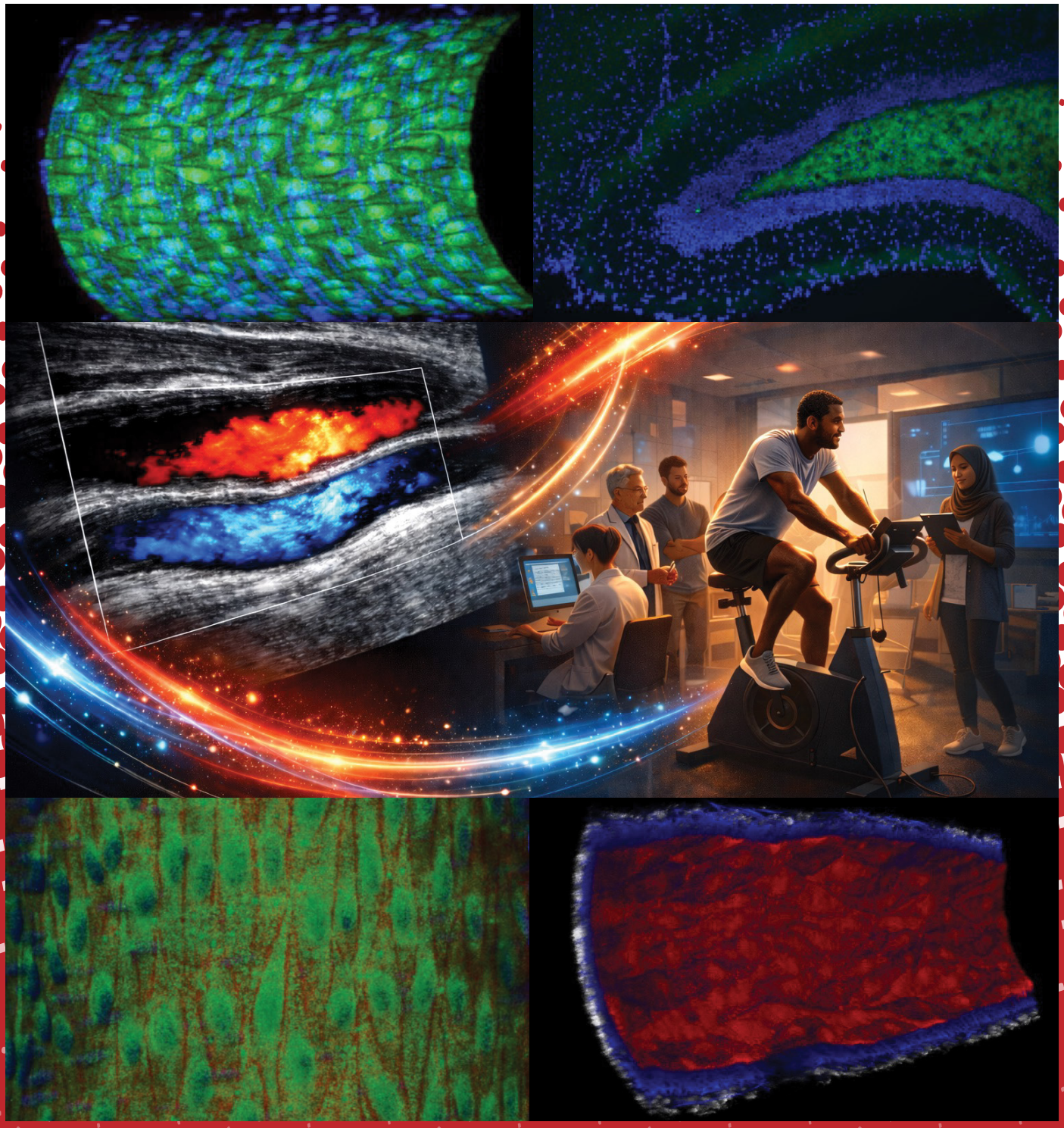
33rd Annual Cardiovascular Day

Program and Abstracts



Wednesday, February 18, 2026
The Hilton Garden Inn Conference Center
Columbia, MO

Mizzou

James O. Davis, MD, PhD

James O. Davis began his career at the University of Missouri—Columbia in the Department of Zoology. He received his Ph.D. in 1942 and subsequently completed an M.D. at Washington University School of Medicine in 1945. In 1947, Dr. Davis accepted a position at the National Institutes of Health. In 1966, he returned to the University of Missouri-Columbia to chair the Department of Physiology. Dr. Davis is considered the pioneer in cardiovascular science at MU and is internationally recognized for his contributions in the area of congestive heart failure and hypertension. His many honors include the Sigma Xi Research Award (MU), Alpha Omega Alpha (Washington University School of Medicine), Golden Apple Teaching Award in Medicine (MU), Modern Medicine Distinguished Achievement Award for "proof of the involvement of the kidney in the production of aldosterone", the MU Faculty/Alumni Award, Outstanding Alumnus Award from Northwestern Oklahoma State University, Distinguished Alumnus Award of the College of Arts and Science (MU), the establishment of the James O. Davis Distinguished Professorship in Cardiovascular Research (MU), and election to the National Academy of Sciences in 1982.

"*Circulation of Knowledge*"

The collection of images on the cover represents the beauty and synergy of cardiovascular research being conducted at the University of Missouri. The central image (Daniel Credeur) depicts a doppler ultrasound view of an artery next to a group of students and scientists engaged in active learning. This image highlights blood flow and vessel structure as central markers of vascular health and is an illustration of how continued education leads to new scientific questions and novel discoveries. Surrounding the central image are fluorescent microscopy images of mesenteric artery endothelial cells (Marc Augenreich) and the dentate gyrus subregion of the hippocampus (Sandy Saunders) depicting the importance of cardiovascular health to the health of other organ systems, and the contributions of scientific discovery to the continuous flow of knowledge.

33rd Annual Cardiovascular Day Schedule of Events

7:30 - 8:30am **Registration and poster set up**
7:45 - 8:30am Breakfast

8:30 - 8:45am **Opening Remarks – Darla Tharp, PhD, CV Day Chair,** Introduction by Scott Rector, PhD

8:45 - 9:25am **Lecture Session I -** Moderators: Jeffery Boychuk, PhD, Alejandro Chade, MD

8:45 - 9:05am Sandy Saunders, PhD, Postdoctoral Fellow, Pathobiology & Integrative Biomedical Sciences, Dalton Cardiovascular Research Center, *"Hyperactive central stress circuits promote autonomic reflex plasticity in SUDEP"*

9:05 - 9:25am Soumiya Pal, PhD, Postdoctoral Fellow, Medical Pharmacology and Physiology, *"Breaking the Calcium Code: Orai Channels Drive Lymphedema Pathophysiology"*

9:25 - 10:00am **FlashTalk Session -** Moderators: Jacqueline Limberg, PhD, and Samuel Martin

Participants: Augustine Udefa, Deepanwita Chakraborty, Eryn Wagoner, Cecile El-Borgi, Yoko Wang, and Ava Fleury

10:00 - 11:00am **Poster Session I**

11:00am - 12:00pm **James O. Davis Distinguished Lecture in Cardiovascular Science**
- introduction by Samuel Martin, Graduate Student, MPP

Julie Freed, MD, PhD, Associate Professor and Executive Vice Chair,
Department of Anesthesiology; Director of Clinical Research, Medical College of Wisconsin.

"Translational Tales of the Human Microvasculature"

12:00 - 1:15pm **Lunch**

1:15 - 2:30pm **Poster Session II**

2:30 - 3:50pm **Lecture Session II -** Moderators: Darla Tharp, PhD, and Charles Norton, PhD

2:30 - 2:50pm Daniel Credeur, PhD, Associate Teaching Professor, Nutrition and Exercise Physiology, *"Sit Less, Learn More: Translating Movement and Vascular Health Research into Classroom Practice"*

2:50 - 3:10pm Teresa Pitts, PhD, Professor and Chair, Speech Language and Hearing Sciences; Dalton Cardiovascular Research Center, *"Pavlov's dog is still hungry- feeding and swallowing as a direct modifier of heart rate and blood pressure"*

3:10 - 3:30pm Marc Augenreich, Graduate Student, Nutrition and Exercise Physiology PhD Program, *"ADAM17-Meditated Insulin Receptor Shedding as a Mechanism of Endothelial Insulin Resistance"*

3:30 - 3:50pm Andrew Behrmann, MD/PhD Student, Integrative Physiology, Translational Biosciences PhD Program, *"A Novel Two-Hit Challenge Induces TRPV4-Dependent Left Ventricular Diastolic Dysfunction in Isolated Hearts of Aged Mice"*

3:50 - 4:20pm **Awards Ceremony**

Laughlin Scholarship Award- Shawn Bender, PhD
Steffen Interdisciplinary Physiology Award – Kerry McDonald, PhD, Daniel Steffen, PhD and Anne Giesecke, PhD
APS Undergraduate Student Poster Award- Jeffery Boychuk, PhD
AHA Pre/Post-doctoral fellowship recognition – Jessica Cayton, DVM
CV Day FlashTalk Awards - Jacqueline Limberg, PhD
CV Day Poster Awards - Alejandro Chade, MD

Wi-Fi information: hgiconference_24 or hgiconference_5g PW: garden24

The James O. Davis Distinguished Lecture in Cardiovascular Science

The highlight of Cardiovascular Day is the James O. Davis Distinguished Lecture in Cardiovascular Science. Dr. Davis was a pioneer in cardiovascular science at MU and he was internationally recognized for research contributions in the areas of congestive heart failure and hypertension.

2026 Keynote Speaker

Julie Freed, MD, PhD

Associate Professor and Executive Vice Chair,
Department of Anesthesiology; Director of Clinical Research,
Medical College of Wisconsin.

"Translational Tales of the Human Microvasculature"

Dr. Freed's research primarily focuses on the role that sphingolipids have in the development of endothelial dysfunction in the human microcirculation. Elevated plasma levels of ceramide, a prototypical sphingolipid, is now considered an independent risk factor for major adverse cardiovascular events in otherwise healthy people. The Freed lab is currently investigating how these bioactive lipids are regulated in human endothelial cells. As a cardiac anesthesiologist, Dr. Freed is also interested in intraoperative blood pressure control including mechanisms and treatments of vasoplegia. Other interests include translating her work in the lab to improve outcomes for surgical patients.

STATE OF MISSOURI

Proclamation

BY THE GOVERNOR

WHEREAS, nearly 685,000 Americans died from major cardiovascular diseases in 2024, which accounts for 1 in 5 deaths in the United States; and

WHEREAS, heart disease is the leading cause of death in Missouri in 2024, and accounts for more than 15,000 deaths statewide; and

WHEREAS, the University of Missouri is home to several hubs of cardiovascular research: the Roy Blunt NextGen Precision Health building, the Dalton Cardiovascular Research Center, the School of Medicine, the College of Veterinary Medicine, and the College of Agriculture, Food and Natural Resources; and

WHEREAS, scientists from the University of Missouri are renowned in areas such as the effects of exercise on the heart, hypertension, and heart failure, and these scientists are committed to the pursuit of further medical advances in the fight against cardiovascular disease; and

WHEREAS, these scientists gather each year in Columbia to share their work with fellow scientists in an event hosted by the University of Missouri, thereby bringing further scientific and medical advances closer to reality.

NOW, THEREFORE, I, MIKE KEHOE, GOVERNOR OF THE STATE OF MISSOURI, do hereby proclaim February 18, 2026, to be

CARDIOVASCULAR DAY

in Missouri.

IN TESTIMONY WHEREOF, I have hereunto set my hand and caused to be affixed the Great Seal of the State of Missouri, in the City of Jefferson, this 8th day of January 2026.

Mike Kehoe
GOVERNOR

ATTEST:

Denny Hoskins
SECRETARY OF STATE

INDEX OF ABSTRACTS

Mericle, Fateme Poster Location: 01 Poster Judging Number: 401

Spinal D1-D2 heteromer formation mediates the involuntary bladder reflex after spinal cord injury

Fateme Khodadadi-Mericle, Christopher Foote, Ruwaida Ben Musa, Zhifeng Qi, Shaoping Hou

Dalton Cardiovascular Research Center, Department of Pathology and Anatomical Sciences, Department of Physical Medicine and Rehabilitation, University of Missouri School of Medicine, Columbia, MO 65211

Micturition dysfunction occurs after spinal cord injury (SCI). We previously disclosed that a spinal dopaminergic machinery emerges to regulate the partially recovered bladder function following SCI. However, this neuronal mechanism is unclear, particularly at the receptor level, in that we found dopamine effects on urinary performance could not be simply interpreted as a result of activation of D1 plus D2 receptors. Based on the preliminary data, we hypothesized that a D1-D2 receptor complex forms in the spinal cord to mediate the spontaneous urination after SCI. Herein, we tested this hypothesis in the micturition reflex. Adult female rats underwent a complete transection at the 10^{th} thoracic (T10) spinal cord. After 3-4 weeks, a cohort of rats was sacrificed and a segment of the L6/S1 spinal tissue, where dopamine neurons controlling the bladder and EUS are located, was dissected for D1-D2 co-immunoprecipitation. Consequently, a protein band for D1-D2 heteromers was detected and the density was increased in the injured cord. In parallel, conscious rats were restrained for continuous bladder cystometrogram (CMG) and external urethral sphincter (EUS) electromyography (EMG) recordings. A serial of doses of the TAT-D1 peptide, an antagonist of D1-D2 heteromers, was i.t. administered. The high dose induced increased volume thresholds (VT) and prolonged voiding intervals (VI), indicating an inhibitory effect on voiding. Furthermore, pharmacological stimulation of D1-D2 heteromers with a combination of SKF 38393, a D1 receptor agonist, and Quinpirole, a D2 receptor agonist, enhanced voiding volume (VV) and reducing VI, which suggests improved voiding capability. Subsequently, injection of TAT-D1 eliminated these effects. The results identified the presence of D1-D2 heteromers in the injured spinal cord and their involvement in regulating the involuntary bladder reflex. This DA receptor heteromer may serve as a therapeutic target for improving voiding in subjects with SCI.

Fundings: NIH/NINDS R01 NS121336, MU SCIDRP2024, and Mizzouforward Initiative

Bryda, Elizabeth **Poster Location: 02**

Advancing Cardiovascular Research Through Rat Models: The Rat Resource and Research Center

Elizabeth C. Bryda **(**Director), Hongsheng Men, Yuksel Agca, James M. Amos-Landgraf, Kevin L. Gustafson

Department of Pathobiology and Integrative Biomedical Sciences, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA

Rats are often the preferred model organism in cardiovascular research because of their genetic and physiological similarities to humans, their ideal size for surgeries and repeated measurements and the availability of genetically modified strains/stocks that model human conditions and diseases. The NIH-funded Rat Resource and Research Center (RRRC) is a centralized repository for maintaining, archiving and distributing rat models and providing rat-related services to the biomedical community. Currently, the RRRC has over 625 rat lines, including more than 100 with cardiovascular phenotypes such as hypertension. Our website (www.rrrc.us) allows user-friendly navigation to identify models of interest. The RRRC provides expertise in rat colony management, health monitoring, genetic assay development/optimization, and can collaborate on investigator projects and grants. As experts in comparative medicine, with access to skilled laboratory animal veterinarians, we can assist with surgical techniques and perform all aspects of in vivo rat studies. Fee-for-service capabilities include a wide variety of genetic analyses, strain rederivation and cryopreservation, isolation of rat tissues, custom breeding/colony management, microbiota analysis and characterization of rat models. The RRRC can make genetically engineered rats from start to finish using state-of-the-art technologies such as genome editing and traditional methods like random transgenesis and embryonic stem cell microinjection. New initiatives include supplying induced pluripotent stem cells (iPSCs) to support new approach methodologies (NAMs). The RRRC provides the in vivo models needed to validate NAMs and support studies that require complex biological systems for appropriate scientific rigor and reproducibility. The University of Missouri is home to the NIH-funded MU Rat Testing Center for Somatic Cell Genome Editing, MU Mutant Mouse Resource and Research Center (MMRRC), National Swine Resource and Research Center (NSRRC), MU Animal Modeling Core, and MU Metagenomics Center. Together, these collaborative groups offer diverse animal model-related services across species to advance biomedical and translational research.

Funded by NIH grant 2P40 OD011062 (ECB).

al Marrawi, Abdullah **Poster Location: 03**

Beyond Clinical Variables: Machine Learning Integration of Clinical and Contextual Factors for Predicting Heart Failure Readmissions

Abdullah al Marrawi [MD/MSc Student, Research Fellow] , Fares Alahdab [MD, MS, MSc, FAHA, Associate Professor]

Affiliations:
Departments of Biomedical Informatics, Biostatistics & Epidemiology and Cardiology, University of Missouri-Columbia

Heart failure patients experience frequent 30-day readmissions, and existing clinical models predict these moderately well. This study used machine learning to integrate clinical and socioeconomic factors to improve prediction accuracy. De-identified electronic medical records from the University of Missouri–Columbia Snowflake system (up to 2025) were used to identify 33,423 adult heart failure patients and 66,804 hospital admissions. Baseline demographic, socioeconomic, clinical, laboratory, medication, comorbidity, and mortality data were extracted. The cohort was split at the patient level into training (70%, n = 46,708), test (20%, n = 6,685), and validation (10%, n = 3,343) sets. After preprocessing, logistic regression, random forest (RF), and XGBoost models were developed. Performance on the test set was evaluated using area under the receiver operating characteristic curve (AUROC), precision–recall AUC (PR-AUC), Brier score, log loss, accuracy, precision, recall, F1 score, specificity, and negative predictive value (NPV), all with 95% confidence intervals (CIs). On the test set, random forest and XGBoost outperformed logistic regression, achieving higher discrimination (AUROC 0.705, 95% CI: 0.688–0.720 and 0.704, 95% CI: 0.688–0.719, respectively) than logistic regression (AUROC 0.684, 95% CI: 0.667–0.701), with improvements in PR-AUC (random forest 0.399, 95% CI: 0.361–0.436; XGBoost 0.394, 95% CI: 0.357–0.426; logistic regression 0.369, 95% CI: 0.332–0.402). Random forest showed superior calibration with the lowest Brier score (0.153, 95% CI: 0.146–0.159) and log loss (0.475, 95% CI: 0.460–0.489). XGBoost achieved the highest accuracy (0.622, 95% CI: 0.612–0.632) and specificity (0.607, 95% CI: 0.592–0.620), while random forest achieved the highest recall (0.760, 95% CI: 0.737–0.782). Overall, preliminary ensemble machine learning models achieved improved prediction of 30-day readmissions (AUROC ≈ 0.70; RF: Brier ≈ 0.15), supporting use for clinical risk stratification. Further feature engineering and model refinement are planned.

Funding: Not Applicable

Shaid, Arooj **Poster Location: 04** **Poster Judging Number: 304**

Sex-specific myocardial dysfunction in cardiometabolic HFpEF

Arooj Shahid[1], Samuel Daughtery[1], Anna E. Burns[1], Mei Methawasin[1]

[1]Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, Missouri

Introduction: HFpEF (Heart Failure with preserved Ejection Fraction) is dominated by the impaired diastolic function, and accounts for ~50% of all heart failure cases. HFpEF disproportionately affects women, with prevalence increasing sharply after menopause. Clinical studies suggest that HFpEF in men and women are distinct, understanding sex-specific pathophysiology in HFpEF is essential for achieving an in-depth understanding of HFpEF and developing personalized therapeutic strategies.

Hypothesis: Sex and hormone status differentially affect myofilament mechanical properties, supporting the need for sex-specific HFpEF therapeutic interventions.

Methods: Menopause was induced in female mice using 4-vinylcyclohexene dieopxide (VCD). Then HFpEF conditions were induced in both male and postmenopausal female mice using a 2-hit regimen (high-fat diet plus LNAME-supplement) for 4 months. We used a myocardial slice mechanical technique to assess the cardiac function at the intact myocardial levels. Left Ventricular (LV) slices (200 μm thickness) were hooked between a force transducer and the piezo length controller. The piezo movement was controlled to generate force-length loops. Key loop parameters were assessed, including end-diastolic stress-length relationship (EDSLR) as a measure of passive myocardial stiffness, isometric relaxation time (IMRT) as an index of relaxation kinetics, stroke length (equivalent to stroke volume), and stroke work (mechanical output). The relative contributions of microtubules, diastolic crossbridge, and titin were assessed using colchicine and 2,3-butanedione monoxime (BDM).

Results: Preliminary findings indicate that the female-HFpEF-like (VCD-2hit) group showed a trend toward increased IMRT and EDSLR, suggesting delayed relaxation increased LV diastolic stiffness. Stroke work and length decrease, suggesting reduced cardiac output. Additional male cohort is currently being included.

Conclusion: Cardiac slice mechanical technique is a useful tool for studying myocardial mechanics in intact cardiac tissue. Preliminary result suggests that the female-2hit-VCD mice develop HFpEF-like condition under metabolic stress, observed as increased myocardial stiffness and delayed relaxation.

Safa **Poster Location: 05** **Poster Judging Number: 305**

Acute Estrogen Treatment Exacerbates Mitochondrial Membrane Potential Depolarization and Cerebrovascular Apoptosis to Oxidative Stress in Ovariectomized but not Ovary-Intact Mice.

Safa, Charles E. Norton
Medical Pharmacology and Physiology.
University of Missouri, Columbia, MO 65212

Stroke and traumatic brain injury increase reactive oxygen species (ROS) production leading to vascular apoptosis. Young females exhibit greater resilience to vascular injury compared to age-matched males. Yet the mechanisms underlying this protection remain undefined. We hypothesized that estrogen protects cerebral arteries from acute oxidative stress by preserving mitochondrial membrane potential ($\Delta\Psi_m$) and thus limiting apoptosis. Posterior cerebral arteries (PCA; ~80 μm diameter) from control (SHAM) and ovariectomized (OVX) female mice (3-6 months old) were isolated, cannulated, and pressurized to 90 cm H_2O at 36°C. Smooth muscle cell (SMC) and endothelial cell (EC) death were quantified using Hoechst 33342 (1 μM; stains all nuclei) and propidium iodide (1 μM; stains dead nuclei) after 50 min exposure to H_2O_2 (200 μM) in the presence and absence of supplemental estrogen (10 μM; 1-hour preincubation). SMC death was significantly higher in OVX *vs.* SHAM mice (16% vs. 7%; $P<0.05$) while EC was unaffected (8% vs. 5%). Loss of $\Delta\Psi_m$ is essential for initiating intrinsic apoptosis. Resting $\Delta\Psi_m$ (JC-1, 5 μM) was unaltered by ovariectomy. We assessed changes in $\Delta\Psi_m$ with TMRM (10 nM). Exposure to H_2O_2 evoked greater depolarization of $\Delta\Psi_m$ in pressurized PCAs from OVX compared to SHAM mice. Contrary to our hypothesis, estrogen treatment did not improve vascular resilience in OVX mice and unexpectedly increased depolarization of $\Delta\Psi_m$ in addition to SMC and EC death. In contrast, acute estrogen treatment had no effect in vessels from SHAM mice. Treating PCAs with the protonophore FCCP (10 μM) led to greater depolarization of $\Delta\Psi_m$ in OVX compared to SHAM vessels; estrogen treatment had no effect on responses to FCCP. We conclude that loss of ovarian hormones heightens susceptibility to $\Delta\Psi_m$ depolarization and apoptosis, whereas estrogen treatment unexpectedly increases damage to oxidative stress in OVX mice. Although there is a protective effect of endogenous ovarian hormones, our findings highlight the potential for estrogen therapy to enhance vascular injury in postmenopausal women.

SUPPORT: NIH R01NS134690

Soumiya Pal **Poster Location: 06** **Poster Judging Number: 406**

Breaking the Calcium Code: Orai Channels Drive Lymphedema Pathophysiology

Soumiya Pal, Adi Patro, Grace A. Pea, Sarah Broyhill, Karen Bromert, Scott D. Zawieja

Department of Medical Pharmacology and Physiology, University of Missouri, Columbia

Background & Aim: Loss of propulsive contractions and lymphatic collecting vessel sclerosis are hallmarks of secondary lymphedema, which implicate lymphatic muscle cell dysfunction in the pathogenesis. Store-operated calcium entry (SOCE) channels, encoded by Orai1-3, are calcium (Ca^{2+}) channels, critical to vascular smooth muscle cell dedifferentiation and proliferation. We investigated SOCE via Orai channels as a novel therapeutic target for lymphedema, focusing on how pathological Ca^{2+} overload through Orai1 channels drives lymphatic paralysis—a hallmark of lymphedema progression.

Methods: Using inducible smooth muscle knockout ($Myh11CreER^{T2}$; ismKO) and global genetic knockout models, we performed isobaric myography and Ca^{2+} imaging on mouse inguinal-axillary lymphatic vessels. Baseline vessel contractility was first evaluated using a pressure step protocol, and SOCE activation was tested via Ca^{2+} depletion/add-back protocol. We then simulated lymphedema-associated Ca^{2+} dysregulation using ryanodine-induced sarcoendoplasmic reticulum Ca^{2+} leak, mimicking the pathological contractile dysfunction observed during lymphedema.

Results: We demonstrate that Orai1-mediated SOCE represents a critical pathological pathway in lymphatic dysfunction. Under simulated lymphedema, control vessels developed catastrophic paralytic constriction, directly mirroring the lymphatic failure seen in patients. Remarkably, genetic deletion or pharmacological inhibition of Orai1 and STIM1, while not affecting baseline contractility, completely prevented this paralysis, maintaining contractions essential for lymph transport. Orai1ismKO and Stim1ismKO vessels showed resistance to Ca^{2+} overload-induced dysfunction, while $Orai3^{-/-}$ vessels remained vulnerable, establishing Orai1 as the primary pathological mediator.

Conclusion: Our findings identify Orai1 as promising therapeutic targets for lymphedema treatment. By preventing pathological Ca^{2+} overload and subsequent lymphatic paralysis, Orai1 inhibition could represent the first mechanism-based therapy to restore lymphatic function rather than merely managing symptoms. This paradigm shift from voltage-gated to store-operated Ca^{2+} channel targeting opens new avenues for developing disease-modifying therapies for lymphedema and related lymphatic disorders. The protective effects of Orai1 ablation against lymphatic failure provide compelling evidence for advancing Orai1 antagonists into clinical development.

Funding: National Institutes of Health (NIH)/National Heart, Lung, and Blood Institute (NHLBI) R01HL175083 to S.D. Zawieja.

Foote, Christopher **Poster Location: 07**

RAS mediated vascular remodeling following spinal cord injury

Christopher A. Foote[1,2], Zhifeng Qi[1,2], Olivia Lesnik[1], Emma Russell[1], Fateme Mericle[1,2], Francesca Pilotti[1], Francisco I. Ramirez-Perez[3], Michael A. Hill[1,4], Shaoping Hou[1,2,5]

[1]Dalton Cardiovascular Research Center, [2]Department of Pathology and Anatomical Sciences, [3]NextGen Precision Health, [4]Department of Medical Pharmacology and Physiology, [5]Department of Physical Medicine and Rehabilitation, University of Missouri, Columbia, MO 65212

Cardiovascular dysfunction is a common sequela of traumatic spinal cord injury (SCI), with affected individuals exhibiting an approximately fourfold increased risk of heart disease and stroke compared to able-bodied populations. Despite this elevated risk, SCI-associated arterial remodeling remains poorly characterized, and the molecular mechanisms driving post-injury cardiovascular pathology are not well understood. We have previously demonstrated that inhibition of enzymes downstream of angiotensin II type 1 receptor (AT1R) activation reduces arterial stiffening in hypertensive patients. Additionally, we have shown that SCI disrupts neural regulation of blood pressure, leading to compensatory upregulation of the renin–angiotensin system (RAS) to maintain organ perfusion. Based on these observations, we hypothesized that chronic RAS activation following SCI results promotes pathological arterial remodeling and stiffening.

To test this hypothesis, adult female rats underwent complete spinal cord transection (T4); sham-operated animals served as controls. Eight weeks post-injury, in vivo imaging showed reduced PWV but decreased aortic strain and distension in SCI animals, findings influenced by reduced transmural pressure, which occurs in our hypotensive SCI model. Ex vivo analysis using pressure myography revealed inward remodeling in both femoral (conduit) and mesenteric (resistance) arteries from SCI rats. Mesenteric arteries also showed increased incremental moduli of elasticity, indicating pathological resistance artery stiffening. To evaluate the contribution of angiotensin II signaling via AT1R to SCI-induced arterial stiffening, mesenteric arteries from SCI and sham animals were incubated overnight with vehicle, the AT1R antagonist losartan, or a LIM kinase inhibitor. Treatment of SCI mesenteric vessels with Losartan or LimKinase inhibitor increased arterial strain relative to vehicle, demonstrating reduced stiffness with AT1R pathway inhibition. Sham vessels showed no significant treatment-related changes. Collectively, these findings support a model in which chronic upregulation of the RAS following SCI drives maladaptive arterial remodeling and stiffening, contributing to the heightened cardiovascular risk observed after spinal cord injury.

Funding: NIH/NINDS R01 NS121336, MU SCIDRP2024, and Mizzouforward Initiative

Lateef, Olubodun Poster Location: 08 Poster Judging Number: 308

Tissue transglutaminase: a stretch mechanosensor in vascular smooth muscle

Olubodun M. Lateef[1,2], Francisco I. Ramirez-Perez[1], Gavin Power[1,3], Marc A. Augenreich[1,3], Manikandan Nagarajan[1], Natnicha Imkaew[1], Min Jeong Cho[1,3], Jaume Padilla[1,3.4], and Luis A. Martinez-Lemus[1,2,5*]

[1]NextGen Precision Health, University of Missouri; [2]Department of Medical Pharmacology and Physiology, University of Missouri; [3]Department of Nutrition and Exercise Physiology, University of Missouri; [4]Harry S. Truman Memorial Veterans' Hospital, Columbia, MO; [5]Center for Precision Medicine, Department of Medicine, University of Missouri

The vascular wall is constantly subjected to hemodynamic forces arising from the pulsatile nature of blood pressure and blood flow across different vascular beds. Vascular smooth muscle cells (VSMCs) detect these mechanical cues through specialized mechanosensing molecules and cellular structures, which activate coordinated mechanotransduction pathways to maintain vascular homeostasis. Tissue transglutaminase (TG2) is a multifunctional enzyme that, when localized to the cell membrane, interacts with several known mechanosensitive molecules, including fibronectin, integrins, and G protein-coupled receptors. However, whether TG2 functions as a stretch-activated mechanosensor that integrates and harmonizes mechanotransduction processes in VSMCs remains unknown. We hypothesized that VSMC mechanical stretching activates TG2 to promote F-actin formation and cellular stiffening. We tested our hypothesis in cultured human aortic smooth muscle cells (HAoSMC) that were either left unstretched or exposed to cyclic uniaxial stretching using a Flexcell tension system with 11% elongation at 1 Hz for 6 hours, simulating conduit artery pulsatility. *TGM2* knockdown was performed in cultured HAoSMC using siRNA alongside scrambled siRNA as a negative control (n=5-6/condition). To evaluate the effects of TG2 overexpression, cells were transfected with an adenovirus expressing human *TGM2* or a control adenovirus (n=6-12/condition). We also tested the role of TG2 activation in isolated mesenteric arteries on their subsequent passive pressure-diameter curves (n=8/condition). All differences reported herein are significant at $P \leq 0.05$. Our results show that mechanical stretching increases VSMC TG2 activity (presence of N-epsilon gamma glutamyl Lysine links, 1.3-fold), F-actin content (phalloidin staining, 1.3-fold), and cortical stiffness (assessed via atomic force microscopy, 1.3-fold) relative to unstretched conditions. Notably, the stretching-induced effects on F-actin content and VSMC stiffness were significantly attenuated by either TG2 knockdown or pharmacological inhibition of LIM kinase (LIMK), an enzyme that promotes cofilin deactivation and actin polymerization. We also found that stretching-induced increases in F-actin and VSMC stiffness in TG2 overexpressing HAoSMCs were blunted by LIMK inhibition. Similarly, in isolated pressurized mesenteric arteries, pharmacological activation of TG2 with L-NAME, a nitric oxide synthase inhibitor and TG2 activator, increased vascular stiffness, which was abrogated by LIMK or TG2 inhibition. We further demonstrate that stretch-induced actin polymerization and VSMC stiffening occur via a TG2/RhoA/LIMK/cofilin/actin signaling axis. Collectively, these findings identify TG2 as a stretch mechanosensor whose activity is essential for the adaptive responses of VSMCs to mechanical stimuli.

Chakraborty, Deepanwita **Poster location: 09** **Poster Judging Number: 309**

FlashTalk Presenter

Microvascular rarefaction contributes to pulmonary hypertension in a mouse model of pulmonary fibrosis

Deepanwita Chakraborty, Gloria Kuebee, and Charles E. Norton
Medical Pharmacology and Physiology
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Pulmonary fibrosis (PF) damages the lungs and leads to pulmonary hypertension (PH). However, it's unclear whether microvascular rarefaction during PF contributes to PH. We hypothesized that PF increases apoptosis of smooth muscle cells (SMCs) and endothelial cells (ECs) leading to PH. We induced PF with intratracheal bleomycin (0.025U; 3wks before study) in male and female C57Bl/6J mice (age, ~5mo), with saline used as a control (sham). In both sexes, bleomycin induced a significant ($P<0.05$) increase in collagen deposition and hypercellularity. Despite similar fibrotic injury, right ventricular systolic pressure (male: 40±2 vs. 19±1mmHg; female: 31±1 vs. 18±1mmHg) and Fulton index (male: 0.58±0.5 vs 0.31±0.12; female: 0.45±0.03 vs 0.31±0.01) were higher in males than females. We quantified the number of capillaries and small pulmonary arteries (≤40μm) in lung sections labeled with lectin and Verhoeff van Gieson stain, respectively. PF mice had fewer capillaries (male: 39±3 vs. 84±7/mm², female: 68 ±6 vs. 102±13/mm²) and small arteries (male: 9±2 vs. 21±4/section; female: 9±1 vs. 23 ±4/section). Given the increase in ROS during PH, we measured SMC and EC death (1μM Hoechst 33342, 2μM propidium iodide) and mitochondrial membrane potential ($\Delta\Psi_m$; TMRM) depolarization in isolated, pressurized (16cmH_2O), pulmonary arteries (diameter 100-150μm) during exposure to H_2O_2 (200μM, 50min). Vascular cell death and $\Delta\Psi_m$ depolarization in response to H_2O_2 were enhanced by PF. Furthermore, mice were treated with the apoptosis inhibitor (Q-VD; 20mg/kg; tail vein injection on days 0, 2, 4, and 7) to determine whether vascular apoptosis drives PH facilitated by PF. Chronic inhibition of apoptosis did not alter fibrosis but significantly reduced RVSP and Fulton index in males, with a trend ($P=0.10$) in females. We conclude that PF increases apoptosis of SMCs and ECs contributing to vessel rarefaction and the development of PH more pronouncedly in males compared to females.

Funding: American Heart Association Career Development Award 931652

El-Borgi, Cecile Poster Location: 10 Poster Judging Number: 310

FlashTalk Presenter

Title: Impact of Prolonged Experimental Sleep Restriction on Indices of Arterial Stiffening in Healthy Humans

Authors: Cecile El-Borgi[1], Naima Covassin[2], Michael J Joyner[3], Virend K Somers[2], Jacqueline K Limberg[1]

Affiliations: [1]Department of Nutrition and Exercise Physiology, University of Missouri, Columbia, MO, USA; [2]Department of Cardiovascular Medicine, Mayo Clinic, Rochester, MN, USA; [3]Department of Anesthesiology and Perioperative Medicine, Mayo Clinic, Rochester, MN, USA.

Background: Insufficient sleep is a key risk factor for the development of hypertension and cardiovascular disease; however, the underlying mechanisms remain to be elucidated. Arterial stiffness is a strong predictor of cardiovascular events. We hypothesized prolonged exposure to restricted sleep would increase carotid-femoral pulse wave velocity (cfPWV), an index of central aortic stiffness, in healthy young adults.

Methods: Ten (5M, 5F) healthy young adults (23±3 yrs, 24±2 kg/m^2) underwent a 16-day inpatient study consisting of 4 days of acclimation followed by 9 days of experimental sleep restriction (4 hours per night). Seven (4M, 3F) adults (23±3 yrs, 23±3 kg/m^2) completed 4 days of acclimation followed by 9 days of normal sleep (9 hours per night, time control). Supine blood pressure was measured via upper arm sphygmomanometry and cfPWV was assessed non-invasively by applanation tonometry (SphygmoCor) on day 2 of acclimation and day 9 of sleep restriction/normal sleep.

Results: Daytime supine blood pressures remained unchanged across both normal (systolic, $p = 0.40$; diastolic, $p = 1.00$) and restricted (systolic, $p = 0.86$; diastolic, $p = 0.37$) sleep. cfPWV did not differ between groups during the acclimation period (5.3±0.5 vs 5.4±0.7 m/s, $p = 0.70$). cfPWV remained unchanged following 9 days of normal sleep (5.3±0.5 to 5.3±0.5 m/s, $p = 0.93$). In contrast, cfPWV increased following 9 days of sleep restriction (5.4±0.7 to 5.7±0.6 m/s, $p = 0.02$). The increase in cfPWV with restricted sleep was coupled with decreased carotid-femoral mean (88±12 to 83±9 ms, $p = 0.03$) and round trip (164±38 to 140±38 ms, $p < 0.01$) time, consistent with faster pulse wave transit time and earlier return of reflected waves to the heart.

Conclusions: Prolonged experimental sleep restriction in young healthy adults increases arterial stiffness independently of blood pressure, potentially contributing to increased cardiovascular event risk.

Funding: NIH HL 114676, NIH HL 083947, NIH TR002377

Reddy, Neeti **Poster Location: 11** **Poster Judging Number: 311**

Chemotherapy versus Radiation for Cardiac Tumors

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Background

Malignant cardiac tumors (MCT) pose substantial treatment challenges due to their close proximity to critical cardiac structures and the common occurrence of delayed diagnosis. Standard treatment approaches often involve chemotherapy and radiotherapy. This study aims to compare the treatment outcomes of these two modalities in patients diagnosed with MCT.

Methods

We reviewed data on 783 patients diagnosed with MCTs from the Surveillance, Epidemiology, and End Results (SEER) program from 2000 to 2021. Survival distribution trends were calculated for variables such as radiation type and chemotherapy type using Kaplan-Meier analysis and Cox Proportional Hazards Regression.

Results

We found a significant difference in survival between the chemotherapy and radiotherapy groups, with chemotherapy showing better outcomes ($p = 0.006$). Most common tumor types were hemangiosarcoma, diffuse large B cell lymphoma and giant cell sarcoma. Chemotherapy had a higher survival rate (16%) than beam (11%) and general radiation (13%). Over 5 years, chemotherapy had the highest survival rates. Notably, no patients received combined treatment.

Conclusion

This study highlights the higher survival rates in MCT patients treated with chemotherapy. These findings underscore the importance of further research to optimize chemotherapeutic treatment strategies for MCT patients. It also opens the question on utility of combined chemo and radiotherapy in highly malignant tumors.

Pearcy, Paige **Poster Location: 12** **Poster Judging Number: 112**

Age modifies the vascular response to β_3-adrenergic receptor agonism in females

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Vascular β-adrenergic receptors (ARs) mediate vessel relaxation. Preclinical work demonstrates enhanced β_3-AR–mediated vasodilation in females which is lost following ovariectomy. Unfortunately, ovariectomy (*i.e.,* surgical menopause) fails to provide insight into the temporality of the menopausal transition which occurs in humans (*i.e.,* perimenopause). We hypothesized β_3-AR–mediated vasodilation declines progressively across stages of menopause. Twelve premenopausal (19-41 yr, 24±3 kg/m^2), five perimenopausal (42-51 yr, 24±3 kg/m^2) and six postmenopausal (57-70 yr, 23±3 kg/m^2) female participants completed two study visits randomized and blinded to oral placebo or vibegron (75mg; a β_3-AR agonist). One hour following ingestion, forearm blood flow (FBF, venous occlusion plethysmography) and blood pressure (BP, finger photoplethysmography) were assessed during a 10-min quiet rest. FBF was normalized for mean BP (forearm vascular conductance, FVC). A two-way mixed effects ANOVA compared FVC across groups and treatments. A change-point analysis implemented via Markov chain Monte Carlo (MCMC) was applied to estimate the age-dependent change point and associated slope shift in ΔFVC (vibegron – placebo). The effect of vibegron on FVC differed by group (group by treatment interaction, p=0.025); β_3-AR–agonism increased resting FVC in premenopausal (p=0.002) but not perimenopausal (p=0.615) or postmenopausal (p=0.680) participants. There was a negative association between age and ΔFVC before 41.4 yr (ΔFVC: B=-0.0895±0.0440, P=0.042). Thereafter, significant associations between age and FVC were not observed (ΔFVC: B=0.0006±0.0361, P=0.987). β_3-AR agonism increased FVC in pre- but not peri- or postmenopausal females, supporting an early decline in β-AR function across the menopause transition. Surprisingly, segmented regression analysis identified an age-associated decline in β_3-AR mediated vasodilation until 41.4 yrs after which it stabilizes. These preliminary data are the first to show β-AR vasodilation in females begins to decline prior to menopause onset. Future analyses will investigate mechanisms responsible and explore strategies to restore vasodilatory responsiveness.

Funding: AHA 909014 (DWJ), HL153523 (JKL), University of Missouri Research Council (NGB, BPB, JKL), APS SURF (VLDV)

Dow, Kaylie **Poster Location: 14** **Poster Judging Number: 314**

Role of Alpha1 Adrenergic Signaling in Nucleus Ambiguus Regulation of Heart Rate

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The brainstem's nucleus ambiguus (NA) is a central regulatory region for heart rate (HR) through cardiac vagal motor neurons (CVNs) that send direct axonal projections to cardiac tissue. Given their quiescent nature, receptor signaling in CVNs is critical for HR regulation and this regulation is dampened in cardiovascular disease (CVD). Adrenergic receptors (ARs) initiate diverse intracellular signaling based on subtype (α1, α2, β). Although α1-ARs were implicated in increased NA excitability, central adrenergic regions elevate heart rate (HR) and α1-AR agonism in CVNs increased inhibitory GABA receptor (GABAR) activity. Both these results are consistent with decreased (not increased) excitability. To clarify the role of α1-ARs in CVN-mediated cardiac regulation, we hypothesized α1A-ARs suppress CVN activity. Molecular biology confirmed expression of all α1-AR isoforms (A, B, D) in NA, including CVNs specifically. In urethane-anesthetized wild-type (WT) mice, bilateral NA nanoinjection of the α1-AR agonist, phenylephrine (PE; 60 nL; 10 nL/s), produced a significant tachycardia (564.2±32.1 vs 625.7±20.9 BPM; n = 5; p = 0.03). In α1A-AR^{Null} mice, PE elicited bradycardia (593.1±7.1 vs 555.5±8.7 BPM; n = 4; p = 0.01), and in mice lacking GABAR activity in NA (ChAT-δ^{Null}), PE induced a smaller, non-significant bradycardia (604.2±31.1 vs 591.4±39.7 BPM; n = 4). HR changes across genotypes differed significantly (WT: +61.3±39.3; α1A-AR^{Null}: −37.6±13.0; ChAT-δ^{Null}: −12.2±26.1; one-way ANOVA, p = 0.001), with post hoc differences between WT and α1A-AR^{Null} (p = 0.001) and WT and ChAT-δ^{Null} (p = 0.01). These findings indicate that activation specifically of α1A-ARs in NA increases HR (by decreased excitability) via increased inhibitory GABAR signaling. Future studies will use optogenetic stimulation of adrenergic terminals in NA and electrophysiological recordings to determine whether α1A-AR effects are direct or indirect. These studies provide new insight into and potential therapeutics to restore central circuit regulation of HR in CVD.

Funding sources
R01HL157366 NIHLB to CRB and Mizzou Life Science Fellowship to KED

McDonald, Matthew **Poster Location: 15** **Poster Judging Number: 315**

Propranolol Enhances Hypoxic Cerebrovascular Reactivity Through Maintained Autoregulation

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PURPOSE: Cerebral autoregulation (CA) allows the brain to maintain steady perfusion when peripheral hemodynamics are altered. β-adrenergic receptor (β-AR) blockade may impair CA by reducing cardiac output and blunting cerebral vasodilation. Hypoxia and hypercapnia are also known to impair CA. Herein we assessed cerebrovascular reactivity (CVR) and CA during hypoxic and hypercapnic stress with and without administration of β-AR antagonist, propranolol. We hypothesized 1) CA would be impaired during hypoxia and hypercapnia, and 2) β-AR blockade would exacerbate the impairments in CVR and CA during both challenges.

METHODS: Middle cerebral artery velocity (MCAv, transcranial Doppler ultrasound) was assessed in 20 young adults (8F/12M, 27±7yrs; 24±3kg/m^2). Individuals completed two randomized visits blinded to oral placebo or propranolol (1 mg/kg). Participants completed a rest period followed by two 5-min conditions: 1) steady-state hypoxia (10.4±0.7 F_iO_2, 81.6±2.1% S_pO_2), 2) hyperoxic hypercapnia (+6.0±1.8 mmHg partial pressure of end-tidal CO_2). CA was quantified using transfer function analysis of blood pressure-MCAv oscillations in the low-frequency (LF) range.

RESULTS: MCAv increased with hypoxia (hypoxia main effect, $p<0.001$) and hypercapnia (hypercapnia main effect, $p<0.001$). Propranolol augmented MCAv reactivity to hypoxia (interaction propranolol and hypoxia, $p=0.006$) but not hypercapnia (interaction propranolol and hypercapnia, $p=0.408$). LF gain increased with hypoxia ($p=0.013$) and hypercapnia ($p=0.002$) during placebo; following propranolol no change in LF gain during hypoxia ($p=0.399$) or hypercapnia ($p=0.581$) was observed.

CONCLUSIONS: β-AR blockade enhances CVR and maintains CA during hypoxia, but not hypercapnia. These findings provide new insight into the role of β-AR signaling in regulating cerebrovascular responses to metabolic stress.

Behrmann, Andrew **Poster Location: 16** **Poster Judging Number: 316**

A Novel Two-Hit Challenge Induces TRPV4-Dependent Left Ventricular Diastolic Dysfunction in Isolated Hearts of Aged Mice

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Introduction: Transient Receptor Potential Vanilloid 4 (TRPV4) is a nonselective cation channel upregulated in cardiomyocytes with aging. TRPV4 activation causes pathological Ca^{2+} influx, arrhythmias, and dysfunction. Angiotensin II (AngII) and selective TRPV4 agonists have shown synergistic effects of fully activating TRPV4 in vascular tissue as reported in recent literature. Herein, we test the hypothesis that combining AngII and specific TRPV4 agonist GSK1016790A (GSK101) elicits a maximal TRPV4 response in isolated hearts of aged mice inducing cardiac dysfunction. The specific TRPV4 antagonist GSK2193874 (GSK219) was used to determine TRPV4 effects. Other TRPV4 antagonists have been deemed safe for human use in other clinical trials.

Methods: Hearts of male and female mice 24-29 months of age were subjected to a modified langendorff perfusion technique. A modified krebs henseleit buffer (KHB) with simultaneous bath ECG and left ventricular pressure monitoring was used. A 15-minute baseline period was performed followed by a 30-minute perfusion of KHB containing: AngII (n=5; 4m,1f), AngII+GSK101 (n=8; 4m,1f), GSK101 alone (n=6; 4m, 2f), or AngII+GSK101+GSK219 (n=6; 4m, 2f).

Results: There were no significant differences in baseline cardiac function between the groups. Following treatment, there were no significant differences in systolic function parameters of maximum pressure development and rate of pressure development between the groups. However, we observed severe impairment in the diastolic parameters of end diastolic pressure (EDP) and rate of ventricular relaxation (dP/dt_{min}) in the AngII+GSK101 group. There was a significant rise in the AngII+GSK101 group's EDP: (GSK101:+0.6±0.5 mmHg, AngII: +2.5±1.1 mmHg, AngII+GSK101: +17.7±4.8 mmHg, AngII+GSK101+GSK219: -0.1±0.9 mmHg; $p<0.01$) as well as dP/dt_{min} (GSK101:-51±169 mmHg/s, AngII:-48±35mmHg/s, AngII+GSK101:+459±54 mmHg/s , AngII+GSK101+ GSK219: -130±103 mmHg/s; $p<0.01$) compared to the other groups.

Conclusions: A Two-hit challenge of AngII and TRPV4 agonists unmasks a TRPV4-dependent phenotype of severe diastolic dysfunction in aged mouse hearts representing a therapeutic target in diastolic heart failure.

Funding: Funded by R01HL136292 (TD), and AHA 25PRE1374407 (AB).

Naeem, Haadi **Poster Location: 17** **Poster Judging Number: 117**

Oxidative stress-induced vascular smooth muscle cell senescence promotes cellular stiffening and actin polymerization via a LIM kinase-dependent pathway

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Aging of vascular smooth muscle cells (VSMCs) is a key pathogenic mechanism in cardiovascular disease (CVD) that promotes arterial remodeling and stiffening. Notably, arterial stiffening is characterized by structural changes in the vascular wall, including increased accumulation of fibrillar (F)-actin in VSMCs, as observed in the arteries of older adults with hypertension and animal models of aging. Oxidative stress has been associated with aging-related increases in arterial stiffness through the induction of VSMC senescence. Indeed, hydrogen peroxide (H_2O_2), a key reactive oxygen species, has been shown to induce VSMC senescence in cell culture, as evidenced by the upregulation of senescence markers such as senescence-associated β-galactosidase (SASP), p53-p21, and p16. However, the mechanisms by which H_2O_2-induced cellular senescence drives VSMC stiffening are unknown. Herein, we tested the hypothesis that H_2O_2-induced VSMC senescence promotes VSMC stiffening by increasing actin polymerization. We tested our hypothesis in cultured primary human aortic smooth muscle cells (HAoSMC) that were either left untreated or treated with 50μM H_2O_2 for 4 hours (n = 6/condition). To determine whether the pharmacological inhibition of LIM kinase (LIMK), an enzyme that promotes actin polymerization by inactivating cofilin severing activity, modulates H_2O_2-induced VSMC stiffening, HAoSMC were pretreated with either vehicle control (dimethyl sulfoxide; DMSO) or the LIMK inhibitor LIMKi3 for 18 hours, followed by exposure to 50μM H_2O_2 for 4 hours (n = 6-12/condition). Our results show that H_2O_2 induced VSMC senescence by increasing the expression of p21. This senescence phenotype was associated with increases in F-actin content and VSMC cortical stiffness (assessed via atomic force microscopy) relative to the control. Providing an understanding of the mechanism by which H_2O_2 increased actin polymerization, we show that H_2O_2 increased cofilin phosphorylation (inactivation) without altering the phosphorylated-to-total cofilin ratio. We further show that LIMK inhibition abrogated the actin polymerization induced by H_2O_2. Collectively, these findings demonstrate that H_2O_2-induced cellular senescence promotes VSMC stiffening via activation of the LIMK/cofilin/actin polymerization pathway.

Mahmud, Sakib Poster Location: 18 Poster Judging Number: 318

Inflammation-Associated Transcriptomic Reprogramming of Perivascular Adipose Tissue in Experimental IBD

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Inflammatory Bowel Disease (IBD) is characterized by chronic intestinal and systemic inflammation and is associated with vascular dysfunction and increased cardiovascular risk. Perivascular adipose tissue (PVAT), an immunometabolic tissue surrounding blood vessels, plays an active role in regulating vascular tone and inflammation. In IBD, PVAT acquires a pro-contractile phenotype, suggesting it may serve as a mechanistic link between intestinal inflammation and vascular pathology. This study employed genomic sequencing approaches to test the hypotheses that PVAT in IBD exhibits altered microbial composition, inflammatory and metabolic transcriptional reprogramming, and the capacity to induce pro-inflammatory gene expressions in macrophages. Using an IL-10$^{-/-}$ + Helicobacter hepaticus mouse model of IBD, with non-gavaged IL-10$^{-/-}$ and IL-10$^{+/+}$ wild-type controls, three complementary studies were performed. Study 1 characterized bacterial communities in mesenteric PVAT (mPVAT), aortic PVAT (aPVAT), visceral adipose tissue, and cecal contents using 16S rRNA sequencing. Study 2 utilized bulk RNA sequencing to identify differentially expressed genes and enriched pathways in mPVAT. Study 3 assessed macrophage responses via bulk RNA sequencing of RAW 264.7 macrophages treated with PVAT-conditioned media. IBD significantly altered microbial communities in both gut and adipose tissues, with beta-diversity analyses demonstrating clear separation among experimental groups. IBD PVAT exhibited increased abundance of inflammation-associated taxa, including Proteobacteria, Fusobacteriota, and Campylobacterota, indicating extra-intestinal dysbiosis. Transcriptomic profiling revealed extensive mPVAT reprogramming, including upregulation of oxidative phosphorylation, cytokine signaling, extracellular matrix remodeling, and immune pathways such as IL-1β production, chemokine signaling, and neutrophil extracellular trap formation. Consistent with these findings, IBD PVAT–conditioned media induced inflammatory gene expression in macrophages, promoting prostaglandin synthesis and immune recruitment. Collectively, these data identify PVAT as a critical mediator linking intestinal inflammation to vascular dysfunction in IBD.

Funding: This work was supported by NIH grant R01HL157038.

Gonsalves, Anna Poster Location: 19 Poster Judging Number: 319

Respiratory modulation of sympathetic activity during hypoxia and impact of obesity

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Muscle sympathetic nerve activity (MSNA) is modulated by respiration, peaking during end-expiration and suppressed mid-inspiration. Given adults with obesity exhibit elevated MSNA and exaggerated ventilatory responses to hypoxia, we sought to examine the relationship between ventilation and MSNA in adults with obesity under normoxic and hypoxic conditions. We hypothesized hypoxia would augment respiratory modulation of MSNA, with greater effects in adults with obesity vs. normal weight. MSNA (microneurography) and minute ventilation (pneumotach) were evaluated in adults with normal weight (8F/12M, 23±2 kg/m^2) and obesity (6F/2M, 35 ±5 kg/m^2) during normoxia (10-min, ~98% SpO_2) and steady-state hypoxia (5-min, ~80% SpO_2). Respiratory modulation of MSNA was assessed via cross-correlation histograms between MSNA spikes and respiration; the modulation index was calculated as the difference between the maximum and minimum number of MSNA spikes across the breath [(maximum-minimum)/maximum×100]. Adults with obesity had greater normoxic minute ventilation (p=0.044) and MSNA (p=0.017) than normal weight adults. Modulation index did not differ between groups during normoxia (p=0.367). Hypoxia increased minute ventilation in both groups (both p<0.001). MSNA increased during hypoxia in normal weight adults (14±5 to 18±6 bursts/min, p=0.004) but not adults with obesity (20±7 to 26±13 bursts/min, p=0.216). Hypoxia increased the modulation index in adults with obesity (57±16% to 76±14%, p=0.005) but not in normal weight participants (61±14% to 66±17%, p=0.333). In summary, respiratory modulation of MSNA does not differ between normal weight adults and adults with overweight/obesity under normoxia. In contrast, adults with obesity exhibit enhanced ventilatory sympathoinhibition during hypoxia, resulting in an amplified respiratory-sympathetic coupling.

Funding: R01HL153523

Nekouian, Soraya **Poster Location: 20** **Poster Judging Number: 320**

Title: Role of CD4+ β2-Adrenergic Receptor Signaling in Cardiac Remodeling in a Pressure-Overload Model of Heart Failure

Background: Heart failure often leads to pathological cardiac remodeling, significantly influenced by immune mechanisms. CD4+ T cells, through β2 adrenergic receptor (β2AR) signaling, are hypothesized to play a crucial role in this process. The impact of β2AR signaling in CD4+ T cells on cardiac remodeling and fibrosis in heart failure conditions remains to be fully elucidated.

Objective: This study investigates the role of CD4+ β2AR signaling in cardiac remodeling under pressure-overload conditions, hypothesizing that deletion of β2AR in CD4+ T cells would impair the remodeling process.

Methods: We utilized a murine model of heart failure induced by transverse aortic constriction (TAC) to study the effects of β2AR signaling in CD4+ T cells. Wild-type mice were compared to CD4+ T cell-specific β2AR knockout mice(tKO). Cardiac function was assessed via echocardiography, fibrosis was evaluated through histological analysis, and inflammatory responses were measured using molecular assays.

Results: The deletion of β2AR in CD4+ T cells led to a marked reduction in cardiac remodeling and fibrosis, indicating that β2AR signaling in these cells is critical in driving the pathological changes observed in pressure-overload heart failure. The results showed that tKO mice exhibited less fibrosis and a reduced inflammatory response, including lower ICAM-1 gene expression, highlighting the significant impact of β2AR signaling on these processes

Conclusion: Our findings demonstrate that β2AR signaling in CD4+ T cells plays a significant role in cardiac remodeling and fibrosis during heart failure. The absence of this signaling dampens these processes, suggesting that modulating β2AR activity in CD4+ T cells could be a potential therapeutic strategy to mitigate adverse cardiac remodeling.

Keywords: CD4+ T cells, β2-adrenergic receptor, cardiac remodeling, heart failure, pressure overload, inflammation, fibrosis

Kammerer, Kyle Poster Location: 22 Poster Judging Number: 322

Unifying Canonical and Non-Canonical Excitability Effects of 5-HT1ARs In Motor Cortex

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Serotonin (5-hydroxytryptamine; 5-HT) is a neurotransmitter that exerts robust effects on neocortical excitability that may be leveraged to promote stroke recovery. Canonically, 5-HT receptor subtype 1A (5-HT1ARs) are expressed in multiple neural structures and function using G protein-gated inward rectifying potassium (GIRK) channels and Gi GPCR intracellular signaling to reduce activity of cells that express them (Polter & Li, 2010). Non-canonically, activation of 5-HT1ARs increases excitability of the motor cortex in vivo and increases probability of action potential firing of this structure's layer V pyramidal neurons (Scullion et al., 2013). As a possible mechanism of these non-canonical effects, we tested whether 5-HT1AR expression is restricted to inhibitory gamma-aminobutyric acid (GABA)-releasing interneurons. Htr1a-IRES2-Cre-D mice (5-HT1AR-Cre) (Daigle et al., 2018) were crossed with Ai14 (TdTomato reporter) mice (Madisen et al., 2010) to express endogenous red fluorescence in 5-HT1AR+ cells. Interestingly, separate populations of 5-HT1AR+ cells co-label with either inhibitory GABAergic or excitatory glutamatergic markers. Adeno-associated virus of retrograde serotype (AAVrg) traced corticospinal cells show less than 5% overlap with 5-HT1AR+ cells indicating 5-HT1ARs affect motor cortex excitability upstream of this cell population. Ongoing experiments are testing the contributions of 5-HT1ARs to excitability of motor cortex using whole cell patch-clamp recordings of corticospinal cells and GABAergic cells in motor cortical layer II/III and V. Measurements include resting motor potential, action potential threshold, firing rate, spontaneous excitatory postsynaptic currents (sEPSCs), spontaneous inhibitory postsynaptic currents (sIPSCs) and tonic GABAAR currents. Preliminary results indicate separate hypoexcitable or hyperexcitable effects of 5-HT1ARs, in a cell type specific manner, in motor cortex. Ongoing testing is increasing sample size and comparing the magnitude of these hypoexcitable versus hyperexcitable effects to unify their net contributions to corticospinal output. Understanding the mechanistic underpinnings for these effects is critical to optimizing this signaling target for stroke recovery.

Funding: NIH RO1NS114651

Barcellos-Filho, Procopio **Poster Location: 23**

Chemo- and visceral afferent activation of Corticotropin Releasing Hormone (CRH) fibers within the nucleus of the Solitary Tract (nTS).

Procopio Gama de Barcellos Filho[1,2], David D. Kline[1, 2, 3].

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Peripheral cardiorespiratory reflexes in response to hypoxia are processed and integrated in the nTS. We and others have previously shown that CRH inputs into the nTS are critical in modulating nTS synaptic and neuronal activity in normoxia and during hypoxia bouts, as well as cardiovascular function. However, the in vivo activation of these CRH fibers within the nTS in coordination with reflex -mediated alterations of cardiorespiratory parameters is unknown. We hypothesize that CRH fibers will increase activity during hypoxia.

To evaluate circuit dynamics *in vivo*, we crossed CRH-Cre mice (B6(Cg)-*Crhtm1(cre)Zjh*/J) with a Cre-dependent GCaMP (B6J.Cg-Gt(ROSA)26Sortm95.1(CAG-GCaMP6f)Hze/MwarJ) reporter line to globally express GCaMP in all CRH neurons in the brain. Mice were instrumented with a femoral arterial catheter to monitor blood pressure and diaphragmatic electrodes for respiratory EMG recording. Furthermore, a bipolar electrode was implanted on the cervical vagus nerve to evaluate visceral afferent integration in the nTS. The dorsal brainstem was exposed, and a fiber optic probe was positioned over the nTS to record calcium activity specifically from CRH-putative fibers and terminals via fiber photometry.

When exposed to a 10% hypoxia challenge (40 sec), mice exhibited a transient increase in mean arterial pressure (MAP) that correlated with a persistent increase in calcium activity in the nTS-CRH terminals. Electrical stimulation of the vagus nerve (20Hz, 20mA) caused a biphasic response during the stimulation; an initial increase in CRH fiber calcium activity at the beginning of the stimulation, followed by a decrease at the end. The decrease occurred alongside reductions in MAP, HR, and respiratory frequency. Overall, these data indicate that CRH-positive inputs in the nTS are actively involved during both systemic hypoxia and vagal afferent stimulation. Further research is needed to clarify their specific roles in influencing MAP, HR, and respiration, and to better understand their functions within the nTS circuitry.

Zhang, Zhe Poster Location: 24 Poster Judging Number: 424

Characterizations of motor neurons derived from iPSCs of a novel sensory motor neuropathy caused by a NAMPT mutation

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Abstract: In mammalian cells, NAD^+ is primarily synthesized through the salvage pathway, where nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme. Increasing evidence suggests a neuronal protective role of NAMPT in a range of neurological diseases such as ischemic stroke, Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS). In our recent study, we discovered a novel sensory motor neuropathy attributed to a homozygous missense mutation (c.472.C>G, p.P158A) in the coding region of the NAMPT gene. To study the functional change of motor neurons caused by the P158A mutation, in current study, we reprogramed the fibroblasts collected from the patient into induced pluripotent stem cells (iPSCs) and subsequently differentiate them into motor neurons (MNs). Using a variety of approach, we investigated the bioenergetic and metabolic effects of P158A mutation in NAMPT on iPSC derived MNs. Our results demonstrated that the P158A mutation in NAMPT disrupts cellular metabolism and causes mitochondrial dysfunction in iPSC-derived MNs. The P158A mutation also leads to aberrant synaptic development and Ca^{2+} signaling and induces cell senescence. Overall, our study reveals an undiagnosed motor neuropathy caused by P158A mutation in NAMPT, confirms NAMPT as a key enzyme in neuronal metabolism and functions and indicates that targeting NAMPT might be a novel therapeutic strategy for motor neuron disease.

Loving, Kate **Poster Location: 25** **Poster Judging Number: 125**

Blood Lactate Removal is Positively Associated with Post-Exercise Neurovascular Coupling

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PURPOSE: Neurovascular coupling (NVC) ensures that increased neuronal activity is met with increased cerebral perfusion. Lactate, a product of anaerobic metabolism and neuronal fuel source, increases during exercise, potentially helping facilitate NVC. This project examined the effect of submaximal exercise on NVC and the relationship between NVC and blood lactate. We hypothesized that 1) NVC would increase following exercise and 2) post-exercise NVC would be positively associated with blood lactate removal.

METHODS: Twelve adults (5M/7F, 25±5y, 28±6 kg/m2) completed a maximal exercise test to determine their lactate threshold (LT). During a subsequent study visit, participants exercised on a cycle ergometer for 30-min at their submaximal LT followed by 30-min of seated recovery. Three cognitive tests [pattern comparison processing (PCP), picture vocabulary (PV), visual reasoning (VR)] were completed prior to and following exercise. Middle cerebral artery velocity (MCAv, transcranial Doppler ultrasound) was measured as an index of cerebral perfusion and NVC was assessed as the peak change (Δ) in MCAv from baseline during each test. Blood was drawn intravenously every five minutes throughout exercise and recovery for measures of lactate levels. Blood lactate removal was assessed as area under the curve during recovery.

RESULTS: The peak change in MCAv did not significantly increase post-exercise for PCP (Δ: 12±4 to 14±6, p=0.43), PV (Δ: 11±4 to 14±8, p=0.11), or VR (Δ: 12±4 to 14±9, p=0.34). Post-exercise NVC showed strong, positive associations with elevated blood lactate levels in all three tests: PCP (r=0.70; p=0.01), PV (r=0.66; p=0.02), VR (r=0.64; p=0.03).

CONCLUSIONS: Contrary to our hypothesis, NVC was unchanged following 30-min of submaximal exercise at the LT for each of the three cognitive tests. However, strong associations between NVC and elevated post-exercise blood lactate levels were observed, supporting a potential role for elevated lactate concentrations in mediating post-exercise NVC.

Funding: University of Missouri

Wagoner, Eryn **Poster Location: 26** **Poster Judging Number: 326**

FlashTalk Presenter

mAKAPβ targeted gene therapy prevents cerebral arteriole endothelial dysfunction and vascular stiffness in female Ossabaw swine with cardiometabolic heart failure

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Heart failure with preserved ejection fraction (HFpEF) is more prevalent in females compared to males, and ~75% of HFpEF patients experience cognitive impairment, increasing their risk of hospitalization and mortality. We previously demonstrated cerebrovascular insufficiency in female Ossabaw swine with cardiometabolic HFpEF (HF) and improved cardiac function and ventricular-vascular interactions by a novel mAKAPβ targeted gene therapy (AAV9sc.AKAP6). AAV9sc.AKAP6 prevents RSK3 and CAMKII binding to mAKAPβ, ultimately preventing cardiac fibrosis and inflammatory $CD3^+$ T-cell infiltration. The goal of this study was to examine the effects of AAV9sc.AKAP6 on cerebral arteriole endothelial function, vascular stiffness, transcriptomic signaling, and protein expression in female Ossabaw swine with HF. Animals were assigned to HF (n=4-5), HF+AAV9sc.AKAP6 (HF+AKAP6; n=4-7), and Lean control (LC; n=5). LC were fed standard chow (12mo.) while HF and HF+AKAP6 were fed a Western diet (9mo.) and aortic banded (6mo.) prior to terminal experiments (12mo.). AAV9sc.AKAP6 was infused immediately after aortic banding. In second order cerebral arterioles isolated from the middle cerebral artery, AAV9sc.AKAP6 prevented dose-dependent reductions in endothelial-dependent dilation (84.6%, HF 53.5%, LC 75% max; Two-way ANOVA, Group x Dose interaction, $p<0.05$) and vascular stiffness (6 ± 1 x10^6, HF 15 ± 2, LC 6 ± 1 x10^6dyn/cm^2; One-way ANOVA, $p<0.05$). RNA sequencing and ingenuity pathway analysis identified decreased endothelial cell movement and cell junction organization and increased immune cell infiltration in HF v LC, decreased immune cell infiltration in HF+AKAP6 v LC, and POR inhibition (upstream regulator of fibrosis) in HF+AKAP6 v HF (z-score <-2,>2). Immunofluorescent POR expression, total and perivascular fibrosis, and cerebral blood flow were unaltered by AAV9sc.AKAP6 in the prefrontal cortex and hippocampus. These findings demonstrate AAV9sc.AKAP6-induced improvement of cerebral arteriole endothelial function is linked with reduced vascular stiffness, but unrelated to POR expression, fibrosis, and cerebral blood flow. Future studies will investigate endothelial cell junction integrity and inflammatory immune cell infiltration in AAV9sc.AKAP6-mediated improvements of cerebral vascular function.

Funding: MU Tier 1 Sequencing Grant and Department of Defense (DOD) Grant W81XWH-18-1-0179

Udefa, Augustine Poster Location: 27 Poster Judging Number: 327

FlashTalk Presenter

Semaglutide enhances vascular relaxation to insulin in Western diet-fed female mice

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Vascular insulin resistance is a hallmark of obesity and contributes to the development of cardiovascular disease. However, therapeutic strategies that restore insulin actions in the vasculature are largely lacking. Glucagon-like peptide-1 receptor agonists (GLP-1RAs), such as semaglutide, were initially approved as glucose-lowering agents in type 2 diabetes and later for chronic body weight management. Subsequent studies have positioned GLP-1RAs in the realm of cardiovascular protective agents. Indeed, large outcome trials consistently demonstrate that GLP-1RAs lower blood pressure, reduce cardiovascular mortality and major adverse cardiovascular events, slow the progression of chronic kidney disease, and decrease hospitalizations for heart failure. Notably, these cardiovascular benefits exceed what would be expected from weight loss alone, suggesting direct tissue-protective effects of GLP-1RAs. Although evidence supports the notion that GLP-1RAs confer cardiovascular protection by mechanisms beyond weight loss, whether semaglutide enhances vascular insulin sensitivity remains less known. Herein, we tested the hypothesis that semaglutide treatment enhances vascular relaxation to insulin in a mouse model of diet-induced obesity. All differences reported are significant at $P<0.05$. Four-week-old female C57BL/6 mice were fed a Western diet for 24 weeks to induce obesity, and treated with daily subcutaneous semaglutide (9 nmol/kg, n=10) or vehicle (n=13) during the last 8 weeks. As expected, semaglutide treatment reduced body weight, improved glucose tolerance, as assessed via glucose tolerance test, and reduced mean arterial pressure, as assessed via tail cuff plethysmography. Insulin-induced relaxation, assessed via wire-myography in aortic rings, was enhanced in semaglutide-treated mice compared to controls. Similarly, acetylcholine-induced relaxation was also augmented in the semaglutide group. Endothelium-independent relaxation, assessed using sodium nitroprusside, was unaltered by semaglutide. These findings indicate that semaglutide improves indices of endothelial function, including vascular insulin sensitivity, in obese female mice. Follow-up experiments are underway to examine the underlying mechanisms by which semaglutide exerts insulin-sensitizing effects in the vascular endothelium.

Funding: American Heart Association (24EIA1248820 to J.P.).

Romero, Chris **Poster Location: 28**

Motor Control of the upper airway is regulated by GABAergic interneurons in the nucleus tractus solitarius

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The upper airway (UAW) is essential for respiration and ingestion. UAW muscles must coordinate to prevent aspiration. In natural ageing, and pathological states such as stroke, UAW loses coordination, leading to aspiration-induced infections that dramatically increase risk of hospitalization and mortality. There is a lack of neural-centric treatments for UAW dysfunction that underscores a need to understand UAW central motor control pathways. The thyroarytenoid muscle prevents aspiration and is controlled by the corticobulbar tract (CBT). The CBT is composed of neocortical upper motor neurons (UMNs) that innervate brainstem lower motor neurons (LMNs) in nucleus ambiguus (nAmb) which then form the neuromuscular junctions of the thyroarytenoid. While GABAergic inhibition regulates CBT LMN coordination, the source of this inhibition is unknown. We hypothesize that GABAergic interneurons located in brainstem's nucleus tractus solitarius (nTS) provide CBT UMN-dependent inhibition to LMNs for spatiotemporal UAW control. We first tested this hypothesis by injecting mice with pseudo rabies virus (PRV)—a polysynaptic retrograde tracer—into the thyroarytenoid for expression of red fluorescence in thyroarytenoid-related UAW motor circuits. After 48, 72, and 96hrs, coronal brain slices containing motor cortex (MC), nTS, and nAmb were imaged for PRV+ neurons. Both nAmb and nTS demonstrated PRV+ labeling at all time points, confirming their primary and secondary hierarchy in CBT respectively. After 48hrs, MC showed no PRV+ signal whereas PRV+ cells were present at 72 and 96hrs, confirming their tertiary location in CBT. To confirm these secondary nTS neurons were GABAergic, transgenic mice expressing GFP in GABAergic neurons were injected with PRV in thyroarytenoid. In these mice, nTS expressed overlapping PRV+ and GFP+ cells. Direct innervation from neocortex to nTS was confirmed by monosynaptic retrograde adeno-associated viral tracing in nTS. Ongoing experiments will determine the identity and role of nTS interneurons in CBT's control of the UAW.

Funding: NIH RO1NS114651

Williams, Cale **Poster Location: 29** **Poster Judging Number: 229**

ACUTE EFFECTS OF INSPIRATORY MUSCLE TRAINING ON CEREBRAL BLOOD FLOW AND COGNITIVE FUNCTION IN HEALTHY ADULTS

Cale Williams, Graduate Student; Matthew McDonald, Graduate Student; Anna M. Gonsalves, Graduate Student; Brian Shariffi, Graduate Student; Jacqueline Limberg, FNES Professor; (Daniel Credeur, FNES Professor)
University of Missouri – Division of Food, Nutrition and Exercise Sciences

Background: Inspiratory muscle training (IMT) has been shown to enhance autonomic regulation and lower arterial blood pressure. While existing evidence focuses on chronic adaptations to IMT, emerging data suggest that even acute bouts may produce beneficial physiological effects, such as increased cerebral blood flow.

Purpose: To investigate the acute effects of IMT on cerebral blood flow and cognitive function in healthy adults.

Methods: Healthy adults (Age=29±7 yrs, N=6, Female=50%) completed a single bout of IMT, consisting of 3 sets of 5 repetitions at 75% of maximal inspiratory pressure, with a 60-sec rest between sets. Middle (MCA_V) and anterior (ACA_V) cerebral artery mean velocity signals were recorded continuously via Transcranial Doppler before and after IMT. Cognitive executive function was assessed using the Stroop color-word interference test and performed before and after IMT.

Results: Stroop performance improved pre- to post-IMT, characterized by a reduction in total time (pre=145±4 vs. post=135±3 sec, $p<0.001$), and average reaction time for both congruent (pre=1222±58 vs. post=1040±65 ms, $p<0.001$) and incongruent trials (pre=1287±83 vs. post=1148±50 ms, $p=0.01$). Cerebral blood velocities did not change pre- to post-IMT; however, there was a tendency for ACA_V to be higher during incongruent Stroop trials (pre-IMT=32±3 vs. post-IMT=38±4 cm/sec, $p=0.08$).

Conclusion: These preliminary findings suggest that a single bout of IMT enhances executive function, potentially through increased cerebral blood flow to frontal cortical regions. Further research is warranted to confirm the mechanisms underlying this response and to explore its clinical significance.

Wang, Yoko **Poster Location: 30** **Poster Judging Number: 430**

FlashTalk Presenter

High fat diet impacts GABAergic neurotransmission in cardiac vagal motor neurons in the nucleus ambiguus

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A 15-day high-fat-diet (HFD) blunts cardiac parasympathetic output, contributing to early cardiac dysregulation. Cardiac vagal motor neurons in nucleus ambiguus (CVN^{NA}) provide parasympathetic control of heart rate (HR), and their activity is regulated by inhibitory, GABAergic neurotransmission. However, how HFD impacts CVN^{NA} GABAergic neurotransmission remains unclear. We hypothesize that $GABA_A$ receptors-containing δ subunits ($GABA_A$δRs) in CVN^{NA} contribute to blunted parasympathetic-mediated HR changes after HFD. To test this, we used ex vivo electrophysiology combined with retrograde cardiac tracing to label CVN^{NA} in mice and examined both modalities of inhibition (phasic and tonic). Since CVN^{NA} receives glycinergic and GABAergic inhibitory inputs, we first characterized the contribution of each to phasic and tonic currents in slice preparation. Application of strychnine (1μM), a glycine receptor antagonist, and $GABA_A$R antagonists (picrotoxin, 100μM and/or gabazine, 100 μM), revealed that glycine contributes to ~91% of phasic current (charge $transfer_{Gly\ vs\ GABA}$=49.6±19.9 vs 1.4±0.7pC, p=0.02, n=13). In contrast, GABA contributed ~68% of tonic inhibition (charge $transfer_{Gly\ vs\ GABA}$=2283±472.3 vs 5336±856.5pC, p=0.005, n=18) and this tonic inhibition was specific to $GABA_A$δRs in CVN^{NA} (~59%; n=5). Furthermore, application of $GABA_A$δR-selective agonist THIP (3 μM) increased tonic current in ~40% of CVN^{NA} (p=0.0005, n=10). Together, these data confirm the importance of GABAergic tonic currents in slice preparation. In HFD mice, phasic current amplitude was increased in HFD group compared with normal fat diet (NFD) (HFD vs NFD=66.0±15.9 vs 34.2±4.5pA, p=0.03, n=7). THIP also induced greater tonic current in HFD compared to NFD (HFD vs NFD=4.4±2.6 vs -2.2±2.1pA), with positive THIP-responses observed in 4/5 cells in HFD and 1/4 cells in NFD. These findings suggest that 15 days of HFD enhanced $GABA_A$δR activity in CVN^{NA}, potentially contributing to early cardiac parasympathetic dysfunction. Future studies will examine how manipulation of $GABA_A$δR-dependent current impacts blunted cardiac parasympathetic output and early cardiac dysregulation.

Funding: R01HL157366 NIHLB to CRB

Shaheen, Sarah Poster Location: 31 Poster Judging Number: 131

Effect of High Protein Intake on Endothelial Function

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Endothelial dysfunction is an early marker of cardiovascular disease and is strongly influenced by dietary factors. Although high protein intake (HPI) is commonly recommended for weight management and metabolic health, its effects on vascular function and underlying mechanisms remain incompletely understood. We hypothesized that HPI impairs endothelial function through postprandial elevations in circulating amino acids and suppression of fibroblast growth factor-21 (FGF-21) signaling.

In a randomized crossover study, participants consumed isocaloric standard-protein or high-protein mixed meals. Postprandial endothelial function was assessed using the reactive hyperemia index (RHI), and plasma amino acid and FGF-21 concentrations were measured before and for 150 minutes after starting the meal. To evaluate the effects of increasing amounts of amino acids and FGF-21 on endothelial cells, we conducted in vitro experiments with human umbilical vein endothelial cells (HUVECs) and mouse vascular tissue.

High protein intake increased postprandial plasma amino acid concentrations and reduced circulating FGF-21, accompanied by a decrease in RHI. In endothelial cells, amino acid exposure increased reactive oxygen species production, reduced stimulatory eNOS phosphorylation
(Ser1177), increased inhibitory eNOS phosphorylation (Thr495), and decreased nitric oxide production. Amino acid treatment also increased inflammatory gene expression and vascular inflammasome activation in mouse arteries. In contrast, FGF-21 treatment enhanced eNOS activation and nitric oxide production in a dose-dependent manner.

These findings suggest that high protein intake impairs endothelial function, likely mediated by amino acid–induced oxidative and inflammatory stress, as well as the suppression of FGF-21 signaling.

Palkar, Rutvi **Poster Location: 32** **Poster Judging Number: 332**

Therapy Development for X-Linked Deletion and Expansion Diseases in Females

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Dosage imbalance of X-linked genes is a central driver in multiple human conditions, contributing to both neurodevelopmental and cardiovascular symptoms. Neurologically, loss of FMR1 gene expression underlies Fragile X Syndrome, the most common inherited cause of intellectual disability and autism. Cardiovascular manifestations are also prominent in X-linked disorders, particularly in Turner syndrome, where haploinsufficiency of X-linked genes contributes to congenital heart disease, vascular dysfunction and aortic abnormalities. Despite these clinical links, strategies to restore dosage of X-linked genes are not studied and no approved drugs are available.

X-chromosome inactivation (XCI) further complicates disease severity in females, as cellular mosaicism and skewed XCI patterns can lead to variable expression of disease-causing alleles. When the active X carries a deletion or silenced gene, the inability to access the intact allele on the inactive X exacerbates functional gene loss. This creates an opportunity to therapeutically target the inactive X (Xi) to restore gene dosage without introducing new genetic alterations.

This project explores targeted reactivation of genes on the inactive X chromosome (Xi) as a therapeutic strategy to compensate for X-linked gene loss. We focus on two complementary molecular approaches: (i) antisense oligonucleotide (ASO) mediated knockdown of XIST, a key regulator of Xi, and (ii) transcriptional activation using dCas9-VPR. Together, these strategies aim to weaken global Xi and directly enhance transcription of X-linked genes such as FMR1.

Initial studies established that X-linked genes show measurable transcriptional responsiveness to XIST modulation, supporting feasibility of this approach. Further studies test female patient-derived iPSCs as relevant models of gene-dosage regulation. Using multiple patients and isogenic iPSC clones only differing in Xi, this platform enables systematic evaluation of X-linked gene reactivation strategies.

Ultimately, this will test whether X-linked gene dosage modulation can stabilize or restore neural and cardiovascular functions, advancing therapies for X-linked disorders.

Qi, Zhifeng **Poster Location: 33**

Neuroendocrine regulation of cardiovascular function after spinal cord injury

Zhifeng Qi[1,2], Fateme Mericle[1,2], Christopher Foote[1,2], Ruwaida Ben Musa[1,2], Michael Hill[1], Eileen Hasser[1,3], Kevin Cummings[1,3], Shaoping Hou[1,2]

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The autonomic nervous system (ANS) normally regulates cardiovascular function, while hormonal mechanisms, particularly the renin–angiotensin system (RAS), also influence hemodynamics. After high-level spinal cord injury (SCI), neural control of blood pressure is reduced while RAS activity increases. It remains unclear whether elevated hormonal regulation contributes to cardiovascular disorders after SCI. Here, we investigated neuroendocrine regulation of resting hemodynamics and autonomic dysreflexia in a rat model of SCI, with particular focus on the role of RAS in baroreflex function and vascular remodeling. Pharmacological interventions included neural blockade (hexamethonium or SB366791), ACE/AngII/AT1R (captopril or losartan) or ACE2/Ang-(1-7)/MasR (A779 or MLN4760) inhibition. SCI elevated plasma AngII levels to imply RAS activation. Resting blood pressure decreased following neural blockade or AngII-AT_1R axis inhibition but was unchanged with MasR axis blockade. Neural blockers, but not RAS antagonists, markedly reduced colorectal distension-evoked autonomic dysreflexia. Baroreflex sensitivity was quantified using phenylephrine and sodium nitroprusside to generate sigmoidal logistic curves. SCI rats exhibited a steeper baroreflex curve and higher peak gain, indicating baroreflex hyperreflexia. Suppression of RAS activity reduced baroreflex gain in SCI rats. After sacrifice, the mechanistic characteristics of femoral and mesenteric arteries were evaluated using pressure myography. It demonstrated increased stiffness in SCI arteries. Subsequent vessel culture tests showed that inhibition of AT1R or its downstream signaling pathway attenuated arterial stiffness. Together, these findings implicate elevated RAS activity as a key regulator of hemodynamic maintenance, baroreflex hypersensitivity, and vascular remodeling after SCI. Targeting this pathway may therefore represent a therapeutic strategy for mitigating cardiovascular disease in SCI patients.

Fundings: NIH/NINDS R01 NS121336, MU SCIDRP2024, and MizzouForward Initiative

Fleury, Ava Poster Location: 34 Poster Judging Number: 134

FlashTalk Presenter

Right atrial calcium homeostasis in a mouse model of obstructive sleep apnea

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Pro-arrhythmic atrial myocyte calcium handling is associated with obstructive sleep apnea (OSA) through poorly understood mechanisms. The aim of this study was to test the hypothesis that atrial calcium homeostasis is altered in a mouse model of OSA induced by intermittent hypoxia (IH). Wildtype C57BL/6 mice (n=19 male) and tamoxifen-treated MerCreMer-Ai95D mice with cardiomyocyte-specific GCaMP6f expression (n=10 male/n=15 female) were exposed to IH (n=9 C57BL/6; n=13 GCaMP6f) or room air (RA, n=10 C57BL/6; n=12 GCaMP6f) for eight weeks. Normalized atrial and ventricular weights were obtained in C57BL/6 mice to assess hypertrophy. Atrial calcium handling was assessed in isolated GCaMP6f hearts at sinus rhythm (SR), 8 Hz, and 16 Hz using high-speed laser scanning confocal fluorescence microscopy. There were no differences in right (P=0.28) or left (P=0.32) atrial weights between groups. However, IH hearts displayed higher right-ventricular weight (P=0.04) and lower left-ventricular weight (P=0.03) than RA hearts. Fulton index, the ratio of right-ventricular weight to left-ventricle+septum weight, was higher in IH hearts (0.28±0.03 IH versus 0.24±0.03 RA, P=0.007). In GCaMP6f hearts, SR rate was higher in IH than RA (306±30 beats/min IH versus 273±39 beats/min RA, P=0.03). No differences in calcium transient amplitude were observed between the two groups at SR (P=0.41) or 8 Hz (P=0.11). Cytosolic calcium decay rate was faster in IH hearts at SR (59±6 ms IH versus 65±7 ms RA, P=0.04) but not at 8 Hz (39±2 ms IH versus 41±3 ms RA, P=0.15). During 16 Hz pacing, IH hearts displayed pro-arrhythmic calcium alternans (Alternans ratio of 0.42±0.38 IH versus 0.17±0.21 RA, P=0.05). Our data reveal altered calcium homeostasis in a mouse model of OSA at high stimulation frequencies, which may predispose the heart to atrial arrhythmias.

Funding: MU Honors College and Peggy and Andrew Cherng, and National Institutes of Health R01HL136292 (TD) and R01HL166617 (MB).

Zhou, Ruimei **Poster Location: 35**

Targeting DOCK2 Preserves Cardiac Function by Reprogramming Post-Infarction Metabolism

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Abstract:

Myocardial infarction (MI) is a leading cause of heart failure and death. Beyond the initial ischemic insult, persistent metabolic dysfunction critically impair recovery from the MI, highlighting the need to uncover molecular pathways that drive these maladaptive responses. Transcriptomic profiling of human and murine infarcted hearts revealed consistent upregulation of Dedicator of Cytokinesis 2 (DOCK2), a Rac-specific GEF, linked to immune cell trafficking and inflammation, prompting investigation into its pathological role in MI. Using a murine model of MI induced by 2 hours of coronary artery occlusion, we found that genetic ablation of DOCK2 (DOCK2-/-) conferred significant and sustained cardioprotection. Transthoracic echocardiography revealed that DOCK2-/- mice exhibited significantly higher left ventricular ejection fraction (LVEF) and improved fractional shortening (FS), along with reduced left ventricular end systolic diameter (LVESD) and end systolic volume (LVESV), indicating preserved systolic function and attenuation of adverse ventricular remodeling. Mechanistically, DOCK2 deletion enhanced myocardial energetic efficiency, as indicated by increased ATP production and activation of the AMP-activated protein kinase (AMPK)–acetyl-CoA carboxylase (ACC)–carnitine palmitoyltransferase 1 (CPT1) regulatory axis, consistent with a metabolic shift away from fatty acid oxidation toward glycolysis, a pathway that yields more ATP per oxygen molecule under ischemic conditions. These metabolic and transcriptional changes converged to promote vascular regeneration, as evidenced by increased proliferation of $CD31^+$ endothelial cells, elevated capillary density in the infarct border zone, and enhanced microvessel formation in Matrigel plug assays. Together, these findings position DOCK2 as a regulator of metabolism in myocardial infarction, highlighting DOCK2 as a compelling therapeutic target for cardiac repair.

Hussein, Samira Poster Location: 36 Poster Judging Number: 336

Tissue-Specific Reduction of IL-10 and Elevated Inflammation in Chronic Intermittent Hypoxia: Potential Implications for Obstructive Sleep Apnea

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Rationale: Obstructive sleep apnea (OSA) a chronic condition and is characterized by intermittent hypoxia (IH), which induces low-grade systemic inflammation that contributes to OSA-related morbidities. Interleukin-10 (IL-10), a critical anti-inflammatory cytokine, is reportedly unaffected by OSA when measured in plasma or serum. However, no studies have comprehensively assessed IL-10 levels across specific tissues in OSA. We hypothesized that IL-10 might be affected by IH in a tissue-specific manner.

Methods: Male C57Bl/6J mice were exposed to IH (FiO_2 cycling from 21% for 90s to 6% for 90s) or room air (RA, 21%) for 12 hours/day over six weeks. After euthanasia, levels of IL-10, interleukin-1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) were measured in plasma, colon, visceral white adipose tissue (vWAT), and aortic tissue using ELISA.

Results: Plasma IL-10 levels did not differ between RA and IH groups (4.4 ± 2.4 pg/ml vs. 3.7 ± 2.3 pg/ml. $p = 0.6$). However, IH significantly reduced IL-10 levels in the colon (24.9 ± 1.8 pg/ml vs. RA: 14.4 ± 4.6 pg/ml, $p < 0.05$), vWAT (10.1 ± 0.9 pg/ml vs. RA: 4.4 ± 0.8 pg/ml, $p < 0.05$), and aorta (15.3 ± 1.3 pg/ml vs. 7.1 ± 2.4 pg/ml, $p < 0.05$). In contrast, plasma levels of IL-1β in mice exposed to IH were significantly higher when compared to control (3.9 ± 1.1 pg/ml vs. RA: 1.8 ± 0.5 pg/ml, $p < 0.05$) as well as colon (14.1 ± 1.7 pg/ml vs. RA: 5.1± 1.1 pg/ml, $p < 0.05$), vWAT (23.1 ± 4.1 pg/ml vs. RA: 2.6 ± 0.5 pg/ml, $p < 0.05$), and aorta (19.2 ± 4.5 pg/ml vs. RA: 4.2 ± 0.8 pg/ml, $p < 0.05$). Similarly, TNF-α levels were significantly elevated in IH-exposed mice plasma (7.1 ± 2.1 pg/ml vs. 2.3 ± 0.6 pg/ml, $p < 0.05$), colon (44.7 ± 3.7 pg/ml vs 21.4 ± 2.1, $p < 0.05$), vWAT (34.4 ± 5.7 pg/ml vs. 11.4 ± 1.9 pg/ml, $p < 0.05$), and aorta (31.3 ± 4.1 pg/ml vs. 14.3 ± 2.9 pg/ml, $p < 0.05$).

Conclusion: IH exposures mimicking OSA reduce IL-10 levels in tissues but not in plasma, while concurrently increasing pro-inflammatory cytokines IL-1β and TNF-α in both plasma and tissues. These results suggest that IH induces a tissue-specific inflammatory imbalance, characterized by reduced anti-inflammatory responses and heightened pro-inflammatory signaling.

Count: 381

Supported by NIH grant HL166617

DeForest, Logan Poster Location: 37 Poster Judging Number:337

Expression of the ERG Transcription Factor in Doxorubicin-Treated Endothelium

Logan DeForest[1], Melissa Cobb[1], Shixin Tao, PhD[1], Eugene Konorev, MD/PhD[1]
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Doxorubicin (DOX) is an anthracycline chemotherapeutic whose clinical utility is limited by dose dependent cardiotoxicity. Emerging evidence suggests endothelial dysfunction contributes to DOX associated vascular injury. The ETS related gene (ERG) is an endothelial transcription factor required for vascular integrity and homeostasis and is regulated by transforming growth factor β (TGF-β) signaling. We investigated whether DOX alters ERG expression in endothelial cells and whether inhibition of TGF-β signaling modulates ERG regulation during drug exposure and post-treatment recovery.

Human umbilical vein endothelial cells (HUVECs) were treated with DOX (16nM), the TGF-β type I receptor inhibitor SB431542 (1μM), DOX plus SB, or control. Treatments were applied either concurrently for 48 hours or followed by a washout period with SB or vehicle to assess recovery following DOX exposure. ERG protein expression was assessed by quantitative PCR and immunoblotting. In vivo, ERG expression was evaluated by immunohistochemical staining of cardiac tissue from male and female C57BL/6 mice treated with saline, DOX, SB, or DOX plus SB.

Under concurrent treatment conditions, DOX with or without SB did not significantly alter ERG expression in HUVECs compared to controls. In contrast, DOX alone decreased ERG expression during the washout period, whereas SB administration during post-DOX washout was associated with partial restoration of ERG expression relative to DOX-only conditions. Analysis of cardiac tissue from both male and female mice revealed no significant differences in ERG staining intensity between treatment groups. Cardiac tissue was harvested three weeks after final DOX injection, which may allow recovery of ERG abundance in endothelial cells.

These findings indicate that ERG regulation following DOX exposure is context and timing-dependent, with recovery phase modulation of TGF-β signaling influencing endothelial ERG expression. Lack of detectable changes in cardiac ERG after completion of Dox therapy may indicate transient suppression followed by recovery of ERG expression.

Funding: Research reported in this presentation was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under Award Number R15HL170243.

Beltran, Jesus **Poster Location: 38** **Poster Judging Number: 438**

Sex-Dependent Vascular and Proteomic Remodeling in Type 1 Diabetic Mice Fed a Western Diet

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A Western diet (WD), high in fats and sugars, is commonly consumed by individuals with type 1 diabetes (T1D) and contributes to the development of obesity. The presence of both conditions may contribute to the high incidence of cardiovascular disease in patients with T1D. We hypothesized that the combination of T1D and WD consumption exerts a more deleterious effect on vascular function than WD consumption alone, irrespective of sex. To test this hypothesis, male and female 8-week-old C57BL/6J and Akita (a genetic T1D model) mice were fed a WD for 20 weeks to induce obesity. Vascular responses were assessed in isolated abdominal aortas, and passive pressure-diameter curves were determined in mesenteric arteries. Proteomic analyses were performed in the thoracic aorta. Female Akita mice were heavier than their corresponding controls, whereas male Akita mice weighed less than male controls. Akita mice exhibited greater hyperglycemia than controls. Male Akita mice exhibited impaired aortic vasodilation to sodium nitroprusside and increased vasoconstriction to the thromboxane A_2 agonist, U46619. In females, aortic vasodilation to insulin was reduced in Akita mice compared with WD-fed female controls. Both male and female Akita mice showed increased modulus of elasticity in mesenteric arteries and higher calculated incremental pulse wave velocity at elevated pressures compared with their respective WD-fed controls. Proteomic network analysis suggests a dysregulation of metabolic (ketone body metabolism), inflammatory, and extracellular matrix pathways in male Akitas compared with WD-fed controls. In contrast, proteomic analysis of female cohorts did not reveal differentially expressed proteins. Taken together, these findings demonstrate that Akita mice exhibit sex-specific vascular dysfunction: males primarily show impaired endothelium-independent vasodilatory responses and molecular remodeling, whereas females display endothelial insulin resistance, indicating divergent mechanisms of diabetic vascular injury between the sexes.

Falayi, Olufunke Poster Location: 39 Poster Judging Number: 339

Non-neuronal Tph1 ablation does not alter basal kidney function but mitigates ischemic kidney insufficiency in adult rats

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The tryptophan hydroxylase (Tph) gene family encodes the rate-limiting enzymes for serotonin biosynthesis, a signaling molecule with diverse physiological and pathophysiological actions. Mammals express two isoforms: Tph1, producing peripheral non-neuronal serotonin, and Tph2, which drives neuronal serotonin synthesis. Both isoforms hydroxylate L-tryptophan to 5-hydroxytryptophan but have distinct biological roles. Tph1 is enriched in enterochromaffin cells and kidney proximal tubules, where serotonin modulates gut motility, secretion, vascular tone, and epithelial ion transport. Tph2 sustains serotonergic neurotransmission in the neurons. We investigated the contribution of peripheral serotonin to kidney function at baseline and during ischemia–reperfusion injury (IRI) using adult transgenic rats harboring a CRISPR-engineered 1-bp deletion in exon 4 of the Tph1 gene. Basal plasma and urinary L-tryptophan concentrations were unchanged in Tph1 knockout (KO) versus wild-type (WT) rats, whereas plasma serotonin and urinary 5-hydroxyindoleacetic acid were markedly reduced, confirming substantial loss of peripheral serotonin. Despite this deficit, mean arterial pressure and heart rate (telemetry), transdermal glomerular filtration rate, renal blood flow, renal vascular resistance, renal function indices, and kidney morphology were unchanged, indicating that non-neuronal serotonin is dispensable for maintaining resting renal hemodynamics and filtration in adult rats. Conversely, Tph1 ablation conferred significant protection during renal IRI. 35 minutes of renal ischemia and 24 hours of reperfusion increased renal Tph1 expression and serotonin production in WT kidneys, without altering intestinal Tph1 expression or serotonin levels, indicating a kidney-specific upregulation of serotonin in response to renal ischemic stress. KO rats exhibited improved renal perfusion, attenuated systemic hemodynamic instability, reduced tubular dilatation, cast formation, and necrosis compared to WT. These results identify Tph1-derived serotonin as a previously unrecognized mediator of renal ischemic insufficiency and implicate serotonin signaling in microvascular dysfunction and tubular injury that exacerbate ischemic acute kidney injury. Therefore, targeting peripheral serotonin may be a promising strategy for treating renal IRI.
Funding: APS Predoctoral Fellowship; NIH: R01HL151735.

Gaw, Skylar **Poster Location: 40** **Poster Judging Number: 340**

Gamma-aminobutyric acid transporter 3 (GAT3) regulates neuronal activity through GABA reuptake in the nucleus tractus solitarii (nTS)

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The nTS is the primary integration site for autonomic and respiratory sensory information. Our group has shown that nTS astrocytes regulate synaptic transmission, in part, via glial transporters and their reuptake of extracellular neurotransmitters. GAT 1 and 3 reuptake the inhibitory neurotransmitter GABA. GAT 3 block reduced spontaneous inhibitory signaling, potentially an effect of GABA increase and its binding to GABA receptors at the synapse. However, less is known for the contribution of astrocytic GABA transporters to nTS action potential discharge activity. We hypothesize blocking GAT-3 decreases neuronal activity in the nTS, driven by an increase in extracellular GABA. To test this, GAD2 tdTomato mice [$GAD2^{tm2(cre)Zjh}$ bred to B6.Cg-$Gt(ROSA)26Sor^{tm14(CAG-tdTomato)Hze}$] were used to distinguish GABAergic from non-GABAergic neurons. Immunohistochemistry for GAT-3 and Glial Fibrillary Acidic Protein (GFAP) quantified transporter and astrocyte expression, respectively, and visualize their interactions with GABA neurons. nTS neuronal activity was recorded using a multielectrode array (MEA). Baseline activity was recorded for 5 minutes, followed by GAT-3 inhibition (SNAP 5114, 50μM, 5 minutes) to assess how GAT-3 influences spontaneous firing. Following a 10-minute wash, the $GABA_A$ receptor antagonist (SR-95531, 25uM, 5 minutes) was applied alone and with SNAP 5114 (5 minutes) to confirm the GABA receptor activated via SNAP 5114 -mediated elevation of GABA. Neuronal activity from the final 2 minutes of each segment was separated into low (<0.3), medium (0.3-1) and high (>1) number of spikes per second and normalized to the initial baseline. Immunohistochemistry revealed GAT-3 and GFAP-labeled astrocytes are closely associated with GABAergic and non-GABAergic neurons. High-firing neurons exhibited the greatest decrease (~47%) in activity from baseline following GAT-3 block. Medium-firing neuron rates also decreased ~18%, whereas GAT-3 block did not alter low firing cells (10% reduction). In the presence of $GABA_A$ receptor block, the SNAP 5114-mediated decrease was ablated in the high-firing cells. These data demonstrate astrocytic GAT-3 tempers nTS inhibition and occurs in an activity-dependent manner.

Steinbach, Derek **Poster Location:41** **Poster Judging Number: 341**

Impaired beta-adrenergic cardiac reserve in a heterozygous Kir6.1 V65M mouse model of Cantú syndrome

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Introduction: Cantú syndrome is a rare genetic condition caused by gain-of-function mutations in the ATP-sensitive potassium channel. This leads to cardiovascular complications including dilated vasculature, cardiomegaly, high-output cardiac remodeling, and exercise intolerance. As mechanisms of exercise intolerance are poorly understood in Cantú, we tested the hypothesis that beta-adrenergic reserve is reduced in a mouse model of Cantú syndrome.

Methods: Hearts from male and female wildtype (n=11) or heterozygous Kir6.1 V65M mutant Cantú mice (n=10) were isolated and perfused with oxygenated Krebs-Henseleit buffer at 37 ºC. A pressure catheter was placed in the left ventricle to monitor heart rate, cardiac pressure development (P_{max}), and relaxation rate (dP/dt_{Min}). Following baseline perfusion, hearts were perfused with 10 nM isoproterenol (ISO) for beta-adrenergic challenge. Data are presented as mean±standard error of the mean, with statistical significance set at $P<0.05$ (unpaired t-test).

Results: Heart weight/body weight ratios were significantly ($P<0.005$) greater in V65M (0.012 ±0.001) than wildtype (0.009±.0004). Heart rates were similar between V65M (333±18 beats/ min) and wildtype (366±21 beats/min) under baseline conditions, but following ISO heart rate was slower ($P<0.05$) in V65M (401±13 beats/min) than in wildtype (446±7 beats/min). Baseline P_{max} was greater ($P<.05$) in V65M (76±2 mmHg) than wildtype (68±1 mmHg), and ISO increased P_{max} in both groups. The ISO-induced change in P_{max} was blunted ($P<0.05$) in V65M (ΔP_{max}:27±7 mmHg) compared to wildtype (ΔP_{max}:58±11 mmHg). Cardiac relaxation rates (dP/dt_{Min}) were similar between V65M (-1,510±154 mmHg/sec) and wildtype (-1,620±188 mmHg/sec) at baseline. However, after ISO, dP/dt_{Min} was slower ($P<0.05$) in V65M (-3,389±214 mmHg/sec) compared to wildtype (-4,542±412 mmHg/sec).

Conclusions: Cardiac hypertrophy and enhanced baseline pressure development in the V65M mouse model are consistent with high-output states observed clinically in Cantú syndrome. Reduced cardiac performance with catecholamine challenge indicates impaired cardiac reserve, which may help explain exercise intolerance and cardiac dysfunction observed in Cantú syndrome patients.

Funding: Supported by NIH R01HL136292 (TLD), NIH R35HL171542 (CGN), and a Margaret Proctor Mulligan Endowed Professorship (TLD).

Wright, Hayden **Poster Location: 42** **Poster Judging Number: 442**

Divergent Synaptic Adaptations in GABAergic vs. Glutamatergic nTS Pathways May Underlie Respiratory Responses to Sustained Hypoxia

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Exposure to hypoxia occurs during physiological (e.g., high altitudes) and pathophysiological (e.g., obstructive pulmonary disease) conditions. The nucleus tractus solitarii (nTS) integrates sensory input from hypoxia-sensitive peripheral chemoreceptors, which release glutamate to initiate reflex responses. Yet, the magnitude of these responses and overall nTS activity is determined through a balance of glutamate (Glu) and gamma-aminobutyric acid (GABA) signaling. This study investigated how sustained hypoxia (SH) affects GABA neurons in the nTS and cardiorespiratory function.

Male and female GAD1-EGFP mice (4-5 weeks) were used to differentiate GABA (GAD +) and presumptive Glu (GAD-) neurons in the nTS. Mice were exposed to SH (10% O_2, 24 hours) or normoxia (21% O_2, 24 hours) before experimentation. Plethysmography revealed increased tidal volume and minute ventilation following SH. Immunohistochemistry showed increased c-Fos in the nTS after SH, primarily in Glu neurons, suggesting increased nTS Glu activity. Whole-cell patch-clamp recordings in nTS slices examined synaptic neurotransmission and electrophysiological properties of GAD+ and GAD- neurons. Neurons were characterized by mono- or polysynaptic connection with the tractus solitarii (TS) via TS stimulation. Under normoxic conditions, GAD+ and GAD- neurons had similar properties with notable exceptions: monosynaptic GAD+ neurons had greater spontaneous excitatory postsynaptic current (sEPSC) frequency than GAD- neurons, while polysynaptic GAD+ neurons showed decreased synaptic throughput during repetitive (20 Hz) TS stimulation. Following SH, monosynaptic GAD- and GAD+ neurons became more depolarized without altered synaptic properties. Conversely, SH differentially affected polysynaptic neurons, altering presynaptic release properties and enhancing GAD+ action potential discharge. SH increased synaptic throughput in both polysynaptic phenotypes at 20 Hz TS stimulation.

These data demonstrate that SH drives differential synaptic remodeling of nTS circuitry by altering transmission and excitability of GABAergic and glutamatergic neurons, possibly contributing to observed respiratory changes.

Funding: NIH HL166183 and MU REP Postdoctoral Scholarship awarded to HW

Fazle, Elahi **Poster Location: 43** **Poster Judging Number: 443**

Female sex protects cerebral arteries from acute oxidative stress by limiting mitochondrial calcium influx

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Reactive oxygen species (ROS) produced following ischemic stroke damage vascular smooth muscles cells (SMCs) and endothelial cells (ECs) to a greater extent in males than females. Increases in mitochondrial calcium concentration ($[Ca^{2+}]_m$) promote cell death. As mitochondrial calcium uniporter (MCU) is the primary pathway for mitochondrial calcium uptake, we hypothesized that cerebral arteries from females have reduced MCU-dependent increases in $[Ca^{2+}]_m$ during exposure to ROS which limits cell death. Posterior cerebral arteries (PCAs; diameter ~80 μm) from mice were pressurized to 90cm H_2O (36°C) and exposed to hydrogen peroxide (H_2O_2, 200 μM) for 50 min. Cell death was evaluated with Hoechst 33342 (1 μM) to label all nuclei and propidium iodide (2 μM) to identify nuclei from dead cells. To assess the role of MCU, PCAs were treated with the MCU inhibitor DS16570511 (50 μM). MCU inhibition decreased SMC death in PCAs in males (26 to 5%; $p<0.05$) but not females (15 to 10%) and decreased EC death in both sexes. Male PCAs had significantly greater increase in $[Ca^{2+}]_m$ in response to H_2O_2 as measured by Rhod2 fluorescence (15 μM). Increases in $[Ca^{2+}]_m$ were nearly abolished by MCU inhibition in both sexes. Activating MCU with kaempferol (15 μM; 30 min) led to a more robust increase in $[Ca^{2+}]_m$ compared to H_2O_2, yet evoked minimal (~10%) SMC and EC death in both sexes. We conclude that greater resilience to oxidative stress in cerebral arteries from female mice is mediated by limiting mitochondrial calcium influx through MCU.

Supported by: NIH R01NS134690.

Calbaza, Gia **Poster Location: 44** **Poster Judging Number: 344**

Neuroprotective Mechanisms Following Episodes of Repeated Mild Hypoxia: A Proteomic Investigation in the Rat Brain

Gia Calbaza OMS-II, Dr. Daniela Ostrowski
A.T. Still University Kirksville College of Medicine

The brain compromises only 2% of body weight yet consumes nearly 20% of the body's oxygen, making it highly susceptible to hypoxic injury. While hypoxia contributes to the pathophysiology of many neurological conditions, mild intermittent hypoxia (IH) has been shown to activate endogenous neuroprotective mechanisms. However, proteomic studies identifying these mechanisms are limited. This study identifies proteins altered by IH in the hippocampus, a region essential for memory and learning, and examines sex-specific neuroprotective responses that may guide targeted therapeutic strategies for brain injury.

Male and female Sprague-Dawley rats (8 weeks old) from the ATSU KCOM facility were maintained under controlled conditions and exposed to either room air (CTL, n = 3/sex) or five 2-hour episodes of sustained hypoxia (HX, 10% O_2, 24 hours apart, n = 3/sex). Hippocampi were dissected, flash frozen, and stored at -80 ℃ prior to proteomic analysis (MetwareBio). Samples underwent protein extraction, enzymatic digestion, chromatography, and mass spectrometry. Data were analyzed using DIA-NN and bioinformatics to identify differentially expressed proteins, pathways, and interactions.

Proteomic analysis identified 11,333 proteins from 124,965 peptides, with consistent abundance across replicates. A total of 91 differentially expressed proteins were found in the male-CTL vs. male-HX group, while 52 were identified in females. Males upregulated energy metabolism and synaptic plasticity proteins (e.g., PANK1, FXYD6) while females enhanced blood-brain barrier integrity and lipid homeostasis proteins (e.g., ENHO, SCP2). Notably, Zdhhc8 expression was reduced in both sexes following hypoxia, suggesting reduced neuronal palmitoylation activity and disruption of trafficking and signaling pathways.

This study successfully used proteomics to demonstrate sex-specific neuroprotective strategies in the rat hippocampus following repeated mild hypoxia, emphasizing the importance of sex-aware therapeutic approaches.

Funding: ATSU Student Research Fund

Wagley, Susmita Poster Location: 45 Poster Judging Number: 245

Sex Dimorphism in Endothelial-to-Mesenchymal Reprogramming by Doxorubicin

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Kansas City University

Over the past decades, the number of cancer survivors has increased due to both population aging and advances in early detection and treatment. Doxorubicin (Dox) is an anthracycline chemotherapeutic drug that is widely used to treat various types of cancers, but is associated with cardiovascular complications, such as cardiomyopathy and heart failure. Endothelial dysfunction is a major contributor to these adverse outcomes, yet the biological mechanisms underlying sex-specific endothelial responses to Dox are not fully understood. This study examines Dox-induced endothelial-to-mesenchymal transition (EndMT) in male and female human umbilical vein endothelial cells (HUVECs). Male and female HUVECs were treated with Dox to assess mesenchymal and endothelial marker expression at the mRNA and protein levels using quantitative polymerase chain reaction (qPCR) and Western blotting. Baseline analysis revealed higher expression of mesenchymal markers and lower expression of endothelial markers in male endothelial cells, as compared to female cells, indicating sex-based differences in untreated cells. Following Dox treatment, both male and female HUVECs exhibited significant upregulation of mesenchymal markers. Notably, female endothelial cells demonstrated a more robust induction of select genes of smooth muscle lineage, including *TAGLN*. In contrast, endothelial markers were downregulated in both sexes, indicating the loss of endothelial identity. The pattern of protein expression was consistent with transcriptional findings, showing increased mesenchymal protein expression and reduced endothelial markers, including eNOS, and claudin-5. Knockdown of Smad3 transcription factor attenuated Dox-induced EndMT, indicating a contributory role of the canonical TGF-beta pathway in endothelial phenotypic switching following chemotherapy. Overall, these data reveal intrinsic sex-specific differences in endothelial reprogramming and Smad3-dependent responses to Dox chemotherapy. Understanding these mechanisms may inform sex-specific strategies to mitigate anthracycline-associated cardiovascular toxicity.

Funding: Research reported in this presentation was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under Award Number R15HL170243.

Yan, Jianxin **Poster Location: 46** **Poster Judging Number: 446**

CYLD Suppresses VSMC Phenotypic Switching into Synthetic VSMCs for Neointimal Formation by Restricting HIF-1α–Dependent Metabolic Reprogramming

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Vascular smooth muscle cell (VSMC) phenotypic switching is central to the development and progression of vascular diseases, such as restenosis and atherosclerosis. However, the molecular mechanisms driving this process remain incompletely understood. Here, we sought to determine the role of a deubiquitinating enzyme cylindromatosis (CYLD) in VSMC phenotypic switching into synthetic VSMCs for occlusive lesion formation in the artery. Under physiological conditions, we found a low level of CYLD expression in the carotid artery primarily localized to VSMCs in the tunica media. After injury, CYLD expression was markedly increased, concomitant with VSMC phenotypic switching into synthetic VSMCs characterized by a reduction in the expression of contractile VSMC markers and an increase in the expression of secretory proteins as well as neointima (NI) formation. Notably, NI formation was significantly enhanced in CYLD knockout (KO) mice, accompanied by increased VSMC proliferation in the injured arteries. Lineage tracing analyses further revealed that CYLD KO promoted phenotypic switching of pre-existing mature VSMCs into synthetic VSMCs and their expansion, leading to increased NI formation in injured arteries. Consistent with these in vivo findings, in vitro studies showed that CYLD deficiency promoted VSMC proliferation and migration, along with a transition from the contractile to the synthetic phenotype. RNA sequencing analysis revealed that CYLD deficiency enhanced HIF-1α–driven transcription of glycolytic genes and a metabolic shift from mitochondrial oxidation toward glycolysis, thereby facilitating VSMC growth and phenotypic switching from the contractile to the synthetic phenotype. Collectively, these findings identify CYLD as a critical suppressor of VSMC phenotypic switching into synthetic VSMCs for neointimal formation in injured arteries, through inhibition of HIF-1α–dependent metabolic reprogramming.

Funding: NIH (R01 HL160541); NIH (R03 RT003610); VA (I01 CX002062).

Augenreich, Marc Poster Location: 47 Poster Judging Number: 347

The iRhom2-ADAM17 Axis as a Mechanism of Endothelial Insulin Resistance

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Endothelial insulin resistance, a hallmark of obesity and type 2 diabetes, plays a key role in the pathogenesis of cardiovascular disease. Our previous work identified ADAM17-mediated shedding of the insulin receptor from the endothelial surface as a novel mechanism contributing to insulin resistance in the vasculature. Because ADAM17 is a highly promiscuous sheddase, understanding its regulation is critical for therapeutic targeting. Part of ADAM17's regulation includes its maturation and trafficking by the inactive rhomboid (iRhom) proteins, of which iRhom2 is strongly associated with inflammatory signaling. Given that vascular inflammation is commonly coupled with endothelial insulin resistance, we hypothesized that iRhom2 promotes ADAM17-dependent insulin receptor shedding. We examined the role of the iRhom2-ADAM17 axis in both immortalized and primary endothelial cells. Data are expressed as mean±standard error and were analyzed using t-test or two-way ANOVA with a post-hoc Bonferroni test, as appropriate. Statistical significance was set at $P<0.05$. We provide evidence that iRhom2 is present in endothelial cells and, when overexpressed (9.31±0.90-fold increase vs control, n=12/condition), promotes the insertion of mature ADAM17 into the plasmalemma (1.71±0.11-fold increase vs control, n=6/condition). Conversely, we found that silencing iRhom2 dampens the insertion of mature ADAM17 into the plasmalemma (0.21±0.08-fold reduction vs control, n=10-12) in cells stimulated with phorbol 12-myristate 13-acetate (PMA), a pharmacological approach that promotes maturation and trafficking of ADAM17. We further show that cells overexpressing ADAM17 (2.33±0.22-fold increase vs control, n=12/condition) have fewer insulin receptors on their membrane (0.82±0.02-fold difference from control, n=6-8/condition), a reduction rescued by incubation with the ADAM17 inhibitor TAPI-0 (0.98±0.03-fold difference from control, n=6-9/condition). These findings suggest that iRhom2 may contribute to endothelial insulin resistance by trafficking ADAM17 to the cell membrane and regulating its sheddase activity. Targeting iRhom2-dependent trafficking mechanisms may represent a promising therapeutic strategy to mitigate ADAM17-mediated disruption of endothelial insulin signaling.

Funding: University of Missouri Life Sciences Fellowship (M.A.A.), National Institutes of Health Grant R01HL151384 (to L.A.M.-L. and J.P.), American Heart Association (23PRE1020897 to G.P., 25DIVSUP1463861 to F.I.R.P., and 24EIA1248820 to J.P.).

Kelly, Kristin **Poster Location: 48** **Poster Judging Number: 248**

α1A-Adrenergic Receptor Expression on Macrophages Elicits a Proinflammatory Response During Heart Failure Progression

Kristin I. Kelly, Kaylie A. Dow, Jesse L. Vandervort, Glenn J. Phaup, Carie R. Boychuk, Laurel A. Grisanti

Heart failure (HF) is the leading cause of death worldwide and inflammation is a key regulator of HF progression. A major regulator of immune response are adrenergic receptors (AR), which mediate the cellular effects of the sympathetic nervous system (SNS). The β2AR subtype is widely expressed on immune cells where it has been shown to modulate a variety of responses while other subtype expression is restricted and their role is poorly defined. Macrophages are vital immunoregulatory cells that influence inflammation based on phenotype. Preliminary findings from our laboratory demonstrates that macrophages upregulate α1A-AR expression under proinflammatory conditions. We hypothesized α1A-AR elicits proinflammatory responses and immune specific deletion of α1A-AR would reduce proinflammatory responses in HF. To examine the effect of α1A-AR expression on macrophages, lipopolysaccride was applied to WT and α1A-AR knockout (KO) bone marrow-derived macrophages (BMDM). α1A-AR expression amplified pro-inflammatory cytokine production, while KO reduced responses. To understand whether the proinflammatory phenotype of α1A-AR in macrophages exists *in vivo*, a chronic isoproterenol (ISO) model of HF was used to mimic the prolonged SNS overactivation that occurs in HF. Mice were irradiated to deplete endogenous bone marrow (BM) and received WT or α1A-ARKO BM transplant (BMT). WT BMT control mice receiving ISO exhibited hallmarks of HF, including increased hypertrophy and fibrosis, which were blunted in α1A-ARKO BMT animals. This corresponded with worsened functional parameters in WT BMT mice, which were improved with α1A-ARKO BMT animals. These findings demonstrate that mice that immune cell α1A-ARKO improves outcomes in a chronic βAR activation model of HF. These findings are significant in that they are the first to show an upregulation of α1A-AR during proinflammatory conditions, which impacts HF development and progression and represent a inflammatory pathway which may be a novel therapeutic target for the treatment of HF.

Nahar, Lutfun **Poster Location: 49** **Poster Judging Number: 349**

Exercise ameliorates obesity-induced cognitive dysfunction in mice

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Background: Obesity is a chronic metabolic condition that also contributes to impairment of brain function, including impairments in learning, memory, and executive function. Obesity is associated with chronic low-grade inflammation, insulin resistance, and cerebrovascular dysfunction, all of which disrupt neuronal signaling and hippocampal integrity. With an increasing burden of obesity-related cognitive decline, there is a critical need for interventions that target both metabolic and neurobiological pathways. Semaglutide is an FDA-approved drug used for the treatment of type 2 diabetes and obesity. Growing research suggests that, independent of its metabolic benefits, semaglutide also has anti-inflammatory, vasoprotective, and neuroprotective actions. Exercise is another well-established intervention that improves metabolic health and cerebrovascular function and enhances neuroplasticity. While semaglutide and exercise are known to have neuronal and vascular protective effects, the synergistic effect of combining them to reduce obesity-induced cognitive dysfunction remains understudied. Herein, we hypothesized that exercise and semaglutide additively reduce cerebrovascular dysfunction, thereby improving cognitive function in a mouse model of diet-induced obesity. ***Methods:*** Obesity was induced in female C57BL/6 mice by feeding a Western diet for 24 weeks. After 16 weeks on the Western diet, mice were treated with a daily subcutaneous injection of semaglutide (9 nmol/kg) or vehicle for 8 weeks, with and without access to running wheels, such that a total of four experimental groups were created (n=10-13/group). Cognitive function tests were performed the week before euthanasia, and vascular function was tested ex vivo in isolated posterior cerebral arteries. ***Results:*** We found that: 1) semaglutide treatment reduced body weight and improved glucose tolerance; 2) None of the treatments affected cerebrovascular function or structure; 3) Exercise reduced anxiety and improved spatial recognition memory, as measured by open field, Y-Maze, and Novel Object Recognition test. ***Conclusion:*** Overall, our findings indicate that exercise and semaglutide treatment improve cognitive function, either alone or when combined.

Ramirez-Perez, Francisco **Poster Location: 50**

Beneficial vascular effects of oral phosphatidylserine supplementation in type 2 diabetes

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Type 2 diabetes (T2D) is characterized by endothelial insulin resistance, a factor contributing to overall vascular dysfunction, including reduced nitric oxide bioavailability, impaired vasodilation, and arterial stiffening. We recently provided evidence supporting the hypothesis that endothelial insulin resistance in T2D may be partly attributed to the shedding of insulin receptors by a disintegrin and metalloproteinase-17 (ADAM17). As prior work by others using cell culture models suggested that exogenous phosphatidylserine (PS) can competitively inhibit ADAM17, we hypothesized that oral PS supplementation would improve overall vascular function in diabetes. First, we corroborated the ability of PS to inhibit ADAM17 activity using in vitro approaches and experiments in isolated arteries. Next, we tested the vascular effects of PS in diabetic mice and subsequently in individuals with T2D through a randomized, double-blinded, placebo-controlled clinical trial. In a cell-free-based assay, endothelial cells (HUVECs), and isolated mesenteric resistance arteries from male mice, water-soluble PS blunted ADAM17 activity, underscoring its capability as an inhibitor. In db/db male mice, oral administration of PS (200 mg/kg/day for 4 weeks) enhanced insulin-induced dilation in isolated resistance arteries and reduced in vivo and ex vivo arterial stiffness indices, including pulse wave velocity, aortic endothelial cell stiffness measured by atomic force microscopy, and the incremental modulus of elasticity in aortas, femoral arteries, and mesenteric arteries, all assessed using pressure myography. Similarly, in individuals with T2D, PS supplementation (900 mg/day for 4 weeks) enhanced leg blood flow responses to an oral glucose load and reduced carotid-femoral PWV. Taken together, this work supports the potential of oral PS as a therapeutic approach to improve vascular function in T2D, and suggests that the beneficial effects of PS may be driven in part by its vascular insulin-sensitizing effects.

Funding: LAM-L and JP are supported by the NIH grant: R01HL151384. FIR-P is supported by the AHA 25DIVSUP1463861.

Patel, Ami Poster Location: 51 Poster Judging Number: 351

Cardiomyocyte Morphology in a Bleomycin-Induced Mouse Model of Type III Pulmonary Hypertension

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Type III pulmonary hypertension (PH) increases pulmonary vascular resistance and induces structural and functional changes in the right ventricle (RV). RV adaptation is a key determinant of survival in PH. While gross RV hypertrophy is well-recognized, the precise pattern of cardiomyocyte remodeling remains unclear. In monocrotaline-induced PH, RV cardiomyocytes undergo morphological changes, becoming shorter and wider, whereas left ventricular (LV) cardiomyocytes remain relatively preserved. Whether similar remodeling occurs in a pulmonary fibrosis model of Type III PH induced by bleomycin, and whether sex influences these changes, is unknown. Given the importance of RV structural adaptation in disease progression, we hypothesized RV cardiomyocyte length-to-width (L/W) ratio would decrease in bleomycin-treated mice. Male and female mice (4-6 mo) received a single dose of intratracheal bleomycin (or saline control) and mice were allowed to develop Type III PH over 3 weeks. Cardiomyocytes were isolated, imaged, and cell dimensions quantified in ImageJ. Cell length was defined as the longest axis perpendicular to sarcomeres, and width as the longest axis parallel to sarcomeres. A sample of 6-20 cells of the LV and RV were taken from 3-4 animals per group, with an average value of length and width calculated from the combined cell population mean from each mouse. Bleomycin treatment did not significantly change cardiomyocyte length, width, or length/width ratio in any groups (male, female, RV, LV). These findings reject the hypothesis that bleomycin-induced Type III PH associates with cardiomyocyte remodeling at this stage of disease progression in mice. This suggests that, unlike monocrotaline-induced PH, early or gross RV structural remodeling in the bleomycin model may occur through mechanisms other than changes in cardiomyocyte length/width. These results highlight the need for further studies to explore alternative contributors to RV adaptation in bleomycin-induced Type III PH.

Funding NIH R01HL136292 (TLD)

Cayton, Jessica Poster Location: 52 Poster Judging Number: 452

Dystrophin deficiency increases calcium mishandling and right atrial remodeling in Mid-Age male mice

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Background: Atrial tachyarrhythmias including atrial fibrillation (AF) and atrial flutter (AFL) are increasing in prevalence and markedly contribute to morbidity and mortality. While it is well-established that dystrophin deficiency in the ventricle leads to calcium handling dysfunction, remodeling, and arrhythmogenesis, the effect of dystrophin deficiency on the atria remains comparatively understudied. This investigation tested the hypothesis that dystrophin deficiency leads to atrial calcium handing dysfunction (trigger), adverse atrial remodeling (substrate), and therefore a predisposition to atrial tachyarrhythmias. *Methods:* To test this hypothesis, isolated hearts of Young (4-6 month) and Mid-Age (12-14 month) male dystrophic (DMDmdx4Cv) and control (C57BL6) mice were cannulated via the aorta for retrograde coronary perfusion. Upright high-speed laser scanning confocal microscopy was used to quantify atrial calcium transient amplitude and calcium transient decay rate. *Results:* In Young, there were no differences between dystrophic and control right-atrial calcium transient amplitude (P=0.252), transient decay rate (P=0.123), or right atrial weight (P=0.217). However, in Mid-Age, dystrophic right atria showed depressed calcium transient amplitude ($\Delta F/F0$) compared to controls ($\Delta F/F_0$=2.04 ± 0.13 dystrophic vs. 2.33±0.29 control, P=0.038). Dystrophic right atria also had slower calcium transient decay compared to controls (68.6±0.3ms dystrophic vs. 62.1±0.1ms control, P=0.006). Measured right atria weights, normalized to tibial length, were higher in Mid-Age dystrophic mice compared to control mice (0.404±0.002 dystrophic vs. 0.307 ± 0.001mg/mm control, P=0.0006), demonstrating atrial remodeling and enlargement. These data indicate that loss of dystrophin with aging predisposes the atrial myocardium to calcium handling dysfunction and remodeling.

Funding: Supported by a MU School of Medicine TRIUMPH Award, a T32 Training Grant 2T32OD011126-43 (Franklin), and an American Heart Association Predoctoral Fellowship (Cayton).

Saheb farman abadi, Mahsa Poster Location: 53 Poster Judging Number: 353

Sex- and Hormone-Dependent Regulation of Arterial Blood Pressure by nTS 5-HT Neurons

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Hypertension is associated with several cardiovascular diseases including heart failure and stroke, leading killers in the USA. Men are more likely to develop hypertension, but after menopause the incidence of hypertension becomes higher in women. In many studies using anesthetized male animals, serotonin (5-hydroxytryptamine; 5-HT) has been shown to influence arterial blood pressure regulation. We previously demonstrated that chemogenetic inhibition of serotonergic neurons innervating the nucleus tractus solitarius (nTS) produces a greater pressor response in males. In contrast, in females, the effect was not significantly different from that of the controls. Herein, we hypothesize that in females, nTS serotonergic neurons regulate arterial blood pressure in a sex hormone-dependent manner. We tested female rats expressing Cre-recombinase exclusively in 5-HT neurons (Tph2-Cre) that received a Cre-dependent, retrogradely transported adeno-associated virus in the nTS expressing inhibitory designer receptors exclusively activated by designer drugs (Gi-DREADDs) to selectively silence projecting 5-HT neurons. Arterial pressure and heart rate were recorded following administration of vehicle or compound 21 (C21; 1 mg/kg) during proestrus or estrus (p/estrus), and again after ovariectomy (post-OVX). Gi-DREADD activation in pre-OVX rats (p/estrus) showed that the change in MAP was not significant. However, female post-OVX rats showed that change in MAP compared to vehicle. These findings show that female sex hormones protect against blood pressure dysregulation caused by a loss of serotonergic drive to the nTS. These data suggest that female sex steroids modulate nTS serotonergic control of arterial pressure. Future studies will assess sympathetic nerve activity and determine whether a specific loss of estrogen signaling in the nTS recapitulates the consequences of OVX on the effect of serotonergic drive to the nTS for arterial blood pressure regulation observed after ovariectomy.

Funding: No external funding was received for this study.

Huang, Minna **Poster Location: 54**

A Critical Role of UCH-L1 in Alleviating Acute Cardiorenal Syndrome type-5 in Sepsis

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Sepsis is a systemic severe inflammatory condition that causes multi-organ dysfunction and has a mortality rate exceeding 25%. Cardiorenal syndrome type 5 (CRS-5) involves concurrent cardiac and renal dysfunction secondary to systemic insults such as sepsis. However, the molecular mechanisms underlying CRS-5 in sepsis and the contribution of CRS-5 to septic death remain poorly understood. Herein, we report a critical role of a deubiquitinating enzyme ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) in the control of sepsis-induced CRS-5 and related death.

Using a lipopolysaccharide (LPS)-induced sepsis mouse model, cardiorenal dysfunction was confirmed by elevated circulating levels of troponin I, blood urea nitrogen (BUN), neutrophil gelatinase-associated lipocalin (NGAL) and a significant reduction in fractional shortening (FS) and stroke volume (SV) in wild type (WT) mice. Moreover, we found that UCH-L1 protein expression in the heart and kidney were correlated with cardiac and renal dysfunction. Interestingly, cardiac-restricted UCH-L1 (CR-UCH-L1) overexpression significantly reduced mortality, improved cardiac function as indicated by increased SV and FS, and alleviated renal injury as reflected by decreased BUN levels in the LPS-induced sepsis model. Notably, vascular cell adhesion molecule-1 (VCAM-1) expression was selectively reduced in both cardiac and renal tissues of CR-UCH-L1 overexpression mice compared with WT mice following LPS challenge, suggesting that cardiac UCH-L1 modulates cardiorenal inflammatory signaling pathways during sepsis.

Together, these findings demonstrate that cardiac UCH-L1 plays a critical role in protecting against acute CRS-5 contributing to death in sepsis via a unique heart-kidney communication.

Funding: This work was supported by grants from the NIH (R01 HL160541), NIH (R03 RT003610) and VA (I01 CX002062) to Taixing Cui (PI).

Ben Musa, Ruwaida **Poster Location: 55**

Repeated autonomic dysreflexia does not aggravate renal function after spinal cord injury

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Cardiovascular dysfunction is a common complication in individuals with spinal cord injury (SCI). One of the most serious consequences is autonomic dysreflexia, a life-threatening condition characterized by sudden and severe hypertension often accompanied by bradycardia, in response to somatic or visceral stimuli. While autonomic dysreflexia is known to adversely affect multiple organ systems, its impact on renal function remains poorly understood. Given the kidneys' sensitivity to blood pressure fluctuations, recurrent hypertensive episodes may compromise kidney perfusion and contribute to progressive renal dysfunction. To address this question, adult female rats underwent a complete transection at the fourth thoracic (T4) spinal cord to eliminate supraspinal control. Two weeks later, a subset of SCI rats was subjected to colorectal distension (CRD) to induce autonomic dysreflexia twice daily for three weeks. SCI rats without CRD or sham-injured served as controls. Subsequently, renal function was assessed by noninvasive transcutaneous measurement of glomerular filtration rate (GFR) using fluorescein isothiocyanate (FITC)-sinistrin clearance. Blood and urine samples were collected to measure kidney injury markers. Furthermore, anesthetized rats underwent renal hemodynamic recordings to monitor renal blood flow (RBF), renal vascular resistance (RVR), and mean arterial pressure (MAP). The results showed that GFR was significantly reduced in both SCI alone and SCI + CRD groups. RVR was significantly elevated in both SCI alone and SCI + CRD groups. However, there was no difference in these parameters between SCI rats with and without CRD treatment. In both SCI groups, biomarkers of kidney injury displayed similar patterns. Urinary neutrophil gelatinase-associated lipocalin and protein levels were significantly increased 24 hours post-SCI, and plasma creatinine was elevated at one week. The results indicate that SCI induces acute kidney injury, while the impairment is attenuated over time. Repeated episodes of autonomic dysreflexia do not worsen renal function despite contributing to cardiovascular instability.

Fundings: NIH/NINDS R01 NS121336, MU SCIDRP2024, and Mizzouforward Initiative

Cho, Min Jeong **Poster Location: 56** **Poster Judging Number: 356**

ADAM17-Mediated Cleavage of CD44 Impairs Hyaluronan-Dependent Shear Stress Mechanotransduction

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Type 2 diabetes (T2D) is associated with endothelial dysfunction and reduced flow-mediated dilation (FMD), suggesting impaired shear stress mechanotransduction. Loss of endothelial glycocalyx components, particularly hyaluronan (HA), may contribute to this impairment. HA functions as a shear-stress mechanosensor through its membrane receptor CD44, which acts as a mechanotransducer. CD44 is also a substrate of a disintegrin and metalloproteinase-17 (ADAM17), a pro-inflammatory sheddase known to be upregulated in T2D. However, whether increased ADAM17 activity promotes CD44 cleavage and impairs HA-dependent mechanotransduction is unknown. Therefore, we hypothesized that increased ADAM17 activity in T2D promotes CD44 cleavage, thereby impairing HA-mediated shear sensing. All differences reported herein are significant at $P < 0.05$. Individuals with T2D have blunted femoral artery FMD compared with age-matched healthy controls. Plasma ADAM17 activity and soluble HA concentrations were also higher in T2D. Consistent with these observations, db/db mice exhibited reduced FMD in mesenteric arteries, increased endothelial ADAM17 expression, and decreased endothelial CD44 content compared with wild-type mice. We also demonstrated that degradation of HA with intraluminal hyaluronidase (15µg/mL, 1hr) reduced FMD in isolated mesenteric arteries, without affecting endothelium-independent vasodilation. Furthermore, in CD44-deficient live cells, acute shear-induced intracellular Ca^{2+} responses were attenuated. Similarly, blockade of the HA-binding site on CD44 using the anti-mouse CD44-LE/AF KM201 antibody (0.25mg/mL, 1hr) impaired FMD ex vivo, supporting a mechanistic role for CD44 in HA-dependent mechanotransduction. Notably, ADAM17 overexpression reduced endothelial surface CD44 levels and attenuated shear-induced Ca^{2+} elevations. Using surface plasmon resonance, recombinant ADAM17 (5ng/µL, 8hr) reduced HA binding to CD44, and intraluminal incubation of isolated arteries with recombinant ADAM17 (1µg/mL, 1hr) impaired FMD without altering endothelium-independent vasodilation. Taken together, these data suggest that ADAM17-mediated CD44 cleavage is a potential mechanism contributing to impaired HA-dependent shear stress mechanotransduction. This ADAM17-CD44-HA axis may represent a novel pathway underlying endothelial dysfunction in T2D.

Funding: R01HL151384 (to LAM-L and JP), R01HL137769 (to JP), R21DK116081 (to CM-A), the Veterans Affairs Merit Grant 1I01CX002399 (to CM-A and JP), American Heart Association (23PRE1020897 to GP, 25DIVSUP1463861 to FIR-P, and 24EIA1248820 to JP) and the São Paulo Research Foundation (FAPESP, 2024/09267-4 to LF-S)

Vedala, Vina **Poster Location: 57** **Poster Judging Number: 157**

Hypoxic vasodilation in pre- and peri-menopausal females: role of β-adrenergic receptors

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Introduction: Hypoxia elicits peripheral vasodilation, which is lower in post-menopausal compared with pre-menopausal females. Whether impairments in hypoxic vasodilation occur earlier in the menopause transition (*i.e.,* peri-menopause) are unknown, and contributing mechanisms are ill-defined. Given β-adrenergic receptors (β-ARs) mediate a portion of hypoxic vasodilation, we hypothesized: 1) hypoxic vasodilation would be lower in peri- vs pre-menopausal females and 2) β-AR blockade would blunt hypoxic vasodilation, with greater effects in pre- vs peri-menopausal participants.

Methods: Fifteen pre-menopausal (27±8 yr, 24±3 kg/m^2, early follicular) and 5 peri-menopausal (47±3 yr, 24±3 kg/m^2) females completed two study visits randomized and blinded to oral placebo or propranolol (β-AR antagonist, 1 mg/kg). Forearm blood flow (FBF, venous occlusion plethysmography) and blood pressure (BP, finger photoplethysmography) were assessed during a 10-min normoxic baseline, followed by 5-min of steady-state hypoxia (80-85% S_pO_2). FBF was normalized for mean BP (forearm vascular conductance, FVC) and a change (Δ) in FVC from baseline was calculated as an index of hypoxic vasodilation.

Results: Steady-state hypoxia elicited vasodilation in pre-menopausal females (main effect of hypoxia, p=0.008), while FVC remained unchanged in peri-menopausal females (main effect of hypoxia, p=0.691). The change in FVC with hypoxia was lower in peri- vs pre-menopausal females (ΔFVC: 0.076±0.173 vs 2.038±2.692 mL/dL/min/100 mmHg, p=0.023). Propranolol lowered FVC in pre-menopausal females (main effect of propranolol, p=0.014) but had no effect in peri-menopausal females (main effect of propranolol, p=0.719). Under propranolol, the change in FVC with hypoxia remained lower in peri-menopausal vs pre-menopausal females (ΔFVC: 0.011 ± 0.414 vs 1.003 ± 0.822 mL/dL/min/100 mmHg; p=0.0198).

Conclusion: These preliminary data support impaired hypoxic vasodilation in peri-menopausal females. Group differences in hypoxic vasodilation persist during β-AR blockade, supporting mechanisms beyond β-AR signaling likely contribute to impaired vasodilatory responses during the menopause transition. Future work should examine receptor-specific mechanisms and pharmacologic strategies to restore vasodilatory responsiveness to hypoxia.

Funding: AHA 909014 (DWJ), HL153523 (JKL), University of Missouri Research Council (NGB, BPB, JKL), APS SURF (VLDV)

O'Connell, Jonathan **Poster Location: 58** **Poster Judging Number: 258**

HIF-2α AND CRLF3 EXPRESSION CHANGES FOLLOWING MILD REPEATED HYPOXIA

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[**Background:** Ischemic stroke remains a major cause of long-term disability and death. Current treatments restore cerebral blood flow but fail to prevent reperfusion injury. In rats, repeated mild hypoxia before stroke reduces neuronal damage, implying activation of endogenous neuroprotective pathways, e.g., hypoxia-inducible factor 2α (HIF-2α), a protein that promotes erythropoietin (EPO) and EPO receptor (EPOR) signaling, enhancing neuronal survival. Studies highlight cytokine receptor-like factor 3 (CRLF3) as an alternative EPOR mediating neuroprotection. We examined CRLF3 expression in the rat brain and whether repeated mild hypoxia upregulates HIF-2α and CRLF3, comparing these molecular responses in male and female rats. **Methods:** Male and female Sprague-Dawley rats (8–10 weeks old) were exposed daily to either normoxia (21% O_2/79% N_2) or normobaric hypoxia (10% O_2/90% N_2) for two hours over three or five consecutive days using a whole-cage hypoxia chamber. Naïve rats were not exposed. In a subset of males, respiratory parameters were recorded using whole-body plethysmography. Protein expression of HIF-2α and CRLF3 in brain tissue was quantified using western blotting. Statistical analyses were performed using GraphPad Prism. **Results:** Rats exhibited increased minute ventilation during hypoxia exposure, but responses did not differ across three or five days. Western blot analysis revealed uniform CRLF3 expression across brain regions and between sexes. After three days of hypoxia, no differences in HIF-2α or CRLF3 expression were observed between hypoxia and control groups. After five days, both HIF-2α and CRLF3 expression increased modestly in males but not females, resulting in significant sex differences. **Conclusions:** CRLF3 is broadly expressed in the rat brain and appears to be regulated by repeated mild hypoxia in a sex-dependent manner. These findings suggest a potential HIF-2α–CRLF3 signaling relationship and highlight greater molecular sensitivity to hypoxia in males, providing a foundation for future studies on CRLF3-mediated neuroprotection in stroke.] (297 words)

Funding:
ATSU – KCOM Master of Biomedical Science Program grant

Grisanti, Laurel **Poster Location: 59**

Interruption of LARP6-collagen mRNA association reduces pressure overload-induced cardiac dysfunction independent of fibrosis in female, but not male, mice

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LARP (La ribonucleoprotein 6, Translational regulator) is a multifunctional RNA binding protein implicated in tissue fibrosis and dysfunction. Prior work in the liver has established that binding of LARP6 to a conserved 5' stem loop (5'SL) in collagen Iα chain mRNA increases collagen I translation and deposition. Despite being expressed in the heart, its role in cardiac remodeling and function are unclear. Therefore, we investigated the hypothesis that interruption of LARP6-collagen mRNA association prevents pressure overload (PO)-induced cardiac dysfunction and fibrosis. Both male and female mice with a knock-in mutation in the 5'SL region that prevents LARP6 association and wild-type (WT) littermate controls underwent PO by transverse aortic constriction, with sham-operated serving as controls. Over 4 weeks post-surgery, male, but not female, 5'SL mice had higher mortality than WT controls. Echocardiography revealed similar reduction in ejection fraction in male 5'SL mice compared to WT mice after PO while female 5'SL mice exhibited blunted reduction in ejection fraction compared to WT. Despite these functional changes, male and female 5'SL mice had similar cardiac hypertrophy and pulmonary edema in response to PO compared to WT mice. Furthermore, the cardiac fibrotic response to PO was not changed by sex or genotype. Bulk cardiac RNA sequencing of female hearts revealed dramatic transcriptomic changes associated with PO that were sensitive to 5'SL mutation. Specifically, the top gene networks in female 5'SL hearts after PO, versus WT mice, were enriched for genes associated with reduced cardiomyopathy and mitochondrial disorders as well as modulation of apoptosis and vasculogenesis. Together, these data suggest that interruption of the LARP6-collagen mRNA interaction lessens cardiac dysfunction induced by PO independent of cardiac fibrosis and in a sex-specific manner. This work provides rationale for interrogation of LARP6 binding partners and actions in the heart beyond its well-described fibrotic action in other tissues.

Funding: AG195557, BX005845, BX004016, R01HL148080, TPA1075016

Zhang, Nannan **Poster Location: 60**

Bone marrow stem cell mobilization decreases brain damage and promotes long-term stroke outcomes after focal ischemic stroke.

Nannan Zhang, Zhe Zhang, Shraddha Amatya, Shinghua Ding

Focal ischemic stroke (FIS) is a leading neural disorder that causes brain damage and human disability and death. Studying therapeutic methods that could reduce brain lesion and promote functional recovery in mouse models could provide therapeutic insights. VEGF (Vascular Endothelial Growth Factor) and BDNF (Brain-Derived Neurotrophic Factor) are vital trophic factors that can promote neural growth, blood vessel formation, working together to support brain health, plasticity, and recovery after FIS. Here we found that plerixafor, a selective antagonist of the CXCR4 chemokine receptor, has been clinically used to mobilize hematopoietic stem and progenitor cells (HSPCs) from the bone marrow into the bloodstream for collection and subsequent autologous transplantation, can significantly increase VEGF and BDNF in the peripheral blood plasma and that plerixafor treated mice exhibited higher numbers of DCX+ cells and NeuroD1+ cells in the dentate gyrus (DG) in hippocampus under control (Ctrl) condition. We further investigated the effects of HSPC mobilization by plerixafor on brain damage, neurogenesis, astrogliosis and motor function recovery after photothrombosis (PT)-induced FIS. Under ischemic conditions, plerixafor treated mice exhibited a smaller infarct volume and enhanced motor function recovery than mice without plerixafor treatment following PT. The plerixafor treated mice also showed a significant increase in the number of GFAP+ (Glial Fibrillary Acidic Protein) reactive astrocytes and GFAP intensity in the penumbra after PT. In summary, our results demonstrated that plerixafor can play a significant role in promoting brain recovery and improving long-term stroke outcomes, and HSPC mobilization by plerixafor may provide a potential therapeutic strategy for stroke therapy.

Bender, Shawn **Poster Location: 61**

Mineralocorticoid receptor blockade prevents cardiac metabolic remodeling in obese Ossabaw swine

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Obesity is associated with cardiac metabolic inflexibility including increased fatty acid uptake/oxidation and reduced glucose oxidation. Inhibition of mineralocorticoid receptor (MR) signaling improves cardiac outcomes in many co-morbid conditions, including obesity, but whether MR signaling contributes to altered cardiac metabolism in obesity is unclear. Indeed, excessive MR signaling has been linked to impaired tissue glucose utilization in non-cardiac tissues. Thus, we investigated the hypothesis that chronic MR blockade with spironolactone (Spiro) will improve obesity-associated attenuation of cardiac glucose uptake and altered cardiac metabolism in obese Ossabaw swine. Lean swine, obese swine fed a high-fat, high-fructose diet, and obese swine treated with Spiro were used in this study. During hypoxemic coronary vasodilation, using an extracorporeal perfusion system, myocardial glucose delivery was directly related to coronary blood flow in all groups. In obese swine, however, the slope of the relationship between myocardial glucose uptake and glucose delivery was reduced compared to lean swine. Chronic MR blockade with Spiro prevented this reduction in myocardial glucose uptake. Myocardial metabolomic analysis revealed pronounced metabolic remodeling in obese hearts enriched, in part, for altered ‘phenylalanine, tyrosine, and tryptophan biosynthesis’ and ‘TCA cycle’ versus lean hearts that was largely prevented by Spiro in obese hearts. Cardiac RNA sequencing further revealed gene signatures associated with reduced cholesterol biosynthesis and glycation signaling in obese, compared to lean, hearts. Hearts from obese swine with MR blockade were not enriched for these pathways, compared to lean hearts, and exhibited transcriptomic changes associated with reduced cardiomyopathy compared to obese untreated hearts. Together, these data suggest that cardiac metabolic inflexibility in obesity is MR-dependent and that prevention of cardiac metabolic alterations may contribute to cardioprotection resulting from spironolactone treatment.

Funding: HL136386

Chou, Chih-Hsuan **Poster Location: 62** **Poster Judging Number: 462**

Transient Developmental Dysregulation of Microglial and Synaptic Pruning Markers in the Nucleus Accumbens of Selectively Bred Low Voluntary Running Rats

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Background: Microglial maturation and their interactions with neurons serve crucial roles during developmental stages, such as synaptogenesis, synaptic pruning, and maintaining brain homeostasis. Evidence suggests that interference with microglial function in the nucleus accumbens (NAc) during adolescence disrupts synaptic pruning and influences adult social behavior in a sex-specific manner. Our previous studies showed that selectively bred low voluntary running (LVR) rats had decreased spine density and numbers of medium spiny neurons (MSN) in the NAc during adolescence, which may underlie altered synaptic function in the motivation for physical activity. However, whether microglia-neuron interactions affect the motivation for voluntary physical activity remains poorly understood. The aim of this study is to determine the developmental profile of microglial and synaptic pruning markers in the NAc of LVR rats.

Methods: We used wildtype Wistar and LVR rats (generations 27-29) in this study. The NAc was collected at postnatal days 0 (P0), 14 (P14), 21 (P21), 35 (P35), and 60 (P60) (N=6-8/sex/timepoint from 2-3 families). We performed quantitative reverse-transcription PCR to examine the RNA expression levels of microglia-specific, dopamine receptor, and synaptic pruning-related genes.

Results: Our preliminary data showed that male LVR rats had significantly increased mRNA expression of microglial reactivity (*Iba1*), neuron-microglial signaling (*Cx3cl1*, *Cx3cr1*), complement regulation (*Csmd1*), and dopamine receptors (*Drd1*, *Drd2*) at P21 and P35 but not P60. There were no differences in the markers of microglial homeostasis (*Tmem119*), astrocyte activation (*Gfap*), or classical complement factors (*C1qb*, *C4A*).

Conclusion: Our preliminary findings indicate a transient, non-inflammatory dysregulation of the neuroimmune axis during the critical adolescence window, coinciding with our previously reported MSN loss in LVR rats. This altered microglia-neuron interaction during development may play a pivotal role in impairing voluntary physical activity motivation.

Foulks, Zachary Poster Location: 63 Poster Judging Number: 363

Impacts of Ketone Bodies on the Heart Do Not Depend on Ketone Body Metabolism

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Heart failure (HF) induces many cardiac metabolic alterations, the primary of which is a decreased rate of fatty acid oxidation which leads to an energetic deficit in heart tissue. Prior studies have demonstrated a protective effect of ketone bodies in HF, potentially due to increased ketone body metabolism to counteract reduced fatty acid oxidation. However, when on a high-fat, low-carbohydrate ketogenic diet (KD), which greatly increases ketogenesis, recent findings suggest that ketone metabolism is downregulated despite increased cardiac ketone delivery. Our objective was to determine whether the observed benefits of a ketogenic diet on the heart were dependent on ketone body metabolism, or whether ketone bodies may instead be having a beneficial regulatory role independent of their metabolism. HF was induced in mice through transverse aortic constriction with apical myocardial infarction (TAC-MI) or cardiac specific mitochondrial pyruvate carrier 2 (csMPC2-/-) knockout, which spontaneously develops HF. Echocardiography was then performed on either a low-fat or KD. In addition to TAC-MI and csMPC2-/- mice, cardiac size and function was assessed in mice with cardiac ketone oxidation knockout (csBDH1-/-) and mice with a double MPC2 and BDH1 knockout (DKO). The cardiac metabolic fate of ketones was also determined by intraperitoneal injection of isotopically labeled beta-hydroxybutyrate. Our gene expression and isotopic tracer results confirmed that cardiac ketone metabolism decreased on KD, and that knockout of ketone oxidation had no overt effect on cardiac function. Additionally, KD was able to improve HF even in csBDH1-/- hearts, suggesting that ketone oxidation is not the mechanism of HF improvement. We further observed that KD enhanced fat oxidation instead, and that ketone body supplementation increased oxidative stress resistance. These findings suggest that ketone body regulatory impacts, rather than metabolism, may be the significant driver of KD-mediated improvement of HF.

Funding: This project was funded by NIH R00, AHA 24TPA1299435, and HL136658 and SLU start up funds to Dr. Kyle McCommis. This work was also supported in part by the Doisy Fund of the Edward A. Doisy Department of Biochemistry and Molecular Biology at Saint Louis University School of Medicine.

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