes, metabolic syndrome (MetS), medication regimens and adherence, fat/salt intake, fasting blood glucose, lipids, leptin, adiponectin, hs-CRP and plasminogen activator inhibitor-1 (PAI-1) were examined for association with HTN control: **Results**: Less than half (178 of 400) of subjects BP was controlled per JNC 7, whereas 67% (268 of 400) were controlled based on JNC 8. Nearly 60% (238 of

400) were diabetic; a DM diagnosis was significantly ($p<0.05$) associated (2.5 fold higher vs non-diabetic) with uncontrolled HTN. Most subjects (93%, 371 of 400) were obese and 90% (360 of 400) met the International Diabetes Federation (IDF) and ATP-III criteria for MetS. Of the biomarkers examined, abnormal levels of PAI-1, adiponectine and hs-CRP were significantly associated with presence of MetS. MetS was associated with a 2.5 and 3.5 fold increase in the odds of having uncontrolled BP based on IDF and ATP-III criteria respectively. Elevated fasting blood sugar was also significantly associated with inadequate BP control. **Conclusions**: While some of this study's findings such as the association with DM and MetS are in agreement with the literature, often reported patient-behavior risk factors such as medication adherence was not. Although HTN control is suboptimal in this population, it is higher than the previously reported national rate (45% vs 39%). This difference may be explained by the higher medication adherence (64%) observed in this population compared with previous national studies (43%).

Disclosures: M. Shara: None. **S. Zayadeen:** None.

Funding: No

Funding Component:

P364

Health Insurance Status Affects Hypertension Control in a Hospital Based General Internal Medicine Clinic

Authors: AYODEJI OSO, Meharry Medical Coll, Nashville, TN; Abiodun Adefurin, Meharry Medical Coll, Antioch, TN; Monique Benneman, Olatunde Oso, Muinat Taiwo, Meharry Medical Coll, Nashville, TN; Oluwafisayo Adebiyi, Indiana Univ Sch of Med, Indianapolis, IN; Olukemi Oluwole, Medical Univ of South Carolina, Charleston, SC

Objectives: Hypertension is a worldwide disorder that contributes significantly to morbidity, mortality, and healthcare costs in both developed and developing communities. No previous US study has evaluated the factors associated with blood pressure (BP) control when easy access to health care is available to hypertensive patients.
Methods: A retrospective cohort study of hypertensive patients attending the Internal Medicine continuity clinic at Nashville General Hospital (NGH) between January and December 2007 was conducted. Given the easy access to health care at NGH and affordable BP medications with a cost of about $4 for a 30 day supply, we explored the ability to achieve optimal guideline directed BP control and evaluated which factors are associated with achieving adequate blood pressure control.
Results: Of the 199 study participants, 59% of the study population achieved optimal guideline directed BP goal < 140/90mmHg similar to the national average. The mean (95% CI) systolic BP (SBP) was 139 mmHg (136 - 141)mmHg while the mean (95% CI) diastolic BP (DBP) was 80 mmHg (79-82)mmHg. Health insurance status was associated with SBP and DBP (All $P < 0.046$). Patients with health insurance had a 2.2 fold increased odds of achieving optimal BP control to less than 140/90mmHg compared to patients without health insurance ($P=0.025$). Furthermore, the number of blood pressure medications used was significantly associated with SBP and DBP (All $P < 0.003$). Patients taking more than three BP medications had a 58% reduced odds of achieving adequate BP control less than 140/90mmHg compared to patients taking one medication ($P = 0.039$). Ethnicity was not associated with achieving BP control in this study.
Conclusion: Our study revealed optimal guideline directed BP control can be achieved when easy access to health care is provided and with use of affordable medications. The number of BP medications used and health insurance status, are factors associated with achieving optimal BP control.

Disclosures: A. Oso: None. **A. Adefurin:** None. **M. Benneman:** None. **O. Oso:** None. **M. Taiwo:** None. **O. Adebiyi:** None. **O. Oluwole:** None.

Funding: No

Funding Component:

P365

Circulating and Kidney EVs in Angiotensin-induced HTN In Mice

Authors: Sabrina La Salvia, Luca Musante, Joanne Lannigan, Sylvia Cechova, Thu H Le, Uta Erdbrügger, Univ of Virginia, Charlottesville, VA

There is emerging evidence that extracellular vesicles (EVs) may be novel bio-markers and bio-activators in the pathogenesis of HTN. We hypothesize that the phenotypes of EVs from the circulation and kidney are altered in Angiotensin II (AII) induced HTN and normalize with anti-hypertensive treatment (Rx). AII was delivered via osmotic mini pumps (500 ng/kg/min) alone, or with candesartan (C) @ 10 mg/kg/day, or with hydralazine, hydrochlorothiazide, reserpine (HHR) @ 30/10/0.2 mg/kg/day in drinking water for 2 weeks to 129S6 mice (n = 5 each). Systolic blood pressure (SBP) was measured daily for 2 weeks by tail-cuff manometry. After 2 weeks, mice were euthanized and citrated blood and kidneys collected. Kidney EVs (KEVs) were isolated after incubation with Collagenase type A and subsequent DC. Both circulating EVs and KEVs were isolated through differential centrifugation and characterized by imaging flow cytometry (AmnisImage-StreamX Mark II) using PECAM-1 (CD31), leukocyte (CD45), platelet (CD41) and endothelial (CD105) markers. AII treated mice had significantly higher SBP (mmHg) (162.05 ± 7.20) than normotensive controls ((N) 120.25 ± 7.12), p=0.02. SBP was similarly reduced with C and HHR (134.12 ± 6.15 and 122.45 ± 5.20, respectively, p=NS), and significantly lower than AII, p=0.0026 and =0.03, respectively). Circulating leukocyte derived EVs (LEVs, CD31+/CD45+) increased after 2 weeks in AII treated mice compared to N (AII 2.01E+0.3, N 2.04E+0.4 particles/mL; p=0.0130). This effect was not observed with endothelial and platelet derived EVs. Numbers of circulating LEVs correlated significantly with SBP [(r^2=0.675); p=0.0029]. Circulating LEVs were reduced after equal normalization of SBP, though no difference in EVs was observed between Rx with C and HHR. CD45+ KEVs in AII treated mice were significantly increased compared to N (AII 7.52E+05 vs N 4.79E+04; p= 0.0257). KEVs also correlated significantly with SBP [(r^2=0.682); p=0.0032]. In conclusion, circulating and kidney derived EVs may play a role in the immune response in HTN and may be influenced by blood pressure threshold, independent of AT_1 receptor activation. Further studies are needed to confirm and to characterize specific subtype(s) of LEVs and dissect their functional role.

Disclosures: S. La Salvia: None. **L. Musante:** None. **J. Lannigan:** None. **S. Cechova:** None. **T.H. Le:** None. **U. Erdbrügger:** None.

Funding:

Funding Component:

P366

Summary Proceedings From The American Heart Association Western Affiliate Commission Of Blood Pressure Task Force: Statement On Institutional And Population Health Strategies To Achieve Blood Pressure Control In Diverse Communities

Authors: Tony Kuo, David Geffen Sch of Med at UCLA, Los Angeles, CA; Steven Chen, USC Sch of Pharmacy, Los Angeles, CA; Sang-Mi Oh, American Heart Association, Western States Affiliate, San Jose, CA; Cevadne Lee, American Heart Association, Western States Affiliate, Los Angeles, CA; Lily Fu, USC Sch of Pharmacy, Los Angeles, CA; Commission of Blood Pressure Task Force AHA Western States Affiliate, American Heart Association, Los Angeles, CA

Objective: The AHA Western States Affiliate (WSA) Commission of Blood Pressure Task Force (TF) sought to establish an evidence base to guide health and population health strategies for blood pressure (BP) control across diverse communities, namely: (1) team care strategies such as pharmacist intervention, engagement of at-risk populations by community pharmacists, and medication management in the neighborhood setting; (2) self-care approaches such as self-monitoring interventions; (3) culturally-tailored diet modification, including DASH and sodium reduction; and (4)

social conditions and other factors that may impede or facilitate medication adherence. ***Methods:*** Along with TF proceedings, a series of systematic reviews were conducted to examine 4 categories of intervention, yielding 51 articles and summary reviews on the impact and implementation contexts of these interventions. Search terms included "hypertension", "blood pressure", "team-based care", "self-monitoring" and "home-monitoring". Synthesis of the evidence is being carried out by a panel of experts who are rating the strength of each identified study using the American Academy of Family Physicians' Strength of Recommendation Taxonomy (SORT) system. SORT is a patient-centered approach to grading evidence in medical literature, offering interpretation of data applicable to practice settings. ***Results:*** WSA reaches 70 million adults comprising 50% diverse adults. The multi-layer review and synthesis of the evidence suggests pharmacist strategies such as coordinated medication therapy management and BP self-monitoring in diverse communities have beneficial impact on helping patients reach AHA/ACC and JNC7 target BP goals. The TF also found that strategy implementation to fidelity can be difficult to achieve in real world settings. ***Conclusions:*** Results from the TF proceedings and synthesis suggest all 4 categories of intervention showed promising outcomes; however, more research is needed to demonstrate their impact collectively or in combination. Additionally, barriers and facilitators to effective use of these interventions may require novel study using emerging models of complex systems and dissemination, implementation, and improvement (DII) sciences.

Disclosures: T. Kuo: None. **S. Chen:** None. **S. Oh:** None. **C. Lee:** None. **L. Fu:** None. **C. AHA Western States Affiliate:** None.

Funding:

Funding Component:

P367

Adherence Assessment Via Comprehensive Identification and Quantitation of Circulating Medications with Significant Correlation to Lower Blood Pressure Observed in Hypertensive Patients

Authors: J Scott Daniels, Ryan D Morrison, Stephen B Milne, Precera Bioscience, Franklin, TN; Candace D McNaughton, Dept of Emergency Med, Vanderbilt Univ Medical Ctr, Nashville, TN; Jeffrey J Sutherland, Data Solutions Consulting, LLC, Lincoln, MA

Employing a novel and proprietary comprehensive precision medicine clinical tool that is a LC/MS/MS-based platform (PrecisCMQ™), we analyzed the serum of HTN patients seeking emergency care (n=293) for the presence and quantity of 42 antihypertensive and cardiovascular medications. Patient self-reported adherence correlated with clinical tool-based adherence. Among patients prescribed $\geq$ 3 medications, clinical tool-based adherence in patients (n=74) who indicate they never miss a dose was 74%, versus 60% in patients (n=69) who reported missed doses ($p = 0.009$, two-sided t-test; median prescribed medications = 3.5). Patients were considered adherent when 80% of prescribed medications were detected and categorized as nonadherent otherwise. Among patients with $\geq$ 3 prescribed medications, we found adherent patients had lower SBP (9.7 mm Hg; 95% CI 1.8 - 17.6; $p = 0.02$) and lower DBP (6.9 mm Hg; 95% CI 1.9 - 12.1; $p = 0.007$) after adjusting for age, sex, BMI, and patient-reported adherence. No significant difference in BP was observed in patients prescribed < 3 medications. By comparing medication clinical tool-based detections to prescribed medications listed in the EHR, 12% of the detected medications (68/549) were not recorded in the patients' EHR. After accounting for the above covariates and the number of medications in the EHR, we observed that the total number of detected medications, including prescriptions not in the EHR, significantly correlated with both SBP (attributed to 4.8% of variation; $p = 0.01$) and DBP (patients prescribed $\geq$ 3medications; explaining 2.5% of BP variation; $p = 0.05$). Additionally, to assess whether patients with higher systemic medication concentrations had lower BP, we compared SBP and DBP to medication concentrations normalized to published reference ranges. After accounting for the above covariates and the number of detected medications, we observed a relationship between medication(s) concentration(s)

and BP across all patients. Together, these results support the utility of clinical tool-based medication monitoring for assessing adherence and improving BP control in HTN patients.

Disclosures: J.S. Daniels: A. Employment; Significant; J. Scott Daniels Employee of Precera Bioscience, Inc., Inc.. 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; J Scott Daniels, Employee Stock Options. **R.D. Morrison:** A. Employment; Significant; Employee Precera Bioscience Inc.. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; Employee Stock Options, Precera Bioscience inc. **S.B. Milne:** A. Employment; Significant; Employee Precera Bioscience, Inc.. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; Employee Stock Options, Precera Bioscience inc. **C.D. McNaughton:** 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); Significant; NHLBI K12 HL109019, NHLBI K23HL125670. **J.J. Sutherland:** A. Employment; Modest; former Precera Bioscience Employee. F. Ownership Interest (includes any stock, stock option, partnership, membership or other equity position in an entity regardless of the form of the entity, or any option or right to acquire such position, and any rights in any patent or other intellectu; Modest; minor stock ownership, Precera Bioscience, Inc..

Funding:

Funding Component:

P368

Predicting the Risk of Refractory Hypertension

Authors: Michael Buhnerkempe, Albert Botchway, Dept of Internal Med, Ctr for Clinical Res, Southern Illinois Univ Sch of Med, Springfield, IL; Carlos E. Nolasco Morales, Vivek Prakash, John M. Flack, Dept of Internal Med, Div of General Internal Med, Hypertension Section, Southern Illinois Univ Sch of Med, Springfield, IL

Background: Refractory hypertension is defined as uncontrolled blood pressure (BP) when taking five or more classes of antihypertensive medication, including a thiazide or thiazide-like diuretic and a mineralocorticoid receptor antagonist. Refractory hypertension represents a potential extreme along the spectrum of hypertension phenotypes. However, due to the rarity and recent definition of refractory hypertension, relatively little is known about quantifying risk of this phenotype. ***Methods:*** Refractory hypertension was retrospectively assessed in patients seen at an urban referral hypertension clinic using a BP goal of 140/90 mmHg. Patients with advanced chronic kidney disease (eGFR < 30) were excluded, and the 1179 included patients had at least one follow-up visit in a window 28 to 240 days after their index visit. Patients were mostly African-American (86%; 1018/1179) and female (65%; 766/1179). Risk for refractory hypertension was estimated using logistic regression with patient characteristics at index visit as predictors (i.e., age, BMI, eGFR, number of adequately dosed antihypertensive medication classes taken at the index visit, BP above goal, diabetes, gender, race, and smoking status). Performance of this risk score at discriminating refractory hypertension over follow-up was assessed using AUC and was internally validated using bootstrapping. ***Results:*** Only 25/1179 (2.1%) developed refractory hypertension. Old age, higher BMI, more antihypertensive medication classes taken at index visit, higher BP, and diabetes were all associated with increased refractory hypertension risk. The risk score discriminated well (AUC = 0.88, bootstrapped 95% CI [0.82, 0.92]). In our patient population, 683/1179 (57.9%) had estimated risks for development of refractory hypertension < 1%. Of these low-risk patients, 682/683 (99.9%) failed to develop refractory hypertension (negative predictive value, 0.997 [0.99, 1.00]). ***Conclusions:*** We created a novel clinical score that discriminates well between those who will and will not develop refractory hypertension and that is particularly useful at identifying patients with little to no risk of refractory hypertension.

Disclosures: **M. Buhnerkempe:** None. **A. Botchway:** None. **C.E. Nolasco Morales:** None. **V. Prakash:** None. **J.M. Flack:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Grant support from Vascular Dynamics, Grant support from Bayer, Grant support from GlaxoSmithKline. G. Consultant/Advisory Board; Modest; serves on the data safety and monitoring board for Rox Medical, Served as a consultant for NuSirt, Served as a consultant for Allergan, Served as a consultant for BackBeat Hypertension.

Funding:

Funding Component:

P369

Non-Adherence to Antihypertensive Medications is Associated with Higher BP and Anxiety Levels.

Authors: Maria El Hachem, Mohammed Siddiqui, Stephen J Thomas, Tanja Dudenbostel, Badhma Valaiyapathi, Eric Judd, Univ of Alabama at Birmingham, Birmingham, AL; Prashanth Patel, Univ of Leicester, Leicester, United Kingdom; Pankaj Gupta, Univ of Leiceter, Leicester, United Kingdom; Maciej Tomaszewski, Univ of Manchester, Manchester, United Kingdom; Suzanne Oparil, David A Calhoun, Univ of Alabama at Birmingham, Birmingham, AL

Background: Anxiety has been linked to higher blood pressure (BP) levels and a higher incidence of cardiovascular outcomes. Anxiety is also associated with antihypertensive medication non-adherence assessed indirectly by questionnaires, pill counts or pharmacy refill rates. In this study, we determine the association between anxiety and antihypertensive medication adherence measured directly by high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS).
Methods: In this prospective study, 237 patients were recruited from the Hypertension Clinic at the University of Alabama at Birmingham. The State-Trait Anxiety Inventory (STAI) questionnaire, 24-hr urine collection for HP LC-MS/MS and 24-hour ambulatory blood pressure monitoring (ABPM) were done in all subjects as part of the study protocol. Patients who did not complete a 24-hr urine collection and/or an STAI questionnaire were excluded from the analysis.
Results: Of the 180 patients included in the study, 137 participants were found to be adherent (taking all antihypertensive medications) (76%) and 43 patients were non-adherent to antihypertensive medications (24%). Group comparison using t-test revealed higher state (p=0.043) and trait (p=0.044) anxiety scores and higher ambulatory blood pressure levels (p<0.001) among non-adherent compared to adherent patients (Table 1).

	Antihypertensive Medication Adherent Patients (n=137)	Antihypertensive Medication Non-Adherent Patients (n=43)	P-Value
Age (Years)	59.5 ± 11.0	54.4 ± 10.4	0.008
Women	57 (41.6 %)	29 (67.4 %)	0.003
African American race	69 (50.4 %)	31 (72.1 %)	0.009
State Anxiety Score	33.5 ± 11.4	37.8 ± 12.2	0.043
Trait Anxiety Score	34.5 ± 11.3	38.8 ± 11.5	0.044
ABPM Systolic BP (mmHg)	137.5 ± 16.2	148.5 ± 25.9	0.001
ABPM Diastolic BP (mmHg)	75.6 ± 9.8	85.6 ± 17.3	<0.001
ABPM Mean Arterial Pressure (mmHg)	96.3 ± 10.8	106.9 ± 19.5	<0.001
ABPM Heart rate (Beats/Min)	72.6 ± 11.7	75.3 ± 11.8	0.195

Conclusion: This is the first prospective study to demonstrate a strong association between antihypertensive medication adherence, higher anxiety levels and poorly controlled hypertension. These findings highlight the importance of a multidisciplinary approach in the management of hypertensive patients.

Disclosures: **M. El Hachem:** None. **M. Siddiqui:** None. **S.J. Thomas:** None. **T. Dudenbostel:** None. **B. Valaiyapathi:** None. **E. Judd:** None. **P. Patel:** None. **P. Gupta:** None. **M. Tomaszewski:** None. **S. Oparil:** None. **D.A. Calhoun:** None.

Funding:

Funding Component:

P370

The Efficacy of Fixed Combination Lisinopril and Hydrochlorothiazide in Obese Hypertensive Women

Authors: Vesna Stojanov, Nenad Radivojevic, Clinical Ctr of Serbia, Belgrade, Serbia; Katarina Paunovic, Branko Jakovljevic, Inst of Hygiene and Medical Ecology, Belgrade, Serbia

Objectives: The aim was to assess the effects of lisinopril combined with hydrochlorthiazide on blood pressure of hypertensive women with normal weight, overweight and obesity. **Methods:** The study comprised 454 hypertensive women, aged 61.50±10.64 years. All patients were treated with fixed combination of 20 mg lisinopril and 12.5 mg hydrochlorthiazide. Body mass index (BMI) was calculated from body weight divided by squared body height. Normal weight was defined as BMI from 18.5 to 24.9 kg/m^2, overweight as BMI from 25.0 to 29.9 kg/m^2 and obesity as BMI exceeding 30 kg/m^2. Blood pressure (BP) was measured by an oscillometric device at the beginning of the study, and after three months of therapy. The differences between the groups were tested with one-way ANOVA. The differences from baseline values were tested with Student's t test for paired samples. **Results:** At the beginning of the study 124 women (27.9%) had normal weight, 217 women (48.8%) were overweight, and 104 (23.4%) were obese. All three groups of women had similar systolic (SBP) and diastolic pressures (DBP) at the beginning of the study as well as at the end of the study. Mean BMI remained stable during three months of antihypertensive therapy. Mean SBP for all patients at the beginning of the study was 161.90±13.26 mmHg, mean DBP was 97.48±8.78 mmHg. After three months of therapy, all women reduced their SBP significantly (mean decrease from baseline = 28.48±12.67 mmHg), as well as their DBP (mean decrease from baseline = 15.48±8.84 mmHg). The average decrease in SBP and DBP during therapy was similar among women with normal weight, overweight and obesity. Women with normal weight reduced their SBP by 29.61±12.33 mmHg, in comparison to overweight women (28.42±11.89 mmHg), and to obese women (26.54±12.74 mmHg), but the difference was not statistically significant. **Conclusion:** Three-month therapy with lisinopril and hydrochlorthiazide is equally effective in hypertensive women with normal weight, overweight and obesity.

Disclosures: **V. Stojanov:** None. **N. Radivojevic:** None. **K. Paunovic:** None. **B. Jakovljevic:** None.

Funding:

Funding Component:

P371

The Effects of Omega-3 Fatty Acid Ethyl Esters on QT Dispersion and Corrected QT Dispersion in Hypertensive Patients With Left Ventricular Hypertrophy

Authors: YoungJae Ki, Joong Wha Chung, Dept of Internal Med, Chosun Univ Sch of Med, Gwangju, Korea, Republic of; Young Uk Seo, Dept of Internal Med, Cheomdan Hosp, Gwangju, Korea, Republic of

Background: Left ventricular hypertrophy (LVH) leads to arrhythmia such as atrial fibrillation, ventricular arrhythmias and known risk factor of sudden cardiac death. Recent study suggests that Omega-3 fatty acid ethyl esters have anti-arrhythmic effects and reduce sudden cardiac death among survivor of myocardial infarction. QT dispersion(QTD) reflects ventricular repolarization heterogeneity and prolonged QTD has been associated with the risk of arrhythmic

death in coronary artery disease patients. However, no study has done to prove the effects of Omega-3 fatty acid ethyl esters on QTD and corrected QTD(cQTD) in hypertensive patients with LVH Objective: We tried to evaluate the effects of Omega-3 fatty acid ethyl esters on QTD and cQTD in hypertensive patients with LVH Design and method: This study included 30 hypertensive patients who confirmed LVH by ECG voltage criteria. The patients were evaluated with 12-leads electrocardiography before and 3~6 months after treatment of Omega-3 fatty acid ethyl esters(1g/day). We measured QTD (maximal QT interval - minimal QT interval) and cQTD (maximal corrected QT interval - minimal corrected QT interval) by Bazett's formula using electronic caliper before and after treatment. Results: QTD (before: 60.86±14, after: 51.84±13.95 P=0.003) and cQTD (before: 63.83±14.18, after: 51.83±13.94 P<0.001) was significantly reduced after treatment of Omega-3 fatty acid ethyl esters Conclusions: The QTD and cQTD significantly reduced after treatment of Omega-3 fatty acid ethyl esters in hypertensive patients with LVH. It may partially explain the anti-arrhythmic effect of Omega-3 fatty acid ethyl esters.

Disclosures: Y. Ki: None. **J. Chung:** None. **Y. Seo:** None.

Funding: No

Funding Component:

P372

The Effect of Periodic Fasting on Patients With Hypertension and Metabolic Syndrome

Authors: Andras Balogh, Experimental and Clinical Res Ctr & Charité Univsmedizin Berlin & DZHK, Berlin, Germany; Nico Steckhan, Charité Univsmedizin Berlin & Immanuel Hosp Berlin, Berlin, Germany; Sofia K. Forslund, Experimental and Clinical Res Ctr & Charité Univsmedizin Berlin & Max Delbrück Ctr for Molecular Med & BIH, Berlin, Germany; Nicola Wilck, Experimental and Clinical Res Ctr & Charité Univsmedizin Berlin & DZHK, Berlin, Germany; Ibrahim Hamad, VIB Ctr for Inflammation Res (IRC), Hasselt Univ, Diepenbeek, Netherlands; Ellen G. Avery, Urša Šušnjar, Experimental and Clinical Res Ctr & Max Delbrück Ctr for Molecular Med, Berlin, Germany; Christoph Hohmann, Charité Univsmedizin Berlin & Immanuel Hosp Berlin, Berlin, Germany; Till Strowig, Helmholtz Ctr for Infection Res, Braunschweig, Germany; Ralf Dechend, Experimental and Clinical Res Ctr & Charite Unviersitätsmedizin Berlin & DZHK & BIH & Helios Hosp, Berlin, Germany; Markus Kleinewietfeld, VIB Ctr for Inflammation Res (IRC), Hasselt Univ, Diepenbeek, Netherlands; Andreas Michalsen, Charité Univsmedizin Berlin & Immanuel Hosp Berlin, Berlin, Germany; Dominik N. Mueller, Experimental and Clinical Res Ctr & Max Delbrück Ctr for Molecular Med, Charite Unviersitätsmedizin Berlin & DZHK & BIH, Berlin, Germany

Periodic fasting may serve as therapeutic strategy for the management of hypertension and metabolic syndrome. We hypothesize that fasting affects the gut microbiome and promotes immune cell homeostasis resulting in lower blood pressure. Patients suffering from metabolic syndrome and hypertension were randomized either to 7-day periodic fasting combined with lifestyle change or Dietary Approaches to Stop Hypertension (DASH) intervention. Data derived from clinical parameters, 16S sequencing of the gut microbiome and immunophenotyping were collected at baseline, at day 7 and 3 months post intervention.
At baseline, 21 out 30 patients in the fasting and 23 out of 31 in the DASH group had elevated systolic office blood pressure (SBP >130 mmHg), which dropped significantly by 10 mmHg after fasting, and remained lower 3 months post intervention (7 mmHg). In the DASH group, SBP significantly decreased by 12 mmHg after 1 week and 8 mmHg after 3 months. At 3 months post intervention, 24-h ABPM confirmed the decrease in fasting, but not in DASH. Fasting, but not DASH significantly reduced body weight and BMI after 7 days compared to baseline, and this effect persisted for 3 months post-intervention. Furthermore, 50 % (15/30) of patients in fasting and 23 % (7/31) in DASH were able to decrease their use of medications for treatment of metabolic syndrome.
Spearman correlation tests showed that fasting, but not DASH, altered the gut microbiome. After fasting *Bacteroides*, *Lachnoclostridium*, *Coprococcus*, and *Ruminococcus* Operational Taxonomic Units (OTUs) decreased, while the

Eubacterium rectale OTU increased. In contrast to DASH, fasting significantly reduced the frequency of CD4+ Th17 cells, active CD69+ mucosa-associated invariant T cells (MAIT) and IFNg+IL-2+ or TNFa+ MAITs indicating the anti-inflammatory effect of fasting. While some of these changes reverted to baseline after 3 months, several persisted. Despite a similar decrease in office SBP in both study arms, the periodic fasting intervention is more beneficial in reducing body weight and BMI compared to baseline. Fasting altered the prevalence of certain gut microbes and influenced immune cell signatures whereas DASH did not, suggesting a distinct role of fasting for improving cardiometabolic health.

Disclosures: A. Balogh: None. **N. Steckhan:** None. **S.K. Forslund:** None. **N. Wilck:** None. **I. Hamad:** None. **E.G. Avery:** None. **U. Šušnjar:** None. **C. Hohmann:** None. **T. Strowig:** None. **R. Dechend:** None. **M. Kleinewietfeld:** None. **A. Michalsen:** None. **D.N. Mueller:** None.

Funding: No

Funding Component:

P373

Design for a Pragmatic Trial Comparing Telehealth Care and Clinic-Based Care for Uncontrolled High Blood Pressure

Authors: Karen L Margolis, Anna R Bergdall, A Lauren Crain, Patrick J O'Connor, Leif I Solberg, MarySue Beran, Jeanette Y Ziegenfuss, Healthpartners Inst, Minneapolis, MN; Beverly B Green, Kaiser Permanente Washington Health Res Inst, Seattle, WA; Pamala A Pawloski, Daniel J Rehrauer, Deepika Appana, Rashmi Sharma, Christine K Norton, Patricia Haugen, JoAnn M Sperl-Hillen, Healthpartners Inst, Minneapolis, MN

The 2017 ACC/AHA hypertension guideline strongly recommends systematic follow-up and monitoring of treatment using team-based care and telehealth, based on Level A evidence. However, different models for organizing team-based care and telehealth have not been compared. We describe the design of a PCORI-funded pragmatic trial with the following objectives: Aim1) compare the effects on BP and patient-reported outcomes of two models of team-based care for uncontrolled hypertension, and Aim 2) study how the two models are carried out in the real-world setting of a large health system. The study is a 5-year cluster-randomized trial in 2000 patients age 18-85 with uncontrolled hypertension cared for in 21 primary care clinics at HealthPartners, a large integrated healthcare system in the Twin Cities area of Minnesota and western Wisconsin. Clinic-based care uses recommended best practices and face-to-face visits primarily with physicians, nurses and medical assistants. The telehealth care approach adapts a research-tested model with systematic use of home BP telemonitoring and home-based telehealth care coordinated by a clinical pharmacist or nurse practitioner. Patients in both groups are recruited directly from primary care clinics using electronic health record (EHR) prompts. Exclusions are few: pregnancy, advanced kidney disease, hospice care, and nursing home residence. The primary outcomes for Aim 1 are:1) change in BP over 12 months, and 2) change in patient-reported outcomes over six months, including treatment side effects, experiences with hypertension care, self-monitoring rates, and confidence in self-care. Secondary outcomes include other heart- and stroke-related risk factors and safety. Patients contributed extensively to the selection of the outcomes. Outcomes are collected over 24 months without reliance on research visits: patient-reported outcomes are measured by surveys and BP and other clinical outcomes are measured using routinely collected data documented in the EHR. We also use EHR data supplemented by qualitative data to assess how the two care models are carried out in practice. The results of this comparative effectiveness trial will assess pragmatic methods for implementing hypertension guideline recommendations.

Disclosures: K.L. Margolis: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **A.R. Bergdall:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **A.L. Crain:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **P.J. O'Connor:** B. Research Grant (includes principal investigator, collaborator, or

consultant and pending grants as well as grants already received); Significant; PCORI. **L.I. Solberg:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **M. Beran:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **J.Y. Ziegenfuss:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **B.B. Green:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **P.A. Pawloski:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **D.J. Rehrauer:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **D. Appana:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **R. Sharma:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **C.K. Norton:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **P. Haugen:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI. **J.M. Sperl-Hillen:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; PCORI.

Funding: No

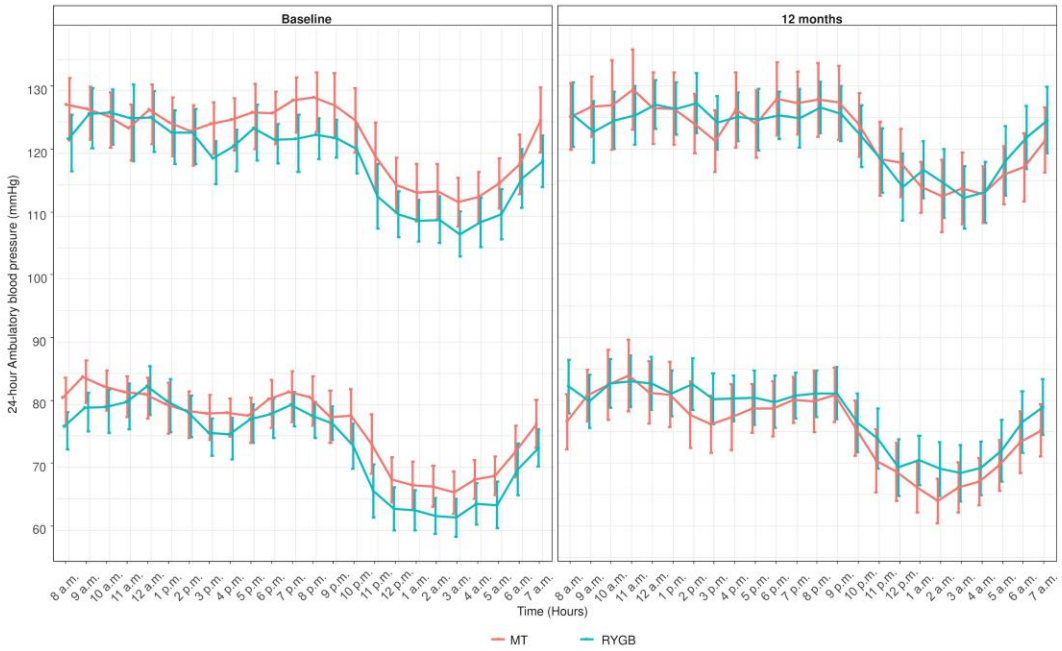
Funding Component:

P374

24-h Blood Pressure Profile, Non-dipping Status And Incidence Of Resistant Hypertension In Patients Randomized To Bariatric Surgery Versus Medical Therapy: The Gateway Randomized Trial

Authors: Carlos Aurelio Schiavon, Dimas Ikeoka, Eliana V Santucci, Renato N Santos, Lucas P Damiani, Juliana D Oliveira, Camila R Torreglosa, Angela C Bersch-Ferreira, Tamiris A Miranda, Heart Hosp, São Paulo, Brazil; Silvana de Barros, HCFMUSP, São Paulo, Brazil; Hélio Halpern, Frederico L Monteiro, Heart Hosp, São Paulo, Brazil; Ricardo V Cohen, Oswaldo Cruz German Hosp, São Paulo, Brazil; Patricia M Noujaim, Heart Hosp, São Paulo, Brazil; Márcio G Souza, Celso Amodeo, Dante Pazzanese Inst of Cardiology, São Paulo, Brazil; Luiz Bortolloto, Heart Inst, São Paulo, Brazil; Otávio Berwanger, Albert Einstein Hosp, São Paulo, Brazil; Alexandre B Cavalcanti, Heart Hosp, São Paulo, Brazil; Luciano F Drager, Heart Inst, São Paulo, Brazil

Background: Bariatric surgery represents an effective strategy for office blood pressure (BP) reduction in obese hypertensive patients. However, no previous study evaluated the impact of bariatric surgery on 24-h BP profile, non-dipping status and incidence of resistant hypertension (RH). **Methods:** This is a sub-analysis of a randomized clinical trial including hypertensive patients with grade 1 and 2 obesity, aged 18 to 65 years, using at least 2 drugs at optimal doses or >2 at moderate doses. Patients were randomly allocated to either Roux-en-Y Gastric Bypass (RYGB) with medical therapy (MT) or MT alone for 12 months. We analyzed the 24-h BP profile, non-dipping status (defined by <10% of systolic BP reduction during sleep as compared to the daytime period) and RH incidence. **Results:** A total of 100 patients were included (76% female, age 43.8±9.2 years, BMI 36.9±2.7 Kg/m^2). The 24-h BP profile was similar at 12 months in both groups, but the RYGB group required less anti-hypertensive classes compared to the MT alone (Figure). The rate of non-dipping BP did not change significantly during the follow-up (RYGB: from 18/48 (37.5%) to 22/48 (45.8%); p=0.30; MT: from 16/33 (48.5%) to 15/33 (45.5%); p=0.80). In an exploratory analysis, the incidence of RH was similar at the baseline (RYGB 10% (5/50) and MT 16% (8/50); p=0.38). After 12 months, it changed significantly in the RYGB group: 0% (0/49) while remained stable in the MT group: 14.9% (7/47) (p<0.001). **Conclusions:** RYGB significantly reduced anti-hypertensive medications while promoting similar 24-h BP profile and non-dipping BP status compared to the MT alone. RYGB may be an attractive strategy to reduce RH incidence in obese patients.



Number of antihypertensive drugs

	Baseline		12 months	
	RYGB (n=50)	MT (n=50)	RYGB (n=49)	MT (n=47)
Median (IQR)	3.0 (2.0 to 3.0)	3.0 (3.0 to 3.0)	0.0 (0.0 to 1.0)	3.0 (2.5 to 4.0)
Mean (SD)	2.8 (0.6)	3.1 (0.7)	0.7 (1.0)	3.0 (0.9)

Figure: 24-Hour Ambulatory Systolic and Diastolic Blood Pressure.

Graphics show mean hourly blood-pressure values for 96 patients at baseline (RYGB = 49, MT = 47) and 81 patients at 12 months follow-up (RYGB = 49, MT = 34). The I bars indicate 95% confidence intervals

Disclosures: **C.A. Schiavon:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Johnson&Johnson Brazil. D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Johnson&Johnson Brazil. E. Honoraria; Modest; Johnson&Johnson Brazil. **D. Ikeoka:** None. **E.V. Santucci:** None. **R.N. Santos:** None. **L.P. Damiani:** None. **J.D. Oliveira:** None. **C.R. Torreglosa:** None. **A.C. Bersch-Ferreira:** None. **T.A. Miranda:** None. **S.D. Barros:** None. **H. Halpern:** None. **F.L.J. Monteiro:** None. **R.V. Cohen:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; Johnson&Johnson Brazil. G. Consultant/Advisory Board; Significant; GI Dynamics. **P.M. Noujaim:** None. **M.G. Souza:** D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Daiichi-Sankio Brasil. G. Consultant/Advisory Board; Modest; Aché Labotatório farmacêutico. **C. Amodeo:** None. **L. Bortolloto:** None. **O. Berwanger:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AstraZeneca, Boheringer Ingelheim, Sanofi, Amgen, Pfizer, Roche, Bayer. **A.B. Cavalcanti:** None. **L.F. Drager:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Philips Respironics. D. Speaker (includes speakers bureau, symposia, and expert witness); Modest; Johnson&Johnson Brazil.

Funding:

Funding Component:

P375

Association Between Use of Alpha-Blockers and Hypotension and Hypotension-Related Clinical Events in Patients With Hypertension

Authors: Swapnil Hiremath, Marcel Ruzicka, Manish Sood, Ottawa Hosp, Ottawa, ON, Canada

Importance Alpha- blockers (AB) are commonly prescribed agents in the treatment of hypertension. Little is known regarding the risk of hypotension and hypotension-related clinical outcomes in patients with advanced age with ongoing treatment for hypertension.**Objective** To assess the risk of hypotension and hypotension-related adverse events (syncope, falls, fractures), major adverse cardiac events and all-cause mortality with AB use compared to other anti-hypertensives. **Methods** Population-based, retrospective cohort study of 933,033 eligible adults of advanced age (> 66 years) prescribed an anti-hypertensive medication between 1995 and 2015 in Ontario, Canada. A high dimensional propensity score was used to match AB prescription to other anti-hypertensives. AB exposure was modeled as a time-varying and cumulative covariate using extended, conditional Cox proportional hazards to examine the association with outcomes. Primary outcome was hospitalization or emergency room usage for hypotension and related complications (syncope, fractures, falls). Secondary outcomes included major adverse cardiovascular events and all-cause mortality. **Results** Among 69,092 matched patients prescribed AB, the incident rate of hypotension related complications were higher compared to other anti-hypertensives (hypotension 1.15 vs. 0.39, syncope 1.47 vs. 0.46, falls 4.37 vs. 1.37, fractures 2.23 vs. 0.69 per 100 person-years of follow-up). In time-varying exposure models with additional adjustment for the total number of anti-hypertensives, the higher risk persisted (hypotension HR 1.34 95%CI 1.26-1.43, syncope HR 1.49 95%CI 1.41-1.57, falls HR 1.27 95%CI 1.23-1.32, HR fractures 1.41 95%CI 1.34-1.48). Secondary outcomes of MACE and all-cause mortality were higher or similar among AB users (MACE IR 7.03 vs. 2.31, mortality 6.54 vs 6.37 per 100 person-years follow-up). The risk was highest among those > 85 and differed by the number of total anti-hypertensives prescribed. **Conclusions** The use of AB is associated with a higher risk of hypotension-related events and other complications. Our findings suggest other anti-hypertensive agents be considered especially among those of advanced age or based on the number of total anti-hypertensive agents.

Disclosures: S. Hiremath: None. **M. Ruzicka:** None. **M. Sood:** None.

Funding: No

Funding Component:

P376

The Monitoring by a Pharmacist Facilitator, of Side Effect, is Useful to Report Adverse Reactions Associated With Antihypertensive Agents in Practical Clinic in South Italy

Authors: Alessia Maretta, AO of Cosenza, Cosenza, Italy; Giuseppina Fersini, Pharmaceutical Policies, Pharmacovigilance and Conventional Pharmaceutical Unit, Regione Calabria, Catanzaro, Italy; Valentina Salerno, Francesca Saullo, Roberta Virno, Pharmacovigilance Regional Ctr, Regione Calabria, Catanzaro, Italy; **Vitaliano Spagnuolo**, Lipid Ctr Cosenza, AO of Cosenza, Cosenza, Italy

Adverse Drug Reactions (ADRs) are a serious health related problem, which can limit the treatment options, compliance and even leads to discontinuation of therapy. Unfortunately not in all countries, the reporting reaches standard levels set by the WHO. As well, in our region (Calabria), located in south of Italy, only few reports are usually reported. For these reasons, the Italian Agency of the Drugs (AIFA) has been promoting and financing regional projects with multidisciplinary groups to increase spontaneous reporting. From first January 2018, we started a survey program finalized to report all adverse events from antihypertensive drugs observed in our Cardiovascular Prevention Clinic. We evaluated all of the 250 outpatients (≥18 years), consecutively visited in our clinic, from 1 January 2018 to 30 April 2018. All of these were in antihypertensive treatment (monotherapy or multiple drugs therapy). A total of 20 ADRs were observed. Of the 20 ADRs, 19 were non-serious events; a serious event required hospitalization (atrial-ventricular complete block). Causality assessment has been estimated with the Naranjo algorithm; the results indicated n=19 possible ADRs and n=1 probable. Incidence of ADRs was found to be higher in the age group between 45 and 54 years old (6 of 20) and males experienced more ADRs than females (35%, 7 of 20). ADRs were more frequently associated with combination therapy (65%, 13 of 20) versus monotherapy. Calcium channel-blockers were found to be the most

frequently associated drugs with ADRs (n=8), followed by angiotensin receptor blockers (n=4). We compared our reports with the reports, of the same period, available for our region obtained from National Pharmacovigilance Network. During this period were reported 27 side effect reactions in all our region (5 reports in the 2017). In conclusion, in the last 4 months, regional projects, with the creation of a multidisciplinary group composed of Medical and Pharmacists Facilitator, was useful in improving and increase quality and quantity of spontaneous reports in Calabria.

Disclosures: A. Maretta: None. **G. Fersini:** None. **V. Salerno:** None. **F. Saullo:** None. **R. Virno:** None. **V. Spagnuolo:** None.

Funding: No

Funding Component:

P377

Effect of Salt Diet on Haplotype Specific Expression of Human Angiotensinogen Gene

Authors: Indu Sivankutty, Brahmaraju Mopidevi, Sravan Perla, Sudhir Jain, Ashok Kumar, New York Medical Coll, Valhalla, NY

Human Angiotensinogen gene (hAGT) is an important component of the Renin- Angiotensin System. The 2.5 Kb promoter of the hAGT gene has ten polymorphisms that form two haplotype (Hap) blocks. Hap-I containing -6A, -20A, -217A, -532T, -793A, -1074T, -1178G, -1561T, -1562C, and -1670A is associated with increased blood pressure whereas Hap-II containing -6G, -20A, -217G, -532C, -793G, -1074G, -1178A, -1561G, -1562G, and -1670G is associated with normal blood pressure in human subjects. In order to understand the role of these SNPs in the transcriptional regulation, we have generated novel transgenic mice with the hAGT gene, targeted to the mHPRT locus with either Hap I or II variants. In the present study, we have examined the effect of high salt diet on the hAGT gene expression and transcriptional regulation in the 8 weeks old male transgenic mice containing these two haplotypes. Quantitative real time PCR analysis revealed an increase in hAGT expression in the liver of Hap-I as compared to Hap-II in basal condition (4.09 fold), Hap-I high salt vs Hap-I basal (2.15 fold), Hap-II high salt vs Hap-II basal (1.9 fold) and Hap-I high salt vs Hap-II high salt (2.47 fold) with $p<0.05$ in these transgenic mice. Immunoblot analysis also indicated the upregulation of the hAGT in plasma in all the study groups, viz; Hap-I compared to that of Hap-II (2.5 fold), Hap-I high salt vs Hap-I basal (1.9 fold) and Hap-II high salt vs Hap-II basal (1.7 fold) with $p<0.05$. Our studies show that the transgenic mice fed high salt diet have increased mRNA levels in the liver for the transcription factors HNF1, FOXO1, FOXA1 and MR (4, 3.13, 3.93 and 5.4 fold increase respectively; $p<0.05$). CHIP assay also complements the above observations. Our results strongly suggest that SNPs in Hap-I promote increased transcription and expression of the hAGT gene in multiple tissues, with resultant elevation of plasma hAGT levels. Our studies would provide new insights into the susceptibility of individuals to salt induced hypertension and explain the novel paradigm in salt induced transcriptional regulation of human angiotensinogen gene.

Disclosures: I. Sivankutty: None. **B. Mopidevi:** None. **S. Perla:** None. **S. Jain:** None. **A. Kumar:** None.

Funding: No

Funding Component:

P378

Dahl Salt-Sensitive Rats Exhibit Aberrant Renal Hemodynamic Responses to a High Salt Diet and Salt-Sensitive Hypertension as Compared to Consomic SS.BN1 Rats

Authors: Aaron J Polichnowski, Jacqueline Potter, Shannon Allen, Rhesa Dykes, Michelle Duffourc, East Tennessee State Univ, Johnson City, TN; Geoffrey A. Williamson, Illinois Inst of Technology, Chicago, IL

Altered renal vascular responses to a high salt diet have been proposed to contribute to salt-sensitive (SS) hypertension. The goals of this study were to assess: 1) SS hypertension and renal injury in Dahl SS vs. Brown-Norway (BN) and consomic SS.BN1 rats and 2) renal hemodynamics in conscious SS vs. SS.BN1 rats during consumption of a low and high salt diet. Systolic BP (24 hrs/day via telemetry), proteinuria and renal injury were assessed in 10 week old SS (n=9), BN (n=8) and SS.BN1 (n=8) rats during a 0.4% NaCl diet and for 3 weeks during a 4.0% NaCl diet. On a 0.4% NaCl diet, BP was different ($P<0.05$) among SS, BN and SS.BN1 rats (160±1 vs. 115±2 vs. 141±2 mmHg) while proteinuria was higher ($P<0.05$) in SS vs. BN and SS.BN1 rats (87±14 vs. 22±3 and 30±5 mg/day). A 4% NaCl diet exacerbated differences ($P<0.05$) in BP (188±3 vs. 122±2 vs. 150±1 mmHg) and proteinuria (259±29 vs. 29±5 vs. 68±15 mg/day) between SS vs. BN and SS.BN1 rats. Abundant uromodulin (UMOD) positive protein casts were only observed in SS rats. Interestingly, strikingly low levels of UMOD, located on chromosome 1, were found in the urine of SS vs. BN and SS.BN1 rats at baseline. Mean arterial BP (MAP, mmHg, via telemetry), renal vascular resistance (RVR, mmHg/ml/min) and renal blood flow (RBF, ml/min, via Transonic flow probe) were assessed in conscious 10 week old SS (n=5) vs. SS.BN1 (n=5) rats for 2-3 hrs/day over 3 days of 0.4% NaCl feeding and over days 3-7 of 4.0% NaCl feeding. While MAP was higher during a 0.4% NaCl diet in SS vs. SS.BN1 rats (137±3 vs. 121±3), interestingly, RVR was lower (16±2 vs. 20±2, $P<0.05$) and RBF higher (9±1 vs. 6±1, $P<0.05$) in SS vs. SS.BN1 rats. During 4.0% NaCl feeding, RVR decreased (17±2, $P<0.05$), RBF increased (8±1, $P<0.05$) and MAP remained unchanged (122±3) in SS.BN1 rats whereas RVR increased (19±3, $P<0.05$), RBF remained unchanged (8±1) and MAP increased (146±4, $P<0.05$) in SS rats. In conclusion, SS hypertension is abolished in SS.BN1 rats. Dahl SS rats exhibit an unexpected low RVR and high RBF when fed a 0.4% NaCl diet vs. SS.BN1 rats. Yet, SS rats display an impaired ability to lower RVR during a high salt diet as compared to SS.BN1 rats. These data warrant future investigations into the role that UMOD may play in altering renal hemodynamics, SS hypertension and CKD in Dahl SS rats.

Disclosures: A.J. Polichnowski: None. **J. Potter:** None. **S. Allen:** None. **R. Dykes:** None. **M. Duffourc:** None. **G.A. Williamson:** None.

Funding: Yes

Funding Component: National Center

P379

Paradoxical Natriuretic Peptide Resetting in Astronauts

Authors: Petra Frings-Meuthen, Jens Jordan, German Aerospace Ctr, Cologne, Germany; Ralf Lichtinghagen, Hannover Medical Sch, Hannover, Germany; Scott Smith, NASA Johnson Space Ctr, Houston, TX; Martina Heer, Univ of Bonn, Bonn, Germany

Human studies in space provide unique and often unexpected findings regarding the role of terrestrial gravity for human cardiovascular and renal regulation. While vascular volume is shifted towards the head, which would be expected to increase natriuretic peptide (NP) release, preliminary observations suggested the opposite. Therefore, we assessed natriuretic peptide regulation on different controlled sodium diets in space and on Earth. Eight male astronauts (50±2.8 yrs.) ingested higher (5.5 g/day) and low sodium (2g/day) diets for five days each on Earth and on the International Space Station (ISS) in alternating order. Dietary nutrient intake was individually tailored and kept constant. At day five of each intervention, midregional-pro atrial NP (MRproANP), N-terminal pro B-type NP (NTproBNP), and aldosterone in blood, urinary sodium excretion, and body mass were measured. On Earth, MRproANP was 71.86±4.39 pmol/L on moderately high and 65.13±6.71 pmol/L on low sodium intake. In space, MRproANP was 44.61±4.26 pmol/L on high and 31.37±4.02 pmol/L on low sodium intake ($p<0.001$ between sodium intakes, $p<0.01$ between Earth and space). Similarly,

NTproBNP responded to changes in sodium intake ($p<0.001$) and was regulated at lower levels in space ($p=0.008$). Serum aldosterone concentrations decreased on higher sodium intake but did not differ between space and Earth ($p<0.001$ between sodium intakes, $p=0.95$ between Earth and space). Body mass did not differ between low and high sodium intake, either on Earth or in space (space: low sodium 83.7±3.1 kg, higher sodium 84.4±3.1 kg, Earth: low sodium 87.1±3.6 kg, higher sodium 87.2±3.2 kg). In conclusion, NP release while responding to changes in sodium intake is paradoxically reset to lower levels in space. The mechanisms may have implications for volume regulation and cardiovascular health during long-term space travel.

Disclosures: P. Frings-Meuthen: None. **J. Jordan:** None. **R. Lichtinghagen:** None. **S. Smith:** None. **M. Heer:** None.

Funding: No

Funding Component:

P380

Inverse Circadian Pattern in the Renal Generation of Tumor Necrosis Factor-Alpha (TNFá) and Angiotensinogen (AGT) in Salt-Sensitive Hypertension (SSH) Induced by Angiotensin II (AngII) in Mice

Authors: Alexander Castillo, Nadia Khan, Isabella Shindler, L.Gabriel Navar, **Dewan S. A. Majid**, Tulane Univ Sch of Med, New Orleans, LA

Individuals with SSH show a non-dipping blood pressure (BP) during the inactive night period, a phenomenon which is significantly associated with enhanced cardiovascular morbidity and mortality. However, the pathophysiological mechanism for this phenomenon is not yet clearly understood. Chronic AngII administration in mice (reversed circadian rhythm) induced SSH with an increase in intra-renal AGT formation, a response that was exaggerated in TNFα receptor type 1 (TNFR1) knockout mice, indicating that renal AGT formation is suppressed by TNFR1 activation by TNFα. The present study examined the hypothesis that AngII-induced TNFα formation follows a circadian pattern with increases during the active period compared to the inactive period, that facilitates less AGT formation during the active period. Experiments were performed in mice chronically treated with or without AngII (25 ng/min; osmotic mini-pump) + high salt (4% NaCl) intake for 4 weeks that increased BP from 90±2 to 108±3 mmHg (tail-cuff method). Circadian rhythm in urinary parameters was assessed by 12 hour collections of urine using metabolic cages during the active night period (7PM to 7AM) and the inactive day period (7 AM to 7 PM). In normal mice (n=6), urinary excretion of TNFα (uTNFα) was higher (0.5±0.2 vs 0.13±0.08 pg/hour) but urinary excretion of AGT (uAGT) was lower (0.28±0.18 vs 0.46±0.14 ng/hour) during the active period compared to that during the inactive period. This inverse relationship between uTNFα and uAGT was exaggerated in AngII+HS treated mice (n=6). From the baseline (0 week) to the 2nd and 4th week period, there were marked incremental changes in uTNFα during active periods (0.68±0.20 to 14.2±5.4 and 44.6±14.6 pg/hour) which were less during inactive periods (0.73±0.48 to 10.3±3.3 and 12.1±4.7 pg/hour). Interestingly, uAGT changed minimally during active periods (0.21±0.14 to 0.05±0.04 and 1.42±0.97 ng/hour) but increased significantly during inactive periods (0.32±0.14 to 1.57±0.71 and 3.98±1.67 ng/hour). These data suggest that an increase in TNFα generation suppresses intra-renal AGT formation during active periods but this phenomenon is inversed during inactive periods. The increase in AGT during the inactive period could lead to a non-dipping BP pattern in SSH.

Disclosures: A. Castillo: None. **N. Khan:** None. **I. Shindler:** None. **L. Navar:** None. **D.S. Majid:** None.

Funding: No

Funding Component:

P381

Deletion of Na^+/H^+ Exchanger 3 Selectively in the Proximal Tubule of the Kidney Augments Acute and Chronic Natriuretic Responses to Saline Volume Expansion in Mice

Authors: Xiao C Li, Univ Mississippi Medical Ctr, Jackson, MS; Manoocher Soleimani, Univ of Cincinnatti, Cincinnatti, OH; Hoang T Nugyen, Jia L Zhuo, Univ Mississippi Medical Ctr, Jackson, MS

The Na^+/H^+ exchanger 3 (NHE3), a ~85 kDa protein encoded by the *SLC9A3* gene, plays a key role in mediating Na^+ reabsorption in the proximal tubule of the kidney and in maintaining blood pressure homeostasis. In the present study, we tested the hypothesis that deletion of NHE3 selectively in the proximal tubule of the kidney, but not globally, augments acute and chronic natriuretic responses to saline volume expansion in mice. Adult male wildtype (*Nhe3*$^{+/+}$), global NHE3 knockout (*Nhe3*$^{-/-}$) and proximal tubule-specific NHE3 knockout mice (PT-*Nhe3*$^{-/-}$) (n=8-13/group) were subject to acute saline administration of 10% body wt., i.p., or a 2% high salt diet for 2 weeks to determine and compare acute or chronic natriuretic responses. In response to 10% acute saline volume expansion, urinary Na^+ excretion increased 6.5 fold from basal 18.9 ± 5.0 µmol/3h to 122.2 ± 7.7 µmol/3h in wildtype mice (p<0.01). By comparison, urinary Na^+ excretion increased 8.4 fold from basal 26.2 ± 3.9 µmol/3h to 206.5 ± 5.7 µmol/3h in PT-*Nhe3*$^{-/-}$ mice (p<0.01), whereas the natriuretic response increased 4.8 fold in *Nhe3*$^{-/-}$ mice (p<0.01). The differences in the natriuretic responses to acute saline expansion between *Nhe3*$^{+/+}$, *Nhe3*$^{-/-}$ and PT-*Nhe3*$^{-/-}$ mice were statistically significant (p<0.01). However, the diuretic response to acute water volume expansion (10% of body wt., i.p.) was not different between *Nhe3*$^{+/+}$ and PT-*Nhe3*$^{-/-}$ mice (*n.s.*). In response to 2% NaCl diet for 2 weeks, 24 h urinary Na^+ excretion increased 19% in *Nhe3*$^{+/+}$ mice (*n.s.*), whereas the natriuretic response increased 43% in PT-*Nhe3*$^{-/-}$ mice (p<0.01). Interestingly, the natriuretic responses to 2% high salt diet were greater in adult female wildtype (Δ66%, p<0.01) and PT-*Nhe3*$^{-/-}$ mice (Δ62%, p<0.01), respectively. By contrast, the natriuretic response to 2% high salt diet was significantly attenuated in global *Nhe3*$^{-/-}$ mice (p<0.01). These data suggest that the deletion of NHE3 selectively in the proximal tubule of the kidney, but not globally, augments the natriuretic responses to acute or chronic saline volume expansion in PT-*Nhe3*$^{-/-}$ mice.

Disclosures: X.C. Li: None. **M. Soleimani:** None. **H.T. Nugyen:** None. **J.L. Zhuo:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; NIH/NIDDK.

Funding: No

Funding Component:

P382

The Role of Atrial Natriuretic Peptide in Cardiac Damage During Salt Sensitive Hypertension

Authors: Daria Ilatovskaya, Medical Univ of South Carolina, Charleston, SC; Kristen Winsor, Adrian Zietara, Mark Paterson, Vladislav Levchenko, Alison Kriegel, Alexander Staruschenko, Medical Coll of Wisconsin, Milwaukee, WI

Atrial natriuretic peptide (ANP), encoded by the *Nppa* gene, is an osmoregulatory hormone that promotes salt excretion. GWAS identified *Nppa* to be associated with hypertension; some studies suggest varying ANP levels as an indicator of salt-sensitivity. The aim of the current project was to assess the effects of ANP deficiency on cardiac damage and function using a knockout of *Nppa* in the Dahl Salt-Sensitive (SS) rat background (SS$^{NPPA-/-}$). A combination of in vivo techniques with ex vivo and biochemical methods were used to test the role of ANP in the development of SS hypertension in male and female rats. IHC analysis was employed to quantify tissue damage; echocardiography was used to test heart function.
SS$^{NPPA-/-}$ rats demonstrated higher blood pressure compared to SS controls when fed a high salt (HS, 4% NaCl) diet: on day 21 of diet MAP was 185.8 ± 9 mmHg in SS$^{NPPA-/-}$ rats compared to 144.6 ± 4 mmHg in SS controls. Heart rate was

found to be on average 40 bpm lower in $SS^{NPPA-/-}$ rats until day 8 of the HS challenge, when it rose up to the rate of wild type counterparts and continued to decline at the same pace. $SS^{NPPA-/-}$ rats showed exacerbated kidney damage, reduced diuresis and lower sodium excretion when fed a HS diet. $SS^{NPPA-/-}$ rats exhibited intensified cardiac damage compared to SS controls, as demonstrated by heart to body weight ratio after HS (3.9 ± 0.13 in wild type vs 5.5 ± 0.09 in KO rats), elevated cardiac fibrosis (up to 6% of fibrotic cardiac tissue area in $SS^{NPPA-/-}$ vs less than 3% in wild types), and a significantly increased cardiac vessel media thickness. Fibrosis and heart hypertrophy were attenuated in female rats. Echocardiography revealed that although $SS^{NPPA-/-}$ rats show heart remodeling, ejection fraction is preserved and they do not exhibit heart failure. Chronic i.v. infusion of ANP in SS rats (100 ng/kg/day) attenuated the HS-induced increase in blood pressure and renal damage, and resulted in less cardiac hypertrophy and fibrosis (1% fibrosis in ANP-infused animals vs 3% in vehicle-treated group).
Therefore, ANP deficiency aggravates SS hypertension and cardiac damage; further work is needed to reveal if ANP deficiency causes heart failure of a HF/PEF phenotype, and what the interplay between heart and kidney is in this setting.

Disclosures: D. Ilatovskaya: None. **K. Winsor:** None. **A. Zietara:** None. **M. Paterson:** None. **V. Levchenko:** None. **A. Kriegel:** None. **A. Staruschenko:** None.

Funding: No

Funding Component:

P383

High Sodium Intake is Independently Associated with Subclinical Hypercortisolism in Hypertensive Patients

Authors: Giacomo Rossitto, Univ of Glasgow, Glasgow, United Kingdom; Giuseppe Maiolino, Silvia Lerco, Valeria Bisogni, Maurizio Cesari, Giulio Ceolotto, Univ di Padova, Padova, Italy; Rhian M Touyz, Univ of Glasgow, Glasgow, United Kingdom; Alessio Pinato, Mario Plebani, Gian Paolo Rossi, Univ di Padova, Padova, Italy; Christian Delles, Univ of Glasgow, Glasgow, United Kingdom

The widely accepted notion of an increase in water intake to compensate for a similar increase in salt consumption has recently been challenged by evidence of long-term renal and metabolic mechanisms favouring endogenous water accrual and body fluid preservation. A glucocorticoid-driven catabolic state is instrumental to this aim and is prevented by increased food intake in rodents. Demonstration of these mechanisms in large human populations is lacking and their relevance for cardiovascular disease is therefore unknown. We explored the association between sodium intake (estimated by 24h urine sodium excretion; USE) and 24h urinary free cortisol (UFC) in 145 patients screened for secondary causes of hypertension in a tertiary referral centre, after washout from drugs affecting the renin-angiotensin-aldosterone system (63 males, 43.3%; age, 48 [38-56] years; BMI, 27.1 [24.3-31.0] kg/m²). USE was directly associated with UFC (Spearman's rho=0.407, p<0.001), as well as male gender (r=0.381, p<0.001), BMI (r=0.243, p=0.006), creatinine clearance (r=0.637, p<0.001) and, borderline, to plasma glucose (r=0.268, p=0.065). Importantly, UFC increased across classes of sodium intake (Low < 2.3g/d, Medium 2.3-5g/d and High > 5g/d) in lean and overweight groups (n=41, ANOVA p = 0.005 and n= 49, ANOVA p < 0.001, respectively), but not in obese subjects (n=35; p = 0.083). Linear regression analysis showed that (square root-transformed) USE was the strongest explanatory variable for UFC after correction for age, gender, BMI, eGFR and aldosterone:renin ratio (β = 0.04, p < 0.0005). A regression model that also included (Log-transformed) BMI as a surrogate of excess food intake (β = -0.51, p = 0.042), explained 23% of UFC variability in the cohort. We conclude that sodium intake, as estimated by USE, is an independent predictor of subclinical hypercortisolism in lean and overweight but possibly not in obese subjects. These data, in a population at increased cardiovascular risk, suggest a link between sodium intake and subclinical metabolic derangement, which could further add to the overall risk profile.

Disclosures: G. Rossitto: None. **G. Maiolino:** None. **S. Lerco:** None. **V. Bisogni:** None. **M. Cesari:** None. **G. Ceolotto:** None. **R.M. Touyz:** None. **A. Pinato:** None. **M. Plebani:** None. **G. Rossi:** None. **C. Delles:** None.

Funding:

Funding Component:

P384

Conditional knockout of Acid Ceramidase in Renal Collecting Ducts Induced Sodium Retention in Mice

Authors: Weili Wang, Pin-Lan Li, Joseph Ritter, **Ningjun Li**, Virginia Commonwealth Univ, Richmond, VA

We have previously shown that renal sphingosine-1-phosphate (S1P), a bioactive sphingolipid metabolite formed by phosphorylation of sphingosine, increases sodium excretion through inhibition of epithelial sodium channel (ENaC) via S1P receptor 1, which is mainly localized in the collecting ducts. However, the role of S1P-producing enzyme in renal sodium excretion remains unknown. The immediate substrate for S1P generation, sphingosine, is formed from the hydrolysis of ceramide by ceramidase, which is a rate-limiting step for S1P production. Acid ceramidase (AC) is the major form of ceramidase in collecting duct. The present study was to test the hypothesis that deletion of AC in the collecting ducts impacts renal sodium handling. Mice with collecting duct-specific AC knockout (CD-AC-KO) were generated by crossing AC floxed mice with Aquoporin 2-Cre mice. The specific knockout of AC in renal collecting ducts was confirmed by immunohistochemistry and Western blot analyses. Functionally, urinary sodium excretion was decreased by 40% in CD-AC-KO mice compared with control mice (Ctrl) after acute IV sodium loading (2.19±0.32 vs. 1.3±0.18 μmole/min.g kwt in Ctrl vs. KO, P<0.05). Furthermore, the natriuretic response to elevated renal perfusion pressure (pressure natriuresis) was severely impaired in CD-AD-KO mice compared with control mice (5.3±0.54 vs. 3.0±0.25 μmole/min.g kwt in Ctrl vs. KO, P<0.05). Moreover, chronic high salt-induced sodium retention was remarkably enhanced in CD-AC-KO mice compared with control animals (2.9±0.21 vs. 5.0±0.30 mmole/100g bw. 24h in Ctrl vs. KO, P<0.05). These results suggest that AC in renal collecting ducts plays an important role in the regulation of renal sodium excretion and that modulation of AC function in the kidneys could be a therapeutic approach for salt-sensitive hypertension.

Disclosures: W. Wang: None. **P. Li:** None. **J. Ritter:** None. **N. Li:** None.

Funding:

Funding Component:

P385

The Enhanced Antihypertensive Effect by a Combination Diet of Allium Bakeri and Capsaicin in 2-Kidney, 1-Clip Renovascular Hypertensive Rats

Authors: Nobutaka Kurihara, Yukiko Segawa, Saki Maruyama, Kobe Women's Univ, Kobe, Japan; Tomoko Osera, Toyo Univ, Itakura, Gunma, Japan; Hiroko Hashimoto, Osaka Seikei Coll, Higashi-Yodogawa, Japan

Objective: Allicin contained in Allium Bakeri (AB) , a transient receptor potential (TRP) vanilloid type-1 (TRPV1) and TRP ankyrin-1 (TRPA1) agonist, increases phosphorylated (p-) endothelial nitric oxide synthase (eNOS). Another TRPV1 agonist, capsaicin (CAP), causes a vasodilatory effect through the increment of p-eNOS. We recently demonstrated the alleviation of blood pressure (BP) elevation by each intake of AB and CAP in 2-kidney, 1-clip (2K1C) hypertensive rats. AB is often used with red hot chili pepper containing CAP in the Japanese cuisine. We hypothesized that a combination diet of AB and CAP enhances the antihypertensive effect compared with each diet. In this study, to test the hypothesis, we investigated the interactive effects of dietary AB and CAP on BP, as well as the p-eNOS/eNOS in the protein expression

of aorta, in 2K1C rats.
Method: Male Sprague-Dawley rats (6wks) were treated with sham operation (SHAM) or clipping the left renal artery (2K1C). After surgery, SHAM rats received a control diet (CTL), and 2K1C rats received CTL, a diet with 1% (10 of 1,000, w/w) freeze-dried AB powder, with 0.006% (60 of 1,000,000, w/w) CAP or with both (AB+CAP) for 6 weeks. The systolic BP (SBP) was measured by a tail-cuff method once per week. At the end of protocol, rats were euthanized and the thoracic aortas were collected for extracting the protein. The p-eNOS/eNOS in the protein expression in aorta was evaluated by Western blotting.
Result: Analysis of variance showed that SBP was significantly higher in 2K1C-CTL than SHAM-CTL through the protocol (123±2.3 vs 165±3.2 mmHg, $p < 0.05$, at 6 weeks after the surgery). SBP was lower in 2K1C-AB and -CAP in 2K1C-CTL (151±2.4 and 139±3.7 vs 165±3.2, $p < 0.05$, each). Compared with 2K1C-AB and -CAP, a significant reduction in SBP was observed in 2K1C-AB+CAP (135±3.1, at 6 weeks) throughout the procedure ($p < 0.05$). The p-eNOS/eNOS was tended to be enhanced in 2K1C-AB and -CAP, but not in 2K1C-AB+CAP, compared to 2K1C-CTL.
Conclusion: A combination diet of AB and CAP may enhance antihypertensive effects compared with each diet of AB and CAP, in 2K1C rats. Although the increase in eNOS phosphorylation may participate in the mechanism of the antihypertensive effects by each diet of AB and CAP, it may not do in that of their combined effect.

Disclosures: **N. Kurihara:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; The Tojuro Iijima Foundation for Food Science and Technology. **Y. Segawa:** None. **S. Maruyama:** None. **T. Osera:** None. **H. Hashimoto:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; The Tojuro Iijima Foundation for Food Science and Technology.

Funding: No

Funding Component:

P386

Effects of Saccharina Japonica Intake on Blood Pressure in 2-Kidney, 1-Clip Renovascular Hypertensive Rats fed a High-Sodium Diet

Authors: Saki Maruyama, Yukiko Segawa, Kobe Women's Univ, Kobe, Japan; Hiroko Hashimoto, Osaka Seikei Coll, Higashi-Yodogawa, Japan; Tomoko Osera, Toyo Univ, Itakura, Japan; Nobutaka Kurihara, Kobe Women's Univ, Kobe, Japan

Objective: One of foods to Japanese cuisine “Washoku” is algae, including Saccharina japonica (SJ). The intake of SJ is reported to decrease blood pressure (BP) in spontaneously hypertensive rats in some studies, and in 2-kidney, 1-clip hypertensive (2K1C) rats in our studies. Water soluble-alginate is rich in SJ. Since alginate has a physiological function to suppress sodium absorption in the gastrointestinal tract, SJ intake may alleviate hypertension to the extent according to the salt intake in 2K1C rats. In the present study, we observed the effects of SJ intake on BP in 2K1C rats fed a normal-sodium (NS) or high-sodium (HS) diet. **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 NS (0.7% NaCl) or HS diet (6.0% NaCl), with or without 5.0% (w/w) SJ for 6 consecutive 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. **Results and Discussion:** Analysis of variance showed that SBP was significantly higher in 2K1C-NS-CTL than in SHAM-NS-CTL through the experiment period ($P<0.001$, Fig), and that SBP was higher in 2K1C-HS-CTL than in 2K1C-NS-CTL ($P<0.05$). It also demonstrated that 2K1C-HS-SJ provided a significant reduction in SBP compared with 2K1C-HS-CTL ($P<0.05$), while there were no significant differences in SBP between 2K1C-NS-CTL and 2K1C-NS-SJ. At the end of the protocol, MAP showed the similar trend to SBP. These results suggested that the intake of SJ decreases BP, probably by suppressing sodium absorption in 2K1C rats.

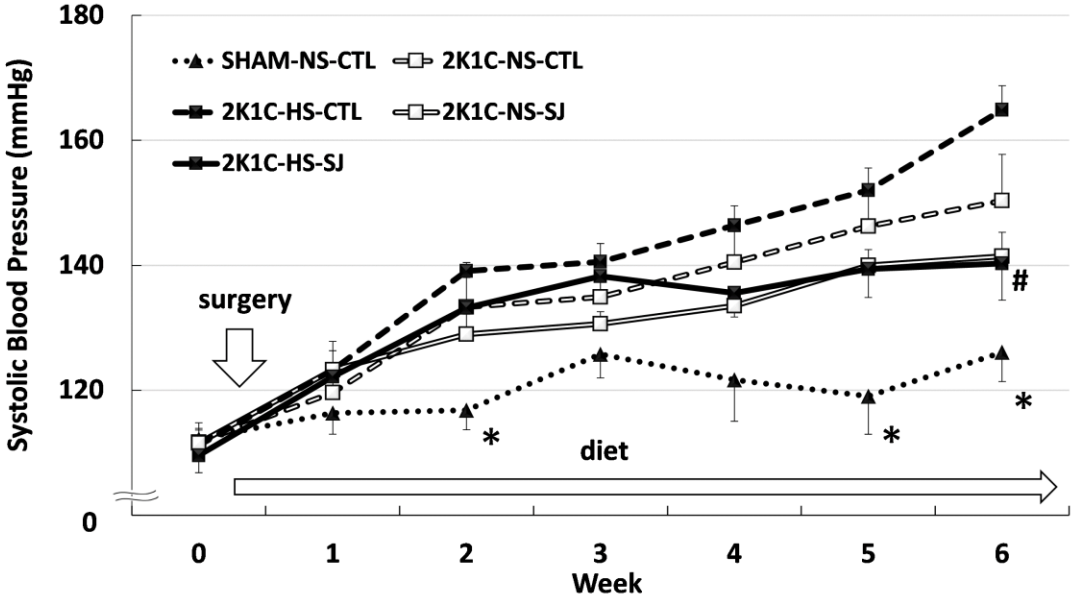


Fig. Systolic blood pressure by a tail-cuff method in SHAM or 2K1C rats fed a NS or HS with or without SJ for 6 weeks.

Values are mean ± SE, n=5-6. Four-way ANOVA: $P < 0.05$ for time, animal (SHAM vs 2K1C), salt (NS vs HS), SJ (CTL vs SJ), time X animal. Two-way ANOVA between 2 groups of 2K1C: $P < 0.05$ for SHAM-NS-CTL vs 2K1C-NS-CTL, 2K1C-NS-CTL vs 2K1C-HS-CTL and 2K1C-HS-CTL vs 2K1C-HS-SJ and $P = 0.10$ for 2K1C-NS-CTL vs 2K1C-NS-SJ. * $P < 0.05$ vs 2K1C-NS-CTL. # $P < 0.01$ vs 2K1C-HS-CTL. SHAM, sham-operated control rats; 2K1C, two-kidney, one-clip hypertensive rats; NS, a normal-sodium diet; HS, a high-sodium diet; CTL, a control diet; SJ, a diet with Saccarina japonica.

Disclosures: S. Maruyama: None. **Y. Segawa:** None. **H. Hashimoto:** None. **T. Osera:** None. **N. Kurihara:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Grant-in-Aid for Scientific Reasearch in Japan. #15K00862..

Funding:

Funding Component:

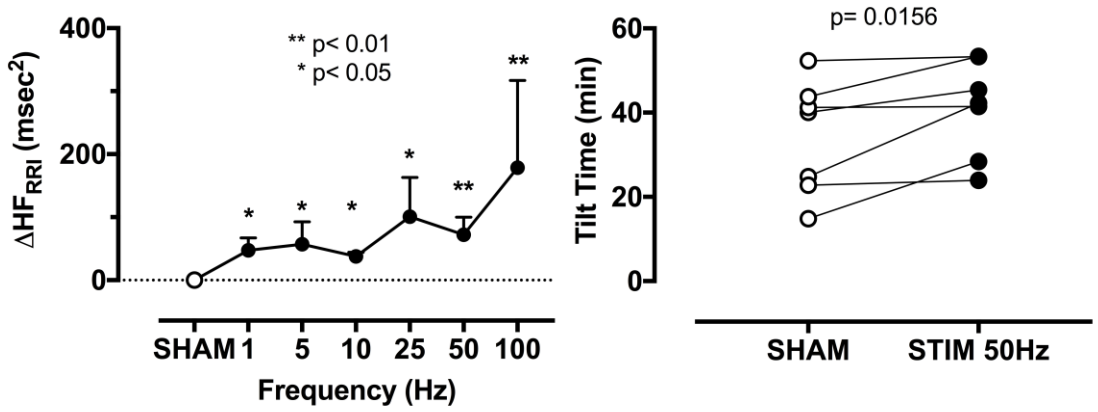
P387

Sub-Perception Transdermal Vagal Stimulation in Postural Tachycardia Syndrome

Authors: André Diedrich, Luis Okamoto, Bonnie Black, Misty D Hale, Italo Biaggioni, Vanderbilt Medical Ctr, Nashville, TN

Postural Tachycardia Syndrome (POTS) is a clinical syndrome characterized by an excessive increase in heart rate (>= 30 bpm) on standing in the absence of orthostatic hypotension, and frequent orthostatic symptoms for more than 6 months. While most researchers focus on the hyperadrenergic features of POTS, these patients also have significant cardio-vagal (parasympathetic) impairment. We tested the hypothesis that transdermal electrical stimulation of the auricular branch of the vagus nerve will enhance cardio-vagal modulation, reduce heart rate, upright symptoms, and improve orthostatic tolerance. We studied 14 patients with POTS (31.5+/-11.7 years, BMI 22.6+/-3.9 kg/m^2). Sham or sub-perception threshold transdermal electrical vagal stimulation was applied in random order to the auricular branch in the right ear in supine and during graded tilt maneuver. Patients with low vagal modulation (low high frequency HF < 200 ms^2) responded to vagal stimulation (Kruskal Wallis p=0.01, n=7, Fig left) with significant increase in HF power (eg @50Hz: +51+/-10 ms^2, p=0.0032). Vagal stimulation during upright tilt tended to reduce orthostatic tachycardia and overall orthostatic symptom score. It improved significantly tilt time (+5.3+/-2.6 min, p=0.0156, Fig right). Patients with higher baseline vagal modulation (HF >= 200 ms^2) did not respond to vagal stimulation (interaction p=0.41). This proof of concept study indicates that auricular transdermal vagal stimulation improves supine cardio-vagal function in POTS

patients with low vagal modulation. Further research will determine if this approach can be used therapeutically, alone or in combination with other therapies.



Disclosures: A. Diedrich: None. **L. Okamoto:** None. **B. Black:** None. **M.D. Hale:** None. **I. Biaggioni:** None.

Funding: No

Funding Component:

P388

Lifestyle Interventions Reduce the Need for Guideline-Directed Antihypertensive Medication

Authors: Alan Hinderliter, Univ of North Carolina, Chapel Hill, NC; Patrick Smith, Andrew Sherwood, James Blumenthal, Duke Univ, Durham, NC

Introduction: The 2017 ACC-AHA Hypertension Guideline recommends lifestyle modification in the initial treatment of hypertension, and therapy with medications based on a threshold blood pressure determined by overall cardiovascular risk. In this secondary analysis of the ENCORE trial, we examined the effects of the DASH diet alone and in combination with weight loss and aerobic exercise on the indications for antihypertensive medication.
Methods: Participants included 129 overweight or obese (BMI=25-40 kg/m^2) men and women between the ages of 40 and 80 years who had blood pressure 130-160/80-99 mmHg and who were not being treated with antihypertensive medications. Participants were randomized to 16 weeks of DASH diet plus behavioral weight management (consisting of nutritional counseling and a behavioral weight management program; DASH+WM), DASH diet alone (consisting of nutritional counseling without exercise or caloric restriction; DASH), or usual care controls (maintenance of usual exercise and dietary habits; UC).
Results: The participants averaged 53.5±8.8 years of age; 68% (88 of 129) were female, and 60% (77 of 129) were white. Nineteen had an estimated 10-year atherosclerotic cardiovascular disease risk ≥ 10% by the Pooled Cohort Equations, and 1 had diabetes. Blood pressure averaged 138±9/86±6 mmHg. Criteria for treatment with antihypertensive medications, as defined in the 2017 ACC-AHA Hypertension Guideline, were present in 53% (68 of 129). Blood pressure fell by 16/10 mmHg in DASH+WM, 11/8 mmHg in DASH, and 3/4 mmHg in UC. The percentage of subjects with indications for antihypertensive medical therapy fell from 54% (25 of 46) to 15% (7 of 46) in the DASH+WM group and from 51% (20 of 39) to 23% (9 of 39) in the DASH group; and did not significantly change (55% [24 of 44] to 48% [21 of 44]) in the UC group (p = 0.001 for active treatments vs UC; p = 0.011 for DASH+WM vs DASH alone).
Conclusions: In overweight or obese men and women with hypertension, lifestyle interventions dramatically decrease the number of individuals for whom guideline-directed antihypertensive medication is indicated.

Disclosures: A. Hinderliter: None. **P. Smith:** None. **A. Sherwood:** None. **J. Blumenthal:** None.

Funding: No

Funding Component:

P389

Lowered Blood Pressure With Hypertension Management Model in a West African Population: Increased Efficiency and Implications for Improved Outcomes

Authors: Isaac Kofi Owusu, Kwame Nkrumah Univ of Science and Technology, Kumsai, Ghana; Fred Adomako-Boateng, Regional Health Directorate, Ghana Health Service, Kumsai, Ghana; Fred Kueffer, Medtronic Cardiovascular, Santa Rosa, CA; Molly Guy, Chemuttaai Lang'at, Teri Whitman, **Douglas Hettrick**, Holloman Keith, Medtronic, Minneapolis, MN; K.O. Opare-Sem, Kwame Nkrumah Univ of Science and Technology, Kumsai, Ghana

Objective: A novel locally appropriate hypertension management model of care was developed and tested in the Republic of Ghana to address specific socioeconomic and community-related barriers to blood pressure (BP) control. **Design and Methods:** Patients with a history of hypertension were enrolled and each agreed to visit a participating nearby community pharmacy at least once per week for 6 months for a BP check, symptom review, and medication monitoring. Weekly pharmacy visit data were manually logged into a mobile application by the pharmacy staff. Guideline based logic in the application provided immediate feedback to the patient and transmitted data to the primary physician. Electronic prescriptions, from the primary physician, were accessible to participating pharmacies. Clinic blood pressure from the prior 6 months was also retrieved from patient records. **Results:** Compliance with weekly BP assessments in the 150 enrolled patients (57± 8 years; 73% Female) was 61% and 2705 total pharmacy BP assessments were conducted. Improvement in overall health awareness was reported in 82% of the patients and 95% indicated a desire to continue using the model of care in the future. During the 6-month voluntary program period, the number of scheduled office visits decreased by 60% compared to standard monthly visits. Despite fewer in clinic visits, average systolic BP decreased significantly from the 6-month pre-trial period baseline (137.4±14.0 to 129.8± 16.3 mmHg, $p<0.01$). The proportion of patients with blood pressure below target (140 mmHg) for least 75% of all readings increased from 41% in the 6 months prior to enrollment to 62% during the study follow up period ($p<0.01$). This improvement was associated with potential cardiovascular risk reduction of 5-21% based on previous randomized trials. The proportion of patients with > 75% controlled BP also improved in the subset of patients with BP below target at enrollment from 52% to 76% ($p<0.01$), implying potential risk benefits even in “well controlled” patients. **Conclusions:** The care model applied in this hypertensive West African resulted in fewer clinic visits and high patient and clinician satisfaction as well decreased systolic blood pressure and increased proportion of time with controlled blood pressure.

Disclosures: I. Kofi Owusu: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Medtronic, Inc. **F. Adomako-Boateng:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Medtronic, Inc. **F. Kueffer:** A. Employment; Significant; Medtronic, Inc. **M. Guy:** A. Employment; Significant; Medtronic, Inc. **C. Lang'at:** A. Employment; Significant; Medtronic, Inc. **T. Whitman:** A. Employment; Significant; Medtronic, Inc. **D. Hettrick:** A. Employment; Significant; Medtronic, Inc. **H. Keith:** A. Employment; Significant; Medtronic, Inc. **K. Opare-Sem:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Medtronic, Inc..

Funding: No

Funding Component:

P391

Ambulatory Blood Pressure Phenotypes and Cardiovascular Target Organ Damage in Adolescents: The SHIP AHOY Study

Authors: 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, Philadelphia, 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 cardiovascular (CV) events. Ambulatory blood pressure (ABP) allows better prediction of TOD than clinic BP in adults, but data in youth are lacking. We aimed to determine if BP phenotype, based on a combination of clinic BP and ABP, predicts underlying CV TOD in otherwise healthy adolescents. We evaluated clinic BP (mean of 6 auscultatory BP's), ABP (Spacelabs OnTrak), left ventricular mass index (LVMI), pulse wave velocity (PWV), diastolic function (E/e'), and systolic function (longitudinal strain) in 244 adolescents (median age 15.7 yrs, 63% white, 54% male). Clinic HTN was defined according to pediatric guidelines, and ambulatory HTN was defined as wake systolic BP $\geq 95^{th}$ percentile for sex and height. General linear models were used to evaluate associations between BP phenotype and TOD. Normal BP phenotype was found in 162 participants (66%), while 44 (18%), 16 (7%), and 22 (9%) had white coat (WCH), masked (MH), and ambulatory (AH) HTN, respectively. Participants with an abnormal BP phenotype had higher LVMI and PWV compared to those with normal BP (table). Participants with AH also had significantly higher PWV than those with WCH or MH. Adolescents with MH or AH had worse diastolic function (higher E/e') compared to those with normal BP or WCH. Only participants with AH had significantly worse systolic function (less negative strain) compared to those with normal BP; there was no significant difference in strain among subjects with normal BP, WCH and MH. In conclusion, ABPM improves TOD risk stratification in adolescents evaluated for HTN.

	BP Phenotype			
	Normal BP (n=162)	**WCH (n=44)**	**MH (n=16)**	**SH (n=22)**
LVMI ($g/m^{2.7}$)	31.6±7.0	35.0±7.1*	34.2±7.8	35.7±6.9*
PWV (cm/sec)	4.8±0.7	5.3±1.1*	5.1±0.7	5.8±1.0*,†
E/e'#	5.8±1.3	5.6±1.2	6.7±1.5§	7.2±1.7§
Strain (%)	-21.2±3.1	-20.3±3.3	-20.4±3.4	-18.7±3.5*

*p<0.05 compared with normal BP
†p<0.05 compared with WCH and MH
#The ratio of transmitral Doppler early filling velocity to tissue Doppler early diastolic mitral annular velocity
§p<0.05 compared with normal BP and WCH

Disclosures: G. Hamdani: B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA grant 15SFRN23680000. **E.M. Urbina:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1 TR001425. B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA grant 15SFRN23680000. **M. Lande:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA grant 15SFRN23680000. **K. Meyers:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; meyersk@email.chop.edu. **J. Samuels:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA

grant 15SFRN23680000. **J.T. Flynn:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Significant; AHA grant 15SFRN23680000.

Funding: Yes

Funding Component: National Center

P392

Ambulatory Arterial Stiffness Index Among Children With Chronic Kidney Disease

Authors: Susan M Halbach, Seattle Children's Hosp, Univ of Washington, Seattle, WA; Megan Carroll, Johns Hopkins Univ, Baltimore, MD; Derek K Ng, John Hopkins Univ, Baltimore, MD; Joseph T Flynn, Seattle Children's Hosp, Univ of Washington, Seattle, WA; Joshua Samuels, McGovern Medical Sch, Univ of Texas Health Science Ctr at Houston, Houston, TX; Susan L Furth, The Children's Hosp of Philadelphia, Philadelphia, PA; Bradley A Warady, Children's Mercy Kansas City, Kansas City, KS

The ambulatory arterial stiffness index (AASI) is a non-invasive measure of arterial stiffness derived from ambulatory blood pressure monitoring (ABPM) data. Studies in children have shown higher AASI is associated with obesity and hypertension, but there are no data examining AASI in children with chronic kidney disease (CKD).
We used data from the Chronic Kidney Disease in Children (CKiD) Cohort Study to evaluate the association between AASI and hypertensive status on ABPM, and to identify predictors of AASI, including BMI, proteinuria, mineral metabolism and BP parameters from ABPM.
Of 639 CKiD participants at their first successful ABPM visit, the mean age was 12 years, 60% (382/639) were male, 15% (96/639) were obese and the mean eGFR was 52 ml/min/1.73m^2. Approximately two-thirds reported antihypertensive therapy use (66%, 422/639) yet 58% (368/639) met criteria for abnormal ABPM (elevated mean awake/sleep DBP/SBP or awake/sleep load >25%). Participants who met criteria for abnormal ambulatory BP had a higher mean AASI than those with a normal ambulatory BP (0.337 and 0.307, respectively, p=0.008). Masked hypertension (MH) was common (42.4%, 284/639) and those subjects had a significantly higher AASI compared to normotensive children (Table). Male sex, age and BMI category were all positively associated with AASI, but presence of proteinuria, abnormal serum calcium or phosphate were not.
AASI is associated with hypertensive status and BMI in children with CKD and may be a useful non-invasive measure of vascular stiffness in this population. The relationship of AASI to other measures of vascular stiffness such as pulse wave velocity requires further study.

Mean AASI scores by hypertensive status at first successful ABPM visit

	N (%)	Mean	SD	p-value[ab]
Hypertensive Status				
Normotensive	252 (39.9)	0.308	0.14	(ref)
White Coat	15 (2.4)	0.288	0.11	0.561
Masked	283 (44.8)	0.351	0.14	<.001
Confirmed	82 (13.0)	0.287	0.15	0.238

[a] 8 participants missing AASI scores and did not contribute to the test for differences
[b] p-values from test for differences between each hypertensive status and the normotensive reference group

Disclosures: S.M. Halbach: None. **M. Carroll:** None. **D.K. Ng:** None. **J.T. Flynn:** G. Consultant/Advisory Board; Modest; Ultragenyx, Inc., Silvergate Pharmaceuticals. **J. Samuels:** None. **S.L. Furth:** None. **B.A. Warady:** None.

Funding: No

Funding Component:

P393

Sex Modifies the Longitudinal Association of Adiposity With Left Ventricular Hypertrophy Among Children With Chronic Kidney Disease

Authors: Tammy McLoughlin Brady, Jennifer Roem, Christopher Cox, Michael Schneider, John Hopkins Univ, Baltimore, MD; Amy Wilson, J.W. Riley Hosp for Children, Indianapolis, IN; Susan Furth, Children's Hosp of Philadelphia, Philadelphia, PA; Bradley Warady, Children's Mercy Hosp, Kansas City, MO; Mark Mitsnefes, Cincinnati Children's Hosp, Cincinnati, OH

Objective: Adiposity, not blood pressure (BP), is associated with left ventricular hypertrophy (LVH) among hypertensive children without chronic kidney disease (CKD). We aimed to determine the longitudinal association of BMI z-score with LVH and left ventricular mass index (LVMI) among children with CKD.
Methods: 696 participants of the Chronic Kidney Disease in Children study with echocardiography results who contributed a total of 1,300 visits were included. Mixed models, adjusting for repeated visits with a random subject effect, were used to determine the longitudinal association of body mass index (BMI) z-score with LVMI and LVH (LVMI> age-sex specific 95th %ile). Models were adjusted for age, sex, race, systolic and diastolic BP z-score, glomerular diagnosis, time with CKD, glomerular filtration rate and calcium*phosphorus product and accounted for informative censoring.
Results: Baseline characteristics are in the table. Among females, a 1 unit increase in BMI z-score was associated with an 8.5% increase in LVMI and 3.4 greater odds of LVH, whereas with boys, a 1 unit increase in BMI z-score was associated with a 5.2% increase in LVMI and a 1.4 greater odds of LVH ($p<0.05$ for all).
Conclusions: Among children with mild-moderate CKD, adiposity is independently associated with LVMI and LVH over time. This association is greater among females, a finding that may have future clinical and research implications.

Characteristics of the 696 participants of the Chronic Kidney Disease in Children (CKiD) study at the first visit with Echocardiography and Body Mass Index obtained concurrently.	
Characteristic	**Median (interquartile range) or % (N)**
Left ventricular mass (LVM), g	83.4 (57.1, 110.9)
LVM index, $g/m^{2.7}$	30.3 (25.3, 36.0)
Left Ventricular Hypertrophy	
LVM index > 95[th] %ile	11% (75)
LVM index > 51 $g/m^{2.7}$	4% (25)
Age-Sex specific Body Mass Index z-score[a]	0.44 (-0.34, 1.20)
Overweight (85[th] ≤ BMI %ile[a] < 95[th])	14% (94)
Obese (BMI %ile[a] ≥ 95[th])	15% (106)
Baseline Age, years	11.0 (7.7, 14.6)
Baseline length of time with CKD, years	8.1 (4.1, 12.4)
Female	38% (266)
African American	21% (143)
Glomerular diagnosis	28% (193)
Age-Sex-Height specific Systolic Blood Pressure z-score[a]	0.25 (-0.42, 0.82)
Age-Sex-Height specific Diastolic Blood Pressure z-score[a]	0.39 (-0.14, 0.92)
Hypertension[a,b]	28% (198)
Calcium*Phosphorus[a], mg/dL	42.7 (38.6, 46.6)
GFR[a,c], ml/min/1.73m^2	52.4 (39.1, 66.1)
Number of visits per participant 1 2 3 4-5	 50% (346) 26% (179) 15% (104) 9% (67)
Renal replacement therapy	30% (206)
Time to RRT (years)	4.5 [2.7, 7.2]
[a] Average of current visit with previous visit measurement. [b] Calculated as SBP or DBP ≥ 95[th] percentile for children < 13 years otherwise SBP or DBP ≥ 130/80 [c] Estimated GFR based on estimating equations using Scr, BUN & Cystatin.	

Disclosures: T.M. Brady: None. **J. Roem:** None. **C. Cox:** None. **M. Schneider:** None. **A. Wilson:** None. **S. Furth:** None. **B. Warady:** None. **M. Mitsnefes:** None.

Funding: No

Funding Component:

P394

Ace Activity and Its Expression as a Hypertension Biomarker in the Urine of Pediatric Renal Transplant Recipients

Authors: Maria Cecilia Pignatari, Fernanda Aparecida Ronchi, Amanda Aparecida Ribeiro, João Tomás de Abreu Carvalhaes, Larissa Miranda, Paulo Cesar Koch Nogueira, Dulce Elena Casarini, Univ Federal de São Paulo, São Paulo, Brazil

Pediatric hypertension is a risk factor for hypertension in adults. Primary hypertension results from genetic, environmental, and behavioral factors. Renin Angiotensin System is important to control cardiovascular and renal functions. Angiotensin Converting Enzyme (ACE) has isoforms: somatic (130-190 kDa) and testicular (90-110 kDa). Casarini et al. described the N-domain isoform of 90kDa, in urine of hypertensive humans as a possible hypertension biomarker. **OBJECTIVE**: Analyze ACE isoform in urine of pediatric renal transplant recipients before and after transplantation and urine of donor, to verify the 90 kDa isoform transmission and correlating with hypertension. **METHODS:** 30 pediatric recipients with living donors; ACE activity was performed before, 1, 3 and 6 months post

transplantation using ZPhe and HHL as substrates and its ratio. **RESULTS:** 69% had pre-transplant hypertension and 85.7% family history. After 6 months 25% remained. From the pre-transplant until the sixth month after, ACE activity was reduced. In the model of linear regression between ACE activity and variables, including pre-transplantation for the substrates ZPhe-HL remained significant, time and uropathy (0.023 more); with HHL, time and uropathy with higher activity of ACE (0.019 more) and for weight, reduction of ACE activity (0.00038 at 1 kg increase in weight). At post-transplant only time and weight remained significant with ZPhe-HL, and for HHL uropathy, proteinuria (0.010 more ACE activity) and weight. Recipients before transplantation had higher urinary ACE activity when compared to controls. Western blotting data showed that all recipients without ACE 90KDa when received a kidney from the donor that had this isoform, started to express it. **CONCLUSIONS:** Increased ACE activity in uropathies may be associated with renal injury or intra-renal production. Proteinuria, a marker of renal injury, can be associated with increase on ACE activity. With nutritional recovery and a healthy kidney after transplantation, patients can reverse the degree of renal damage and decrease the production of ACE.

Disclosures: M. Pignatari: None. **F. Ronchi:** None. **A. Ribeiro:** None. **J. Carvalhaes:** None. **L. Miranda:** None. **P. Nogueira:** None. **D. Casarini:** None.

Funding: No

Funding Component:

P395

Blood Pressure Screening in Teenage Blood Donors Using the New American Academy of Pediatrics Guidelines

Authors: Laurie Sutor, Univ of Texas Southwest Medical Ctr, Dallas, TX; Stephen J Eason, Shankar Goudar, Jeff Centilli, Carter Bloodcare, Bedford, TX; Merlyn H Sayers, Carter BloodCare, Bedford, TX

Background Screening of youngsters is valuable in identifying current or future health risk. Since approximately 20% of all blood donated in the U.S. comes from teenagers, blood donation is an opportunity to screen younger individuals. We decided to capitalize on this opportunity and investigate the prevalence of abnormal blood pressure in teenagers using the new American Academy of Pediatrics (AAP) guidelines which lowered the threshold for what could be considered a raised blood pressure. Since comparative data are limited in this age group we also decided to report our results by gender and race.
Study Participants were volunteer donors at high schools, aged 16-19 years, who donated between 2015 and 2017. Blood pressure was measured using automated equipment (Welch Allyn, ProBP). Blood pressure was classified as suggested by the AAP: normal (<120/<80), prehypertension (120/<80 to129/<80), Stage 1 hypertension (130/80 to139/89), and stage 2 hypertension (≥140/90). Donors were invited to declare their ethnicity at the time of donation.
Results During the study period there were 80,950 individual donors,10.8% (8,774) were African American, 4.2% (3,410) Asian, 51.9% (42,040) Caucasian and 33.0% (26,726) Hispanic. More males than females, in all ethnicities, had blood pressures above normal.
% 16 - 19 year old blood donors (donating from 2015-2017) categorized by the AAP blood pressure guidelines, by ethnicity and gender (N=80,950)
Conclusion Blood donation is confirmed as an opportunity to screen blood pressure in an ostensibly healthy subset of the younger population. This scrutiny does identify teenagers deserving follow up and also reveals ethnic and gender differences.

	African American		Asian		Caucasian		Hispanic	
	Male	Female	Male	Female	Male	Female	Male	Female
Normal	52.7	70.9	57.3	82.6	52.3	74.7	53.7	79.3
Elevated BP	19.0	10.6	15.7	5.2	19.3	9.2	18.6	7.2
Stage 1 Hypertension	19.9	16.1	21.0	11.2	20.9	14.3	20.7	12.2
Stage 2 Hypertension	8.4	2.4	6.0	1.1	7.5	1.9	7.3	1.7

Disclosures: L. Sutor: None. **S.J. Eason:** None. **S. Goudar:** None. **J. Centilli:** None. **M.H. Sayers:** None.

Funding: No

Funding Component:

P396

Vitamin D Supplementation Improves Cardiovascular Response to Head Up Tilt in Adolescents Suffering from Syncope

Authors: Hossam A Shaltout, Wake Forest Univ, Winston Salem, NC

Background:
In previous work we identified a group of adolescents whose diagnostic workup for chronic unexplained nausea revealed underlying cardiovascular instability manifesting as orthostatic intolerance (OI) and syncope. These patients exhibited impairment in autonomic function and excessive release of catecholamines (Epi / NE) and vasopressin (AVP) upon head up tilt compared to pre tilt. They also had low vitamin D level that correlated with the severity of symptoms on tilt. In this pilot study we hypothesized that vitamin D supplementation to normal levels will improve the cardiovascular response to tilt and reduce NE/Epi and AVP release.
Methods:
A cohort of seven Adolescents (mean age= 16.2 years) who are vitamin D deficient had a head up tilt at baseline and after two months of vitamin D supplementation (2000-5000 IU daily based on baseline level). Heart rate, blood pressure, NE/Epi and AVP were measured in supine and standing position. Total time on tilt was also recorded.
Results:
As see in the table. Compared to baseline, vitamin D supplementation reduced the HR elevation post HUT and reduced NE/Epi baseline levels and ameliorated the elevation in AVP after HUT.
Conclusions:
Vitamin D supplementation for two months restored vitamin D to normal levels and was associated with less nausea symptoms during tilt and lower level of baseline catecholamines. It was also associated with less release of NE/Epi and vasopressin post head up tilt and longer duration of standing on tilt table. These data provides evidence of potential therapeutic benefit of vitamin D supplementation for patients suffering from syncope who are vitamin D deficient

	Baseline		Post Vitamin D		
	Supine	HUT	Supine	HUT	p value
Systolic (mm Hg)	115	115	107	112	>0.1
Diastolic (mm Hg)	63	63	68	64	>0.1
Heart Rate (BPM)	71	73	67	65*	0.012
Minutes on tilt		19		35	0.15
Vitamin D	21.5		32		0.2
NE/Epi (pg/ml)	325	535	242*	443	0.1
AVP (pg/ml)	19.9	81	7.6	4	0.4

Disclosures: H.A. Shaltout: None.

Funding: Yes

Funding Component: Mid-Atlantic Affiliate (Maryland, North Carolina, South Carolina, Virginia & Washington, DC)

P397

Obesity Modifies the Association between Food Insecurity and Hypertension in Children and Adolescents

Authors: Callie Brown, **Andrew Michael South**, Deepak Palakshappa, Wake Forest Sch of Med, Winston Salem, NC

Background: Over 16% of children in the United States live in households with food insecurity (FI), the limited or uncertain availability of nutritionally adequate foods. FI is associated with several health disparities and, along with obesity, is independently associated with an increased risk of hypertension. However, it is not known if obesity modifies the association between FI and hypertension. **Objective:** To determine if obesity moderates the association between FI and high blood pressure (BP) in children and adolescents. **Design/Methods:** We performed a cross-sectional study of low-income children aged 2-17 years who presented for a well visit between March 1, 2016 and February 28, 2017. Data were collected via automated and manual electronic chart review. FI was assessed using a validated 2-item FI questionnaire. Height, weight, and BP were measured by nursing staff according to clinic protocol. We calculated BMI and categorized weight status per CDC guidelines and defined high BP as i) systolic BP or diastolic BP ≥90th %ile for age <13 years or ≥120/80 mmHg for age ≥13 years. Extracted covariates included age, sex, race/ethnicity, and geocoded address (to assess whether the child lives in a food desert). We used logistic regression to assess the association between FI and high BP, adjusting for race/ethnicity and living in a food desert and stratifying by overweight/obesity (BMI ≥85th %ile). **Results:** FI was present in 9.0% (242 of 2688) of subjects, 10.6% (284 of 2667) had high BP, and 43.5% (1298 of 2984) had overweight/obesity. Stratification by weight status revealed that in children with overweight/obesity, FI lowered the likelihood of high BP (β: -0.088, 95% CI -0.177 to -0.0004) while in healthy-weight children, the relationship was attenuated (β: -0.005, 95% CI -0.054 to 0.043). **Conclusions:** We found that FI was inversely associated with high BP in children with obesity, an effect that was attenuated in healthy-weight children. This could be due to differential effects of FI on dietary intake in children with obesity compared to those without. More research is needed to elucidate the role diet plays among high BP, FI, and obesity in children.

Disclosures: C. Brown: None. **A.M. South:** None. **D. Palakshappa:** None.

Funding:

Funding Component:

P399

Abnormal Ankle-Brachial Index Values are Associated With a Greater Prevalence of Left Ventricular Hypertrophy, Renal Impairment and Relevant Vascular Findings in Diabetic Hypertensive Patients

Authors: Jose C Pompeu Filho, Univ de Fortaleza, Fortaleza, Brazil; Ricardo P Silva, Faculdade de Medicina , Univ Federal do Ceará, Fortaleza, Brazil; Raquel P Pompeu, Hosp Leiria de Andrade, Fortaleza, Brazil; Wandervânia G Nojoza, Jedson V Gomes Filho, João L Falcão, Faculdade de Medicina , Univ Federal do Ceará, Fortaleza, Brazil; Sandra N Falcão, Univ de Fortaleza, Fortaleza, Brazil; **Luiz Bortolotto**, Heart Inst (InCor), São Paulo, Brazil

Few studies have examined the correlation between the presence of peripheral artery disease diagnosed by ankle-brachial-index (ABI) and cumulative target organ damage (kidney, heart and vessels) in hypertensive patients with diabetes. For this purpose, we evaluated the prevalence of target organ damage as evidenced by an echocardiogram, Doppler carotid scan, retinography and glomerular filtration rate according to ABI values in 99 hypertensive diabetic patients. The patients were classified into a normal ABI group ($1.4 \geq ABI > 0.9$) and abnormal ABI group ($ABI \leq 0.9$ or $ABI > 1.4$). From the data obtained, we analysed cumulative number, mean and frequency (mean) of lesions in target organs. The mean age of the patients was 65.4 ±7 years, and 61.6% were women. There were no differences concerning age, gender, race, body mass index, and metabolic profile between the normal (49 patients) and abnormal ABI group (50 patients), but higher levels of systolic blood pressure were observed in abnormal ABI group compared to normal ABI group (170.3 ± 26 vs. 153.3 ± 18; p = 0.002). In patients with abnormal ABI, it was observed a higher prevalence of left ventricular hipertrophy (56% vs. 26,5%, p = 0,003), internal carotid stenosis > 50% (13,7% vs. 3,2%, p = 0,009) and glomerular filtration rate < 60ml/1,73m^2/min (33,3% vs. 12,8%, p = 0,018). Abnormal ABI group has a higher frequency of 2 or more lesions (54% vs. 20.4%, p < 0.001), a greater mean number of lesions (2.37 ± 1 vs. 0.71 ± 0.7, p < 0.001), as well as a higher frequency of target organs damage (0.36 ± 0.31 vs. 0.19 ± 0.19, p = 0.001). Multiple linear regression analysis revealed abnormal ABI as an independent factor related to the higher frequency of target organ damage (OR = 13.22; 95% CI = 1.81-24.63; p = 0.024). In conclusion, hypertensive diabetic patients with abnormal ABI values had a higher prevalence of single and cumulative target organ damage.

Disclosures: J.C.J. Pompeu Filho: None. **R.P. Silva:** None. **R.P.N. Pompeu:** None. **W.G. Nojoza:** None. **J.V. Gomes Filho:** None. **J.L.A.A. Falcão:** None. **S.N.S.R. Falcão:** None. **L. Bortolotto:** None.

Funding: No

Funding Component:

P400

Screening for Hypertension Using Retinal Vascular Calibre in Ultra-Widefield Fundus Imaging

Authors: Gavin Robertson, Alan Fleming, Optos PLC, Dunfermline, United Kingdom; Michelle C Williams, Ctr of Cardiovascular Science, Univ of Edinburgh, Edinburgh, United Kingdom; Emanuele Trucco, The Vampire project, Computer Vision and Image Processing Group, Sch of Science and Engineering, Univ of Dundee, Dundee, United Kingdom; Nicola Quinn, Ruth E Hogg, Gareth J McKay, Ctr for Public Health, Queen's Univ Belfast, Belfast, United Kingdom; Enrico Pellegrini, Optos PLC, Dunfermline, United Kingdom; David E Newby, Ctr of Cardiovascular Science, Univ of Edinburgh, Edinburgh, United Kingdom; Edwin J van Beek, Ctr of Cardiovascular Science, Univ of Edinburgh, Edinburgh, UK, Edinburgh, United Kingdom; Tunde Peto, Ctr for Public Health, Queen's Univ Belfast, Belfast, United Kingdom; Baljean Dhillon, The Vampire project, Ctr for Clinical Brain Sciences, Univ of Edinburgh, Edinburgh, United Kingdom; Jano van Hemert, Optos PLC, Dunfermline, United Kingdom; Tom MacGillivray, The Vampire project, Ctr for Clinical Brain Sciences, Univ of Edinburgh, Edinburgh, United Kingdom

Fundus images from the left eyes of 440 subjects aged 50-59 years enrolled in the Northern Ireland Cohort of Longitudinal Ageing were analyzed. Subjects were categorized as normotensive or hypertensive, according to thresholds on systolic/diastolic blood pressure measurement (140/90 mm Hg) averaged over two sitting measurements in a clinical setting. A fully automatic system to analyze each image used conventional and deep neural network machine learning techniques to locate retinal landmarks and detect, classify and measure retinal vessels. From this data, a measure of the arteriolar-venular ratio (AVR) in the peripheral retina was calculated. Semi-automatic analysis was also performed. Results are presented in Table 1. Subjects had mean age of 54.6 ± 2.9 years; 56.1% (247 of 440) females, with 34.3% (151 of 440) subjects categorized as hypertensive. Narrower arterioles and smaller AVR were observed in subjects with hypertension. This was also observed in fully-automated analysis, however 4% (17 of 440) subjects failed to be processed by the system. In fully-automated analysis the area under a receiver operator characteristic curve of AVR for hypertensive status was 0.69 (95% CI, 0.63 to 0.74).

Analysis	Subjects				Two sample t-test (hypertensive –normotensive)			Area under curve
	Total	Hypertensive	Female	Age (years)	Arterioles (µm)	Venules (µm)	AVR	AVR
Semi-automated	440	151	247	54.6 ±2.9	-8 [-10, -6]*	1 [-2, 3]	-0.08 [-0.10, -0.06]*	0.73 [0.68, 0.78]
Automated	423	146	236	54.5 ±2.8	-7 [-9, -5]*	-1 [-3, 2]	-0.06 [-0.08, -0.04]*	0.69 [0.63, 0.74]

Table 1 - Results for semi-automated and automated analysis of retinal vessel parameters. *p<0.005

Automated measurement of AVR in ultra-widefield fundus imaging was associated with hypertension. With further development, such as evaluation against diagnosis of hypertension obtained from ambulatory blood pressure monitoring clinics, this system could become a test for undiagnosed hypertension in people attending routine eye health check-ups.

Disclosures: G. Robertson: A. Employment; Significant; Optos PLC. **A. Fleming:** A. Employment; Significant; Optos PLC. **M.C. Williams:** None. **E. Trucco:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Optos PLC. **N. Quinn:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Optos PLC. **R.E. Hogg:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Optos PLC. **G.J. McKay:** None. **E. Pellegrini:** A. Employment; Significant; Optos PLC. **D.E. Newby:** None. **E.J.R. van Beek:** None. **T. Peto:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Optos PLC. **B. Dhillon:** None. **J. van Hemert:** A. Employment; Significant; Optos PLC. **T. MacGillivray:** B. Research Grant (includes principal investigator, collaborator, or consultant and pending grants as well as grants already received); Modest; Optos PLC.

Funding: No

Funding Component:

P401

The Difference in Improvement of Kidney Function Between Febuxostat and Topiroxostat in Hypertensive Patients

Authors: Yuta Tezuka, Fumitoshi Satoh, Kei Omata, Yoshikiyo Ono, Ryo Morimoto, Masataka Kudo, Yasuhiro Igarashi, Sadayoshi Ito, Tohoku Univ Hosp, Sendai, Japan

[Introduction] Control of uric acid level (UA) plays an important role in the protection of organs in patients with hypertension (HTN). Newly developed xanthine oxidase inhibitors, febuxostat (FBX) and topiroxostat (TPX) are expected

to lead to more reduction of organ damages. However, the effects of them in a clinical situation remain unclear. We hypothesized these drugs have different effects based on the difference in action mechanism. [Objective] To reveal the effects of FBX and TPX on clinical parameters and compare them between the drugs. [Method] We retrospectively collected stable HTN patients with hyperuricemia (HU) who newly received a prescription of FBX or TPX and continued at least 12 weeks. Those who had other uric acid-lowering drugs or severe proteinuria were excluded. Participants were divided into FBX and TPX groups matched for age and sex. [Result] The almost baseline characteristics were not significantly different between both groups except the usage of renin-angiotensin inhibitors (Table). Administration of FBX or TPX lowered UA significantly ($p<0.0001$). Contrary to previous reports, blood pressure and urinary albumin-to-creatinine ratio were not significantly changed between both groups. However, eGFR in only TPX group was significantly increased from 57.2 ±15.0 to 60.6 ±15.7 mL/min/1.73m^2 ($p=0.006$), while FBX group was not. In TPX group, there was no significant difference in change of eGFR between patients using high and low amount of TPX. [Result] Our current study revealed TPX, not FBX, could improve eGFR in HTN patients. Moreover, the effect seemed to be independent of the amount of TPX. TPX could be 1st-line choice in HTN patients with HU.

Characteristics of FBX and TPX groups

		FBX (n = 47)	TPX (n = 47)	P value**
The amount of FBX or TPX at 12 weeks (mg/day)		13.4 ± 6.0	51.9 ± 18.5	
Age (year)		62.3 ± 11.9	62.4 ± 11.0	matched
sex (%, M/F)		57.4 (27/20)	57.4 (27/20)	matched
Body Mass Index		26.5 ± 4.8	25.8 ± 3.6	0.43
The number of AHT (n)		3.2 ± 1.3	2.9 ± 1.1	0.20
The Usage of RA inhibitors (%, n)		72.3 (34/47)	93.6 (44/47)	**0.01**
SBP (mmHg)	BL	128.3 ± 15.2	123.5 ± 16.2	0.17
	12 weeks	124.2 ± 13.6	125.3 ± 13.8	0.72
DBP (mmHg)	BL	77.4 ± 9.8	74.1 ± 10.9	0.15
	12 weeks	76.5 ± 9.3	76.0 ± 10.1	0.81
eGFR (mL/min/1.73m^2)	BL	58.7 ± 17.7	57.2 ± 15.0	0.66
	12 weeks	58.4 ± 15.0	60.6 ± 15.7	0.49
UACR (mg/gCr)*	BL	9.8 (1.4, 172.9)	10.9 (2.4, 247.5)	0.67***
	12 weeks	9.5 (1.3, 164.3)	10.4 (2.2, 481.6)	0.68***
Uric acid level (mg/dL)	BL	7.6 ± 1.3	7.4 ± 1.2	0.51
	12 weeks	5.6 ± 1.4	4.9 ± 1.1	**0.008**

BL: baseline, SBP: systolic blood pressure, DBP: diastolic blood pressure, AHT: anti-hypertensive agents, RA: renin-angiotensin, UACR: urinary albumin-to-creatinine ratio *Median (Q1 – Q3) **t-test ***Mann-Whitney U test

Disclosures: Y. Tezuka: None. **F. Satoh:** None. **K. Omata:** None. **Y. Ono:** None. **R. Morimoto:** None. **M. Kudo:** None. **Y. Igarashi:** None. **S. Ito:** None.

Funding: No

Funding Component:

P402

A New Oral Salt Tolerance Test has Acceptable Consistency for its First Volunteer

Authors: Zhiyi MA, Yuanyuan CHEN, People's Hosp,Peking Univ, Beijing, China; Zhechun ZENG, Beijing An Zhen Hosp of Capital Medical Univ, Beijing, China; Ningling SUN, People's Hosp,Peking Univ, Beijing, China

Objectives: One of the cores of salt restriction is to keep balance of salt intake and excretion. There is still no method to identify salt tolerance based on salt intake and excretion. In order to evaluate the ability to absorb and excrete salt, we established a new method Oral Salt Tolerance Test (OSTT). We firstly evaluated its consistency for one person among repeated tests. **Methods:** After signing informed consent, the 40-year-old healthy woman volunteer was asked to keep fasting after 9PM and empty bladder before sleep on the day before test. On the morning for test, she emptied bladder firstly and then drank 500ml 0.9% saline in 15 minutes. Urine volume and urine samples for sodium test were collected at 30min, 60min, 120min and 180min time points. This test was repeated twice on the other two days. **Results:** 1. According to sodium concentration and urine volume at each timepoints, we calculated equal sodium chloride amount. The baseline urine sodium concentration before test 1 is higher than those before test 2 and test 3. Accordingly, the 30min sodium concentration and urine volume are lower and higher respectively than those of the other two tests, while the 30min equal sodium chloride amounts are similar among the three tests. 2. The Intraclass Correlation Coefficient for equal sodium chloride amount among the three tests is 0.94 (95% Confidence Interval 0.6810 to 0.9958) and this indicates that the consistency among the three tests is acceptable. **Conclusion:** The acceptable consistency makes this new OSTT possible to screen more subjects with various clinical conditions. In the future, we hope this new method would give us a novel insight for hypertension or renal diseases patients.

samples	sodium concentration (mmol/l)	urine volume (ml)	equal sodium chloride amount (g)
test 1			
baseline	70.9	-	-
30min	24.7	110	0.16
60min	17.0	150	0.15
120min	69.9	150	0.61
180min	168.0	80	0.79
test 2			
baseline	113.1	-	-
30min	36.9	75	0.16
60min	19.5	225	0.26
120min	63.8	170	0.63
180min	206.8	70	0.85
test 3			
baseline	126.2	-	-
30min	32.1	60	0.11
60min	26.7	180	0.28
120min	62.1	220	0.80
180min	170.7	70	0.70

Disclosures: Z. Ma: None. **Y. Chen:** None. **Z. Zeng:** None. **N. Sun:** None.

Funding: No

Funding Component:

P403

Mortality of Hypertensive Men is Affected by Pneumonia and Lung Cancer

Authors: Yuechun Shen, Xinchun Li, Jun Li, First Affiliated Hosp of Guangzhou Medical Univ, Guangzhou, China

Background: Both morbidity and mortality of hypertension are high. Whether pneumonia and lung cancer affect the mortality of men with hypertension is not well studied. The aim of the study was to study the association between pneumonia/lung cancer and the mortality among male individuals with hypertension. **Methods:** A cross-sectional study was performed on hypertensive men, who admitted into our hospital and lived in eligible downtown areas prior to admission. Variety statistical analyses were performed, including logistic regression to assess the association between pneumonia/lung cancer and the mortality. **Results:** 14354 patients were enrolled. Mean age of them was 68.9±12.4 year old (y) and dead ones was 75.9±9.5y. The hospitalized mortality was 5.9%, which was increased with age: the mortality of group ≦49y, 50-59y, 60-69y, 70-79y, 80-89y and ≧90y was 0.7%, 2.2%, 2.9%, 7.1%, 11.1% and 16.6% respectively. The increased mortality was significantly positively correlated with the prevalence of pneumonia in different age, $P<0.05$, r=0.99. Pneumonia was prone to involved in men with older age and severer organ damage by hypertension. Similar to traditional risks coronary heart disease and stroke, pneumonia and lung cancer were also significantly associated with the mortality. OR (95% CI) for pneumonia and lung cancer were 6.18 (4.35-8.78) and 1.55 (1.14-2.11) respectively. **Conclusions:** The study provides evidence that pneumonia and lung cancer significantly affect mortality of hypertensive men in downtown areas, bringing alert that in order to reduce the mortality of hypertension in mordent life, theses lung diseases should be prevented and treated intensively. **Key words:** hypertension, hospitalized mortality, pneumonia, lung cancer

Disclosures: Y. Shen: None. **X. Li:** None. **J. Li:** None.

Funding: No

Funding Component:

P404

Differential Expression of miR-146b-5p Modulates Sex-Specific Cardiac Phenotypes in a Rat Model of Chronic Kidney Disease

Authors: Mark Paterson, Alison J. Kriegel, Medical Coll Wisconsin, Milwaukee, WI

Type 4 cardiorenal syndrome is a complex disorder in which primary chronic kidney disease (CKD) contributes to secondary cardiovascular disease (CVD). Although the pathological link between the kidney and heart are well-characterized, the molecular switches which drive cardiac pathology are not well understood. miRNAs have the potential to be master regulators of cellular signaling networks through modulation of RNA and protein expression profiles and have been shown to be important in both cardiovascular health and disease. Previous work in our laboratory identified a significant increase of miR-146b-5p expression in both the heart and the kidney in a 5/6 nephrectomy (5/6Nx) model of CKD in the Sprague-Dawley rat. To evaluate how miR-146b-5p is involved with pathological changes in the heart following 5/6Nx, echocardiography, blood pressure and left ventricle (LV) pressure-volume analyses were performed on male and female wild-type (WT) and miR-146b-5p null mutant ($146b^{-/-}$) rats for seven weeks following 5/6Nx or sham surgery. Structural analysis of the heart revealed that male and female rats exhibited significant hypertrophy of the LV wall in both the WT (+21% [1.50 vs. 1.24g] and +24% [1.00 vs. 0.81g] vs. sham, respectively; $p<0.001$) and $146b^{-/-}$ (+17% [1.36 vs. 1.17g] and +26% [1.03 vs. 0.82g] vs. sham, respectively; $p<0.001$) genotypes. Furthermore, dilation of the LV chamber was observed in WT male (+9% LVIDd [9.45 vs. 8.67mm] vs. sham; $p=0.032$) and female (+29% LVIDd [9.39 vs. 7.30mm] and +36% LVIDs [5.80 vs. 4.86mm] vs. sham; $p<0.005$) rats 7 weeks post-5/6Nx. However, LV dilation was attenuated in $146b^{-/-}$ females (-18% LVIDd [8.18 vs. 9.39mm] and -26% LVIDs [4.88 vs. 5.80mm] vs. WT; $p=0.07$) but not in $146b^{-/-}$ males. Functionally, LV pressure-volume analysis revealed $146b^{-/-}$ females exhibit an increase in stroke work (+59% vs. sham; $p=0.063$) and a significantly elevated mean arterial blood pressure (+23mmHg vs. sham; $p=0.02$). These alterations in cardiovascular function are not observed in miR-$146b^{-/-}$ males. Taken together, these data suggest $146b^{-/-}$ female rats may be resistant to eccentric dilation despite increased afterload and highlight the sex-specific modulation of cardiorenal phenotypes by miR-146b-5p following 5/6Nx.

Disclosures: **M. Paterson:** None. **A.J. Kriegel:** None.

Funding: Yes

Funding Component: National Center