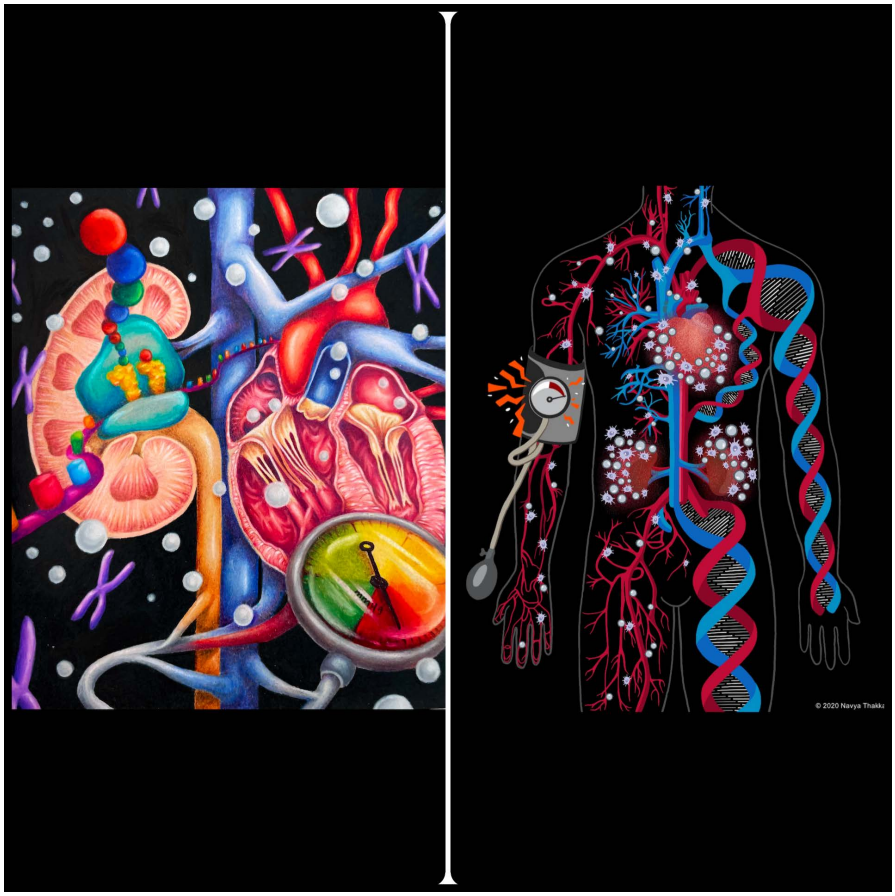
32nd Annual Cardiovascular Day

Program and Abstracts



Tuesday, March //, 2023

The Hilton Inn, Garden Conference Center

Columbia, MO

University of Missouri

32nd 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** – Scott Rector, PhD, Professor, Nutrition & Exercise Physiology, Medicine; Director, NextGen Precision Health Building; Associate Dean for Basic Sciences and Research Infrastructure, MU School of Medicine; Interim Director, Dalton Cardiovascular Research Center - introduction by Kerry S. McDonald, PhD

8:45 - 9:45am **Lecture Session I -** Moderators: Drs. Camila Manrique and Jessica Cayton

8:45 - 9:05am Randi Foraker, PhD, Professor and Chair, Biomedical Informatics, Biostatistics, and Medical Epidemiology, *"From Data to Decisions: AI's Impact on Personalized Healthcare Delivery"*

9:05 - 9:25am Jeffery Boychuk, PhD, Assistant Professor, Biomedical Sciences, *"Adjusting Neocortical Microcircuits for Complex Motor Recovery After Stroke"*

9:25 - 9:45am Natasha Boyes, PhD, Postdoctoral Fellow, Nutrition & Exercise Physiology, *"Autonomic Support of Blood Pressure: Sex, Obesity, and Sleep Apnea"*

9:45 - 10:00am **FlashTalk Session I -** Moderators: Drs. Camila Manrique and Jessica Cayton
Sandy Saunders, PhD, Samuel Martin, Victor Chen, Mikayla Fraunfelder

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

11:00am - 12:00pm **James O. Davis Distinguished Lecture in Cardiovascular Science**
- introduction by Charles E. Norton, PhD

Meena Madhur, MD, PhD, Associate Professor of Medicine
Division Director of Clinical Pharmacology, Indiana University School of Medicine
"Between a Rock and a Hard Place: Immune Modulation in Hypertension"

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

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

2:15 - 2:30pm **FlashTalk Session II -** Moderator: Drs. Jaume Padilla and Carie Boychuk
Anna Gonsalves, Eryn Wagoner, Soraya Nekouian, Zachary Foulks

2:30 - 3:50pm **Lecture Session II -** Moderator: Drs. Jaume Padilla and Carie Boychuk

2:30 - 2:50pm Dwight Towler, MD, Professor of Medicine, Division Director – Endocrinology, Diabetes and Metabolism, *"Aging and Arteries: Noncanonical Wnt Signaling in Arteriosclerosis"*

2:50 - 3:10pm Taixing Cui, MD, PhD, Professor, Medical Pharmacology & Physiology, *"Janus-faced Nrf2 in heart failure"*

3:10 - 3:30pm Shaoping Hou, PhD, Associate Professor, Physical Medicine and Rehabilitation; Pathology and Anatomical Sciences, *"Transplanting neural progenitor cells combined with exercise enhances tolerance of cardiac arrhythmias after spinal cord injury"*

3:30 - 3:50pm Methajit (Mei) Methawasin, PhD, Assistant Professor, Medical Pharmacology & Physiology, *"Reducing granule formation alleviates cardiac dysfunction caused by a Rbm20 genetic variant"*

3:50 - 4:10pm **Award Ceremony**
Laughlin Scholarship Award
Steffen Interdisciplinary Physiology Award
CV Day FlashTalk Awards
CV Day Poster Awards

wifi 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.

"Between a Rock and a Hard Place: Immune Modulation in Hypertension"

H025 Keynote Speaker

Meena Madhur, MD, PhD

! ăă2ŏλl·ű$ tNJ2ŦŜăă2N 2Ŧ a ŜŘλŏλyŜ
5λØλăλ2ϒ 5λNJŜŏű2N 2Ŧ /fλyλŏl·f tKl·NJϒl·Ŏ2f2∃è
LyŘλl·yl· ¦ yλØŜNJăλűè {ŎK22f 2Ŧ a ŜŘλŏλyŜ

5N¢ a l·ŘKdzNQă NJŜăŜl·NJŎK λyűŜNJŜăűă l·NJŜ Ŧ2ŏdzăŜŘ 2ϒűKŜ λyűŜNJLJfl·è ōŜűøŜŜϒűKŜ λϒϒdzyŜ ăèăűŜϒ l·yŘ KèLJŜNJűŜyãλ2yΣ LJl·NJűλŏdzfl·NJfè űKŜ NJŜyl·fΣ Øl·ăŏdzfl·NJΣ l·yŘ Ŏl·NJŘλl·Ŏ Ŏ2ϒLJfλŏl·űλ2yã 2Ŧ KèLJŜNJűŜyãλ2y¢ l ŜNJ f2y∃πűŜNJϒ ∃2l·f λă ű2 λŘŜyűλŦè y2ØŜf Řλl·∃y2ăűλŏ l·yŘ űKŜNJl·LJŜdzűλŏ ăűNJl·ûŜ∃λŜă Ŧ2NJ ûKλă ŘλăŜl·ăŜ¢ l ŜNJ Ŝl·NJfè λyØŜăűλ∃l·ûλ2yã NJŜØŜl·fŜŘ l· ŎNJλűλŏl·f NJ2fŜ Ŧ2NJ ûKŜ LJNJ2πλyŦfl·ϒϒl·ü2NJè Ŏèű2ʅλyŜΣ λyűŜNJfŜdzʅλϒмт ! óL[πмт ! úΣ λϒl·yƎλ2űŜyãλϒLL λyŘdzŎŜŘ KèLJŜNJűŜyãλ2ϒl·yŘ Øl·ăŏdzfl·NJ Řèă Ŧdzyŏűλ2y¢ l ŜNJ fl·ō2NJl·ű2NJè Kl·ă ŘŜŦλyŜŘ LJl·ûKøl·èă ōè øKλŎK L[πмт ! NJŜƎdzfl·ûŜă NJŜyl·f ă2Řλdzϒ ûNJl·yãLJ2NJűŜNJă l·yŘ K2ø ăl·fű λyŦfdzŜyŎŜă ¢ ŎŜftă λϒKèLJŜNJűŜyãλ2y¢ wŜŎŜyűfè KŜNJ fl·ō Kl·ă λyØŜăűλ∃l·ûŜŘ λyyl·ûŜ λϒϒdzyŜ ϒŜŎKl·yλăϒă 2Ŧ KŜl·NJű Ŧl·λfdzNJŜ øλűK LJNJŜăŜNJØŜŘ ŜƨŜŎűλ2ϒŦNJl·Ŏűλ2ϒl·yŘ ŜfdzŎλŘl·ûŜŘ K2ø l· Ŏ2ϒϒ2ϒƎŜySűλŎ LJ2fèϒ2NJLJKλăϒ λϒ[bYκ {Kнōo Ŏ2yűNJλōdzűŜă û2 KèLJŜNJűŜyãλ2y¢ 5N¢ a l·ŘKdzNJ λă l·ϒl·ŎűλØŜ ∃NJl·yű NJŜØλŜøŜNJ Ŧ2NJ ûKŜ bl·ûλ2yl·f Lyãűλűdzűŝă 2Ŧ l Ŝl·fűK l·yŘ ! ϒŜNJλŎl·ϒ l Ŝl·NJű ! ăă2ŏλl·ûλ2y¢ {KŜ l·fă2 ăŜNJØŜă l·ă ! ăă2ŏλl·ûŜ 9Řλű2NJ 2Ŧ /fλyλŏl·f {ŏλŜyŏŜ l·yŘ DdzŜăű 9Řλű2NJ 2Ŧ l èLJŜNJűŜyãλ2y¢ {KŜ Kl·ă l·dzűK2NJŜŘ 2ØŜNJ pл LJl·LJŜNJă λϒKλ∃K λϒLJl·Ŏű ƨ2dzNJyl·fă λyŏfdzŘλy∃ ûKŜ W2dzNJyl·f 2Ŧ /fλyλŏl·f LyØŜăűλ∃l·ûλ2ϒóW/LúΣ W/L Lyãλ∃KűΣ /λNJŎdzfl·ûλ2ϒwŜăŜl·NJŎKΣ /l·NJŘλ2Øl·ăŏdzfl·NJ wŜăŜl·NJŎKΣ l·yŘ l èLJŜNJűŜyãλ2ϒ

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

STATE OF MISSOURI

Proclamation

BY THE GOVERNOR

WHEREAS, nearly 695,000 Americans died from major cardiovascular diseases in 2023, which accounts for 1 in 5 deaths in the United States; and

WHEREAS, heart disease is the leading cause of death in Missouri in 2023, and major cardiovascular disease accounts for 15,400 deaths statewide; and

WHEREAS, the University of Missouri is home to three centers for cardiovascular research: the Dalton Cardiovascular Research Center, the School of Medicine, and the College of Veterinary Medicine; 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 March 11, 2025, 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 3rd day of February 2025.

Mike Kehoe

GOVERNOR

ATTEST:

Denny Hoskins

SECRETARY OF STATE

INDEX of ABSTRACTS

Martin, Samuel Poster Location: 01 Poster Judging Number: 101

Title: Impact of adiposity on vascular adrenergic receptor sensitivity in young males and females

Authors: Samuel A Martin[1], Haylen A McKnelly[1], Brian Shariffi[1], Dain W Jacob[1], Arun Kumar[2], Brian P Bostick[2, 3] Natasha G Boyes[1], Jacqueline K Limberg[1, 4]

Affiliations: [1]Department of Nutrition & Exercise Physiology; [2]Department of Medicine - Division of Cardiovascular Medicine; [3]Department of Medical Pharmacology and Physiology; [4]Dalton Cardiovascular Research Center

Introduction: Obesity increases the risk of hypertension, however mechanistic studies examining the role of adiposity on vascular adrenergic receptor (AR) control of blood flow are limited by mixed-sex cohorts. We sought to examine the impact of adiposity on α-AR vasoconstriction and β-AR vasodilation in males and females. We hypothesized greater adiposity would be associated with greater α-AR vasoconstriction and lower β-AR vasodilation in males and females.

Methods: Twenty-four young adults (12F, 25±7 yrs, 24±3 kg/m^2; 12M, 29±6 yrs, 25±2 kg/m^2) were instrumented for measurement of forearm blood flow (FBF, venous occlusion plethysmography) and underwent brachial artery catheterization for local drug infusion and blood pressure (BP) monitoring. Isoproterenol (non-specific β-AR agonist) was infused to assess β-AR vasodilation. Following β-AR blockade (propranolol), norepinephrine was infused to assess α-AR vasoconstriction. Forearm vascular conductance was calculated (FVC=FBF/mean BP) and reported as the relative change from baseline (%ΔFVC). Pearson *r* correlations assessed associations between %ΔFVC and indices of adiposity [body mass index (BMI), waist circumference, percent body fat (dual energy x-ray absorptiometry), android: gynoid fat ratio (A:G ratio)].

Results: In females, α-AR vasoconstriction (*i.e.,* decreasing %ΔFVC) was negatively associated with BMI (r=-0.670, p=0.017), waist circumference (r=-0.666, p=0.018), and A:G ratio (r=-0.583, p=0.047), but not total body fat (p=0.207), demonstrating greater α-AR vasoconstriction with increased central adiposity in females. No significant associations between α-AR vasoconstriction and indices of adiposity were observed in males (all $p\geq0.240$). β-AR vasodilation was not associated with indices of adiposity in males (all $p\geq0.313$) nor females (all $p\geq0.068$).

Conclusion: These preliminary data show α-AR vasoconstriction is exacerbated by increasing central adiposity in females independent of total body fat. In contrast, β-AR vasodilation is not associated with adiposity in males nor females. Thus, adiposity-mediated mechanisms of vascular adrenergic function may impact chronic conditions affecting females with obesity (*e.g.,* polycystic ovarian syndrome).

Lateef, Olubodun **Poster Location: 02** **Poster Judging Number: 302**

The essential role of transglutaminase 2 in the cytoskeletal responses of vascular smooth muscle cells to mechanical stretching

Olubodun M. Lateef[1,2], Francisco I. Ramirez-Perez[1], Gavin Power[1,3], Marc Augenreich[1,3], Jaume Padilla[1,3,4], and Luis A. Martinez-Lemus[1,2,5]

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

The hardening of the vasculature, otherwise known as arterial stiffening, often precedes the onset of hypertension. Notably, arterial stiffening increases pulsatile pressure and modulates circumferential stress-strain relationships in vascular smooth muscle cells (VSMC). This triggers a cascade of events that leads to structural changes, including accumulating VSMC fibrillar (F)- actin, as shown in the arteries of hypertensive humans and animal models. We and others have also demonstrated that tissue transglutaminase 2 (TG2) activity promotes arterial stiffening and inward remodeling. TG2 is a crosslinking enzyme that works as a G-protein and cellular receptor scaffold. However, it remains unclear how TG2 is activated in the arterial wall. Due to its structural conformation and location at the cell membrane, it has been suggested that mechanical forces activate TG2. Accordingly, we hypothesized that VSMC cytoskeletal responses to mechanical stretching require TG2 activity. We tested our hypothesis in cultured human aortic smooth muscle cells (HAoSMC) that were either left unstretched or exposed to cyclic uniaxial stretch using a Flexcell tension system with 11% elongation at 1 Hz for 6 hours simulating conduit artery pulsatility. TG2 activity was inhibited by treating HAoSMC with the nitric oxide (NO) donor s- nitrosoglutathione (400µmol/L for 6 hours) or cystamine (200µmol/L for 6 hours) under stretched conditions. Additionally, *TGM2* knockdown was performed in cultured HAoSMC using siRNA alongside scrambled siRNA negative controls under static and stretched conditions. We also assessed ex vivo TG2 activity in abdominal aortic rings under unstretched or stretched (12mN for 1 hour) conditions. All differences reported herein are significant at $P<0.05$. Our results show that in vitro mechanical stretching increases VSMC TG2 activity (presence of N-epsilon gamma glutamyl Lysine links), F-actin stress fibers (phalloidin staining, and ex vivo TG2 activity (cadaverine incorporation) relative to unstretched conditions. Notably, the increase in TG2 activity and F-actin content occurring in response to stretching was inhibited by the NO donor, cystamine, or TG2 knockdown. Accordingly, VSMC stiffening induced by mechanical stretching was significantly prevented by TG2 inhibition as determined using atomic force microscopy. These findings demonstrate that stretch-induced VSMC F-actin formation and stiffening require TG2 activity. They also offer valuable insights into the potential targetable mechanisms by which TG2 activation in response to mechanical stimuli contributes to arterial stiffening and remodeling.

Keywords: Arterial stiffening, cytoskeleton, vascular mechanotransduction, hypertension, vascular remodeling.

Nekouian, Soraya **Poster Location: 03** **Poster Judging Number: 303**

Title: Role of β2-Adrenergic Receptor in CD4+ T Cells in Modulating Vascular Stiffness During Hypertension

Authors: Soraya Nekouian, Miles A. Tanner, Chastidy A. Bailey, Shawn B. Bender, Laurel A. Grisanti

Background: Hypertension is a major global health concern, contributing to cardiovascular events such as stroke and heart failure. Immune cells, particularly T cells, have emerged as key players in the inflammatory mechanisms underlying hypertension. Recent evidence indicates that β2-adrenergic receptor (β2AR) signaling alters CD4+ T cell function but its role in hypertension remains unexplored.

Objective: This study aims to investigate how the deletion of β2AR in CD4+ T cells impacts immune responses and vascular remodeling in an angiotensin (Ang) II-induced hypertension model. We hypothesize that β2AR deletion will reduce the recruitment and activation of pro-inflammatory T cells, leading to decreased vascular stiffness and fibrosis.

Methods: We employed a murine model with a CD4+ T cell-specific β2AR knockout (tKO) and induced hypertension using Ang II infusion. Wild-type mice were compared to tKO mice to assess changes in blood pressure, vascular stiffness, and immune cell populations. Vascular stiffness was measured through pressure myography, while immune responses were analyzed using flow cytometry and molecular assays.

Results: Deletion of β2AR in CD4+ T cells significantly influenced both immune responses and vascular function in Ang II-induced hypertension. β2AR-deficient mice exhibited reduced recruitment and activation of pro-inflammatory T cells within the aorta, resulting in less vascular stiffening and fibrosis compared to controls. This reduction was associated with a dampened Th1/Th17 response and fibrosis markers in vascular tissues. β2AR-deficient mice had a less pronounced increase in blood pressure, suggesting a protective effect of β2AR deletion against the hypertensive response. Pressure wire myography demonstrated greater arterial compliance and reduced stiffness in these mice, highlighting the role of β2AR in modulating vascular remodeling during hypertension.

Conclusion: β2AR signaling in CD4+ T cells plays a crucial role in regulating vascular stiffness and inflammation during hypertension. Targeting β2AR signaling could offer new therapeutic strategies for managing hypertension-induced vascular remodeling.

Dow, Kaylie **Poster Location: 04**

Contribution of $GABA_A(\delta)R$ to Heart Rate Regulation by Nucleus Ambiguus

Kaylie E. Dow and Carie R. Boychuk

University of Missouri, Dalton Cardiovascular Research Center, Department of Biomedical Sciences, College of Veterinary Medicine

Abstract body: Nucleus ambiguus (NA) contains cardioinhibitory cardiac vagal neurons (CVNs) that decrease heart rate (HR) through axonal projections to the epicardial fat pad. Despite robust evidence for the role of glutamate to regulate CVN activity, the role of the primary fast inhibitory neurotransmitter, gamma-aminobutyric acid type A receptors ($GABA_AR$), is less clear. $GABA_AR$ ligand binding and kinetic properties are determined by the subunit composition of an individual receptor. $GABA_ARs$ containing a δ-subunit ($GABA_A(\delta)Rs$) are typically seen in the extra synaptic space and possess a high affinity for GABA with slow rates of desensitization, making them well suited to contribute to long duration, or tonic current. Given the signal integrating nature of $GABA_A(\delta)Rs$, we hypothesized that $GABA_A(\delta)Rs$ in NA contribute to resting HR regulation. To determine this, we used pharmacological manipulations of GABAA(δ)Rs in NA through bilateral microinjections (60nl; 10nL/s; from bregma: AP: -7.0, ML: 1.3 DV: -4.5). All experiments were under urethane in adult mice (6-12 weeks) with equal sex distributions. Initial experiments microinjected THIP (4,5,6,7-tetrahydro isoxazole[5,4-c]pyridin-3-ol); a GABAA(δ)R super agonist). THIP produced a dose dependent change in HR (n=4/group) where 20uM produced significant tachycardia (30.7 BPM ± 9.6; p=0.05). To specifically isolate $GABA_A(\delta)R$ contributions to HR, we utilized a unique transgenic mouse line, δ* KI, which possesses $GABA_A(\delta)R$ insensitive to picrotoxin, a GABAAR antagonist (n=8). Picrotoxin (3uM) significantly decreased HR (97.6 ± 9.7 BPM) compared to vehicle. Sequential administration of gabazine (1mM) further decreased HR (76.3 ± 50.4 BPM) compared to vehicle. Therefore, understanding the plasticity of $GABA_A(\delta)R$ signaling in CVNs is critical to our understanding of cardiac physiology. Future studies will follow-up these results using electrophysiology in CVNs. Additional experiments will turn to disease models, namely high fat diet, where the pathology results in a decrease in cardiac vagal drive, thought to be mediated by changes in $GABA_AR$ in CVNs.

Funding Sources: R01HL157366 NIHLB to CRB

Aher, Aman Poster Location: 05

Environmental Cardiomyopathy: Mechanisms, Triggers, and Preventive Strategies

Abstract (300 words)

Environmental cardiomyopathy encompasses cardiac dysfunction from exposure to environmental factors such as pollutants, toxins, extreme temperatures, and socioeconomic conditions. Chronic alcohol consumption, exposure to chemotherapy agents, and toxins can directly damage the myocardium, while fine particulate matter (PM2.5) pollution exacerbates risks of heart failure and cardiomyopathy. Psychosocial stressors, high altitudes, and noise pollution further aggravate preexisting cardiac conditions through stress-induced cardiomyopathy, arrhythmias, and hypertension. Climate extremes, including heatwaves and cold weather, impose additional burdens by inducing dehydration and vasoconstriction, leading to myocardial infarction and stroke.

Mechanistically, environmental triggers contribute to cardiovascular mortality via chronic low-grade inflammation, oxidative stress, epigenetic alterations, vascular dysfunction, and direct cardiotoxicity. Air pollutants activate systemic inflammation and disrupt the balance of reactive oxygen species (ROS), resulting in endothelial dysfunction and atherosclerosis progression. Epigenetic modifications, such as DNA methylation and microRNA expression, impair vascular repair and nitric oxide bioavailability, promoting hypertension and arrhythmias. Direct cardiotoxicity from heavy metals and particulate matter leads to myocardial apoptosis and mitochondrial dysfunction, increasing the risk of heart failure and sudden cardiac death.

Epidemiological evidence links pollutant exposure with elevated cardiovascular mortality, highlighting increased troponin levels and myocardial injury markers in high-pollution areas. Preventive strategies include air quality regulations, antioxidant-rich diets, and biomonitoring for high-risk individuals. Lifestyle interventions, such as stress management, smoking cessation, and regular physical activity, further mitigate risks. This multidisciplinary approach underscores the significance of environmental triggers in cardiovascular health, necessitating targeted policies and public health measures to address these challenges.

Authors

Dr.Aman Aher, M.B.B.S , University of Missouri , Columbia , United States of America.

Netra Shah, MS2, Krishna Institute of Medical Sciences , Karad , India

Safa Poster Location: 06 Poster Judging Number:306

Alzheimer's Disease Augments Cerebrovascular Apoptosis to Oxidative Stress in Males and Females at Different Stages of Disease Progression

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

Alzheimer's disease (AD) is characterized by vascular rarefaction, oxidative stress, and mitochondrial dysfunction. While healthy aging enhances vascular resilience to oxidative stress, we hypothesized that AD increases apoptosis of smooth muscles (SMC) and endothelial cells (EC) to acute oxidative stress by enhancing depolarization of mitochondrial membrane potential ($\Delta\Psi_m$). Posterior cerebral arteries (PCA; ~80 μm diameter) from male and female 3xTG AD mice—examined at 3-time points: young (YH; 2-3 months), cognitively impaired (CI; 4-5 mo) and amyloid β–plaques (Aβ; 6-8 mo)—were isolated, cannulated, and pressurized to 90 cm H_2O at 36°C. Mitochondrial ROS production in PCAs was evaluated with MitoSox (5 μM). ROS production was modestly augmented ($P<0.05$) in CI mice and further augmented in Aβ mice of both sexes. SMC and EC death were quantified using Hoechst 3342 (1 μM; stains all nuclei) and propidium iodide (1 μM; staining dead nuclei) after 50 min exposure to H_2O_2 (200 μM). SMC and EC death were <10% in YH PCAs from males and females. Apoptosis was enhanced in both SMCs and ECs from male Aβ mice (~ 20%) compared to YH and CI (<10%). While SMCs from female CI mice exhibit significantly ($P<0.05$) higher death (~ 20%) compared to YH, SMCs from Aβ mice are more resilient to H_2O_2 (<10% cell death). EC death in females showed similar trends. As depolarization of $\Delta\Psi_m$ initiates apoptosis, we assessed changes in $\Delta\Psi_m$ during H_2O_2 exposure with TMRM, 10 nM. For males, exposure to H_2O_2 exasperated depolarization of $\Delta\Psi_m$ in PCAs from Aβ compared to CI or YH mice. In females, H_2O_2 evoked the greatest $\Delta\Psi_m$ depolarization in PCAs from CI mice consistent with the high cell death. We conclude AD enhances sensitivity to ROS-mediated cell death in cerebral arteries at different timepoints in males and females.

Support: AARG-NTF-23-1148948

Kelly, Kristin **Poster Location: 07** **Poster Judging Number: 207**

Loss of Myeloid Beta-2-Adrenergic Receptor is Protective in a Non-Ischemic Heart Failure by Enhancing Reparative Mechanisms

Kristin I. Kelly, Master's Student
Laurel A. Grisanti, PhD
Miles A. Tanner
(Laurel A. Grisanti)
Kristin I. Kelly, Miles A. Tanner, Kun-Eek Kil, Priscila Y. Sato, Laurel A. Grisanti
Biomedical Sciences Department

Introduction

Heart failure (HF) is a leading cause of death resulting in the inability for the heart to meet the body's metabolic demands. Inflammation regulates HF progression. Adrenergic receptors (AR) mediate the effects of the sympathetic nervous system and are important regulators of cardiovascular function, including immune responses. β2AR is the most widely expressed subtype in the immune system. Previous studies implicate global β2AR deletion in suppressing macrophage infiltration following cardiac injury. Our laboratory has demonstrated a protective role for myeloid cell β2AR deletion in a sepsis model, suggesting a protective role for mβ2ARKO through enhanced phagocytosis and reduced inflammatory cytokine production. The myeloid cell specific role of β2AR in models of heart failure is unknown.

Methods

$Adrb2^{flox/flox}$ and LysM-cre mice were crossed to create a myeloid-specific β2AR knockout mouse (mKO). Transverse aortic constriction (TAC) was used to model non-ischemic HF. Cardiac parameters were monitored following sham or TAC surgery, and pathology was assessed at the termination of the study. In vitro assessment of macrophage function was assessed using standard techniques.

Results

Acutely, mKO TAC mice had a larger left ventricular mass and decreased contractility compared to wild-type (WT) TAC animals. Chronically, mKO animals had improved contractility and reduced cardiac hypertrophy. Assessment of cardiac macrophage function demonstrated enhanced efferocytosis in β2ARKO cells and a reduced pro-inflammatory cytokine profile.

Conclusion

mβ2ARKO is protective in non-ischemic HF, however, increased hypertrophy acutely following TAC suggests a distinct role for myeloid β2AR at different phases of disease progression. Enhanced efferocytosis and suppression of inflammatory cytokines suggests a reparative macrophage role with β2ARKO. While βAR has been studied thoroughly in cardiomyocytes, the implication of the protective effect in mβ2ARKO identifies a novel therapeutic target in the treatment of HF.

Weiss, Rachel **Poster Location: 08** **Poster Judging Number:308**

Loss of mitochondrial pyruvate transport initiates cardiac glycogen accumulation and heart failure

Rachel C. Weiss[1], Kelly D. Pyles[1], Kevin Cho[2], Michelle Brennan[1], Jonathan S. Fisher[3], Gary J. Patti[2], Kyle S. McCommis[1]

[1] Department of Biochemistry & Molecular Biology, Saint Louis University
[2] Departments of Chemistry and Medicine, Washington University in St. Louis
[3] Department of Biology, Saint Louis University

Heart failure metabolic changes involve decreased fat and glucose oxidation and increased glycolysis. The mitochondrial pyruvate carrier (MPC) transports pyruvate into the mitochondrial matrix and previous studies show that heart failure decreases MPC protein expression and cardiac-specific MPC2-/- mice (csMpc2-/-) spontaneously develop heart failure. However, it is unknown why loss of the MPC results in heart failure. The objective of this study was to investigate the link between MPC loss and heart failure. Targeted metabolomics and isotope tracing was performed in fl/fl and csMpc2-/- hearts after i.p. injection of U-^{13}C-glucose to measure cardiac ^{13}C enrichment into glucose metabolites by LC-MS/MS. Cardiac glycogen was assessed biochemically and by electron microscopy. Glucose uptake was measured in cardiac muscle fibers by ^{3}H-2-deoxyglucose. Glycogen regulating enzymes were measured by western blot. fl/fl and csMpc2-/- mice were fed low fat control, or ketogenic diet. From our metabolomic analyses, the failing csMpc2-/- hearts contained normal levels of both ATP and phosphocreatine high-energy phosphate pools. The failing csMpc2-/- hearts displayed increased enrichment from ^{13}C-glucose throughout the glycolytic pathway, increased glycolytic metabolite pool size, and ~6X increase in glycogen levels, despite enhanced inhibitory phosphorylation of glycogen synthase. csMpc2-/- hearts also displayed increased glucose uptake both basally, and with insulin stimulation. Glycolytic enrichment was also increased in non-failing csMpc2-/- hearts of young mice, yet glycogen levels remained unchanged compared to fl/fl. Two weeks of ketogenic diet completely normalized the cardiac glycogen and reversed the HF. Lastly, mice were treated twice daily for two weeks with 100 mg/kg MZ-101, a glycogen synthase-1 inhibitor, which significantly reduced the size of failing csMpc2-/- hearts. These results suggest the loss of MPC in the heart increases glycolytic metabolism and ultimately glycogen accumulation and heart failure. Thus, the loss of MPC in the heart results in a glycogen storage disease phenotype, causing heart failure.

Hollomon, Hunter **Poster Location: 09** **Poster Judging Number: 109**

Low Circulating FGF-21 after High Protein Intake Likely Mediates the Adverse Effect of High Protein Intake on Endothelial Function

Hunter Hollomon, Alan Fappi, and Bettina Mittendorfer. Departments of Medicine and Nutrition and Exercise Physiology, University of Missouri at Columbia.

Endothelial dysfunction is an early marker of cardiovascular disease (CVD). A Western-type diet is a major risk factor for CVD, presumably because of high intake of simple sugars and red and processed meats with high salt, saturated fat, and cholesterol contents. Results from animal models and population studies suggest high protein intake as part of a Western-type diet also contributes to CVD burden, but the mechanisms involved are unknown. We tested the hypothesis that high protein intake adversely affects endothelial vasodilator function, and this effect is partially mediated by the inhibitory effect of high protein intake on FGF21 production.

First, we evaluated the effect of consuming a high-protein (HP) mixed meal (22% of energy as protein), compared with an isocaloric standard protein (SP) meal (15% of energy as protein), on plasma FGF21 concentration and reactive hyperemia, an established marker of endothelial function, in nine middle-aged (49±14 years-old) people with overweight/obesity (body mass index: 28±3 kg/m^2). Secondly, we evaluated the effect of escalating doses (0-50 ng/ml) of FGF21 on endothelial nitric oxide synthase (eNOS) phosphorylation at Ser1177 (stimulatory) and Thr495 (inhibitory), and nitric oxide (NO) production in cultured human umbilical vein endothelial cells (HUVEC).

Plasma FGF21 concentration and the reactive hyperemia index were not different after ingesting the SP meal compared with basal conditions (before meal). After the HP meal, plasma FGF21 concentration decreased by 70±2 % (mean ± SEM) and the reactive hyperemia index decreased by 21±3 %. Recombinant human FGF-21 treatment of HUVECs significantly increased Ser1177 eNOS phosphorylation, decreased Thr495 eNOS phosphorylation, and stimulated NO production in a dose-dependent manner.

In conclusion, high protein intake impairs endothelial function, at least in part by lowering circulating FGF-21. These findings provide a potential mechanism for the increased CVD risks associated with high protein intake.

Fraunfelder, Mikayla **Poster Location: 10** **Poster Judging Number:310**

ENDOGENOUS SURFACTANT PROTEIN A PROMOTES VASCULAR SMOOTH MUSCLE CELL PHENOTYPE-SWITCH DURING ABDOMINAL AORTIC ANEURYSM

Mikayla M. Fraunfelder, B.S., MD/PhD Student, M1

(Shiyou Chen, DVM, Ph.D.)

MU School of Medicine

Introduction: Abdominal aortic aneurysm (AAA) is characterized by stretching and weakening of the vascular wall that is driven, in part, by vascular smooth muscle cell (VSMC) phenotype switch. We previously demonstrated that in response to inflammation or injury, VSMCs will express surfactant protein A (SPA), an inflammatory collectin found in multiple tissues. Using global and VSMC-specific SPA knockout (SPA-/-) mouse models we reveal a link between SPA expression and VSMC phenotype switch during AAA.

Methods: 8-week-old male ApoE-/-, SPA-/- ApoE-/- (SPA-/-), and ApoE-/- MyhIICre+ SPAfl/fl mice were used to test the effect of global and SMC-specific knockout of SPA on AAA formation, respectively. ApoE-/- MyhIICre+ SPAfl/fl mice ($SPA^{VSMC-/-}$) were subjected to tamoxifen injection at 6-weeks of age. AAA was induced via 4-week angiotensin II (AngII) infusion, and AAA development was assessed via ultrasound at D0 and at D28. Aortas were analyzed via immunofluorescent, H&E, and elastin staining.

Results: Following 4 weeks of Ang II infusion, SPA-/- mice exhibited a reduction in aortic diameter change, dissection incidence, and elastin fragmentation compared to the controls. Immunofluorescent staining reveals decreased expression of inflammatory cytokine but increased expression of VSMC contractile markers. Consistently, $SPA^{VSMC-/-}$ mice showed similar attenuation of aneurysm and VSMC phenotype switch. These data suggest a role for endogenous SPA in VSMC inflammatory response.

Conclusion: Our data suggest a role of endogenous SPA in VSMC phenotype switch during AAA development. As VSMC phenotype switch is a major driver of inflammation and weakening in the aortic wall, SPA may provide a potent target for future therapeutics. *In vitro* studies are underway to elucidate a potential mechanism through which SPA might promote VSMC phenotype switch.

Hogue, Sam **Poster Location: 11** **Poster Judging Number: 311**

The effects of age and acclimation temperature on mitochondrial ROS production, respiration, and structure in western painted turtles (Chrysemys picta bellii)

Sam Hogue, Kyle McCommis, Daniel Warren

In mammalian hearts, reperfusion following ischemia upsurges reactive oxygen species (ROS) production via succinate-driven reverse electron transport (RET). Yet, adult painted turtles, which can survive ~170 days of anoxia at 3°C, can avoid damage upon reoxygenation following anoxia, making it an ideal model to study this clinically relevant insult. Hatchlings are less anoxia-tolerant, surviving ~40 days at 3°C, and their handling of ROS production during reoxygenation may reflect this difference. We aimed to determine the effect of development on mitochondrial ROS production and respiration in adult and hatchling painted turtles. Because reoxygenation naturally occurs during cold temperatures, when turtles emerge from overwintering in anoxic ponds, we also examined the effects of cold acclimation on mitochondrial function. To test the hypotheses that cold acclimation decreases mitochondrial respiration more in adults than hatchlings, and that hatchlings show a higher rate of maximal reactive oxygen species production, turtles of both age groups were acclimated to 20°C and 3°C, and ROS production of isolated cardiac ventricular mitochondria was measured during succinate-driven RET. Additionally, respiration rates were measured with pyruvate/ malate, palmitoylcarnitine, glutamate and succinate. Samples from the 20°C group were fixed in glutaraldehyde, embedded in epoxy, and sectioned onto grids for imaging using transmission electron microscopy (TEM). We found that hatchlings had lower rates of succinate-induced ROS production via RET and State III respiration compared to adults. Cold acclimation reduced ROS production in both age groups. TEM images showed decreased cristae membrane area in hatchlings compared to adults, providing a structural explanation for the observed differences in mitochondrial function. In conclusion, the variation of anoxia tolerance within painted turtles does not appear to correlate with the functional properties of the mitochondria.

Behrmann, Andrew **Poster Location: 12** **Poster Judging Number: 312**

Right Ventricular Cardiomyocyte Excitation-Contraction Coupling Defects in a Mouse Model of Type 3 Pulmonary Hypertension:

Andrew J. Behrmann, Scott Roselli, Arooj Shahid, Charles E. Norton III, Timothy L. Domeier. Medical Pharmacology and Physiology, University of Missouri, Columbia, MO, USA.

Type 3 pulmonary hypertension (PH) occurs when lung disease causes hypoxic vasoconstriction, increased right ventricular (RV) afterload, and progressive right heart failure. Changes in RV cardiomyocyte excitation-contraction coupling (ECC) in the setting of PH are poorly defined. We tested the hypothesis that RV cardiomyocyte ECC is impaired in the bleomycin-induced PH mouse model. To test this hypothesis, male and female C57BL/6 mice (4-7-month age) received a single bolus of intratracheal saline (*sham; N=11 male/9 female*), or bleomycin (0.025U, *PH; N=12 male/8 female*), followed by 21 days to develop fulminant Type 3 PH. Cardiomyocytes were enzymatically isolated from the RV of excised hearts and loaded with fura-2 for Ca^{2+} and contractility assessments over a range of stimulation frequencies (0.5-4 Hz at 37 °C). Bleomycin treatment induced RV hypertrophy (Fulton index) in male and female mice ($P<0.0001$), with more severe hypertrophy observed in *PH* males vs. *PH* females ($P<0.05$). Isolated RV cardiomyocytes of male *PH* mice exhibited elevated diastolic Ca^{2+}, decreased Ca^{2+} transient amplitude, and prolonged Ca^{2+} reuptake kinetics vs. *sham* across all frequencies ($P<0.05$). Furthermore, changes in Ca^{2+} handling in males was associated with shorter diastolic sarcomere length, decreased shortening, and prolonged relaxation in *PH* vs. *sham*. In RV cardiomyocytes of females, Ca^{2+} and contraction parameters were similar between *PH* and *sham* at low frequencies (0.5-1 Hz). However, increased diastolic Ca^{2+}, prolonged Ca^{2+} reuptake, shorter diastolic sarcomere length, and prolonged relaxation were unmasked in RV cardiomyocytes of *PH* females at stimulation frequencies of 2-4 Hz ($P<0.05$). In conclusion, bleomycin-induced PH impairs Ca^{2+} handling and contractile activity in RV cardiomyocytes, with more pronounced effects in males vs. females. Alterations in RV cardiomyocyte ECC may represent an underlying mechanism of RV dysfunction observed clinically in PH.

Hayden, Matthew **Poster Location: 13** **Poster Judging Number: 313**

TRANSIENT RECEPTOR POTENTIAL VANILLOID 4 INHIBITS CARDIAC AUTOPHAGY IN AGING & ISCHEMIA-REPERFUSION INJURY

Myocardial infarction results in ischemia-reperfusion (I/R) injury and pathological cardiac remodeling. Cellular autophagy is vital to cardiomyocyte repair in response to stress, and dysregulated autophagy associates with adverse cardiac remodeling following I/R injury. Expression of the Transient Receptor Potential Vanilloid 4 (TRPV4) ion channel increases in cardiomyocytes with aging and contributes to cardiomyocyte dysfunction after I/R. The objective of this study was to test the hypothesis that TRPV4 inhibition alters cardiomyocyte autophagy with aging and I/R. Left ventricular myocytes were isolated from *Young* (3-6 mo) or *Aged* (25-31 mo) male and female mice and treated with the TRPV4 antagonist HC067047 (HC, 1 uM) or vehicle control for 2-hrs at room temperature. After treatment, immunocytochemistry was performed with antibodies for pATG16L1, LC3, and Cathepsin D. Autophagic organelles were classified as phagophores (pATG16L1 puncta), autophagosomes (LC3 puncta), lysosomes (Cathepsin D puncta), and autolysosomes (LC3 and Cathepsin D co-localized puncta). To assess the role of TRPV4 in I/R injury, *ex vivo* Langendorff-perfused *Aged* hearts were exposed to 45-min global ischemia followed by 2-hrs reperfusion, and immunohistochemistry was performed to assess LC3 puncta. In *Young* myocytes HC did not affect the number of phagophores (p=0.911), autophagosomes (p=0.181), lysosomes (p=0.356), or autolysosomes (p=0.237). However, in *Aged* myocytes, HC led to accumulation of phagophores (p=0.015), autophagosomes (p<0.001), and autolysosomes (p=0.001), with no change in lysosomes (p=0.323). In *ex vivo Aged* hearts following I/R injury, HC led to a significant accumulation of LC3 puncta (p=0.0391). In conclusion, these data indicate TRPV4 inhibition promotes the pathway of autophagy in cardiomyocytes with aging and following I/R injury. Consistent with a growing body of preclinical literature, pharmacological TRPV4 inhibitors may emerge as a viable therapy to reduce reperfusion injury following myocardial infarction with aging.

Pharmacological Inhibition of Galectin-3 Suppressed Aortic Stiffness in Smooth Muscle Cell Specific Beclin-1 Deficient Mice

Authors: Arun Kannan[1], Eddie Downey[1], Venkateswaran Subramanian[1].

[1] Department of Medicine, Division of Cardiovascular Medicine, University of Missouri, Columbia, MO.

Introduction: Aortic stiffness, a hallmark of aging, is a significant risk factor for cardiovascular diseases like hypertension and atherosclerosis. In our preliminary studies, smooth muscle cell-specific Beclin-1 deficiency in mice accelerated aortic stiffness which is associated with a significant increase in Galectin-3. An increased level of Galectin-3, a galactoside-binding lectin, is shown to be associated with arterial stiffness in cardiovascular patients. In this study, we aim to investigate the effect of galectin inhibition on aortic stiffness development in SMC-Beclin-1 deficient mice.

Methods and Results: Tamoxifen-inducible SMC-Beclin-1 deficient mice were generated by breeding Acta2-CreERT2 hemizygous mice with Beclin-1 floxed mice. Male SMC Beclin-1 wild type (Cre-) or deficient (Cre+) mice (n=7-8/group) were administered with either vehicle (VC) or galectin inhibitor (GI-100 mg/kg/day) in drinking water for 7 weeks. Post 8 weeks of tamoxifen injection, as measured by ultrasound, SMC-Beclin-1 deficiency (Cre+) in mice spontaneously accelerated aortic stiffness as evidenced by a significant increase in aortic pulse wave velocity (PWV;Cre-:2.2±0.05 vs Cre+:3.5±0.2 m/s; $P<0.001$) with decreased radial strain and distensibility compared to Cre- controls. Interestingly, administration of galectin inhibitor (GI) partially but significantly suppressed aortic PWV in Cre+ mice compared to the Cre- and vehicle control groups (Cre- VC:2.2± 0.05; Cre- GI:2.1±0.02; Cre+ VC:3.5±0.2; Cre+ GI:2.8± 0.04 m/s; $P<0.01$). Furthermore, *ex-vivo* aortic ring analysis with pin myography in response to contractile (serotonin) and relaxation [acetylcholine ($Ach10^{-5}M$) and sodium nitroprusside ($SNP10^{-5}M$)] agents showed significantly decreased contraction and relaxation in Cre+ mice compared to Cre- controls (Contraction:Cre+VC:9.1 ±0.9; Cre-VC:17±2.5mN; Relaxation Ach: Cre+VC:5% vs Cre-VC:66%; SNP: Cre+VC:70% vs Cre-VC:97%). However, galectin inhibition in Cre+ mice showed minimal response to Serotonin or Ach/SNP-induced aortic contraction and relaxation compared to vehicle or WT controls (Contraction: Cre-VC: 17±2.5& GI:10±1; Cre+VC:9±0.9&GI:7±0.2mN); Relaxation: Ach-Cre-VC:66%&GI:63%; Cre+VC:5%&GI:-7%/SNP-Cre-VC:97% &GI:97%; Cre+VC:70%&GI:58%).

Conclusion: These findings suggest that pharmacological inhibition of galectin partially suppressed SMC-Beclin-1 deficiency accelerated aortic stiffness in mice, as evidenced by decreased PWV.

Kwok, Amy **Poster Location: 15** **Poster Judging Number: 315**

Expression of Mesenchymal Marker Proteins in Doxorubicin-Treated Endothelial Cells

Amy Mo Kwok[1], Kass Sjostrom[2], Shixin Tao[1], Melissa Cobb[1], Eugene Konorev[1]
[1]College of Osteopathic Medicine, Kansas City University; [2]College of Biosciences, Kansas City University

Abstract:
Doxorubicin (Dox), an anthracycline chemotherapeutic agent, is widely used in cancer treatment. However, increasing evidence suggests that cancer survivors treated with Dox are at a higher risk of developing cardiovascular conditions. Dox specifically harms endothelial cells, which are exposed to high concentrations of the drug following intravenous administration. This accumulation results in cellular senescence, suppression of endothelial proliferation, and impaired vascular network formation. Previous studies have identified the involvement of the canonical TGF-beta pathway in endothelial damage induced by Dox, but the downstream mechanisms remain unclear. Given that the TGF-beta pathway promotes endothelial-to-mesenchymal transition (EndMT), we hypothesize that Dox activates this pathway, causing long-term mesenchymal reprogramming in endothelial cells.

We utilized human umbilical vein endothelial cells (HUVECs) to assess the expression of mesenchymal markers such as N-cadherin, vimentin, alpha-smooth muscle actin (aSMA), and fibronectin. These markers were normalized to beta-actin and analyzed following either 48-hour Dox treatment or 48-72 hours after Dox withdrawal (washout). To investigate the role of the TGF-beta pathway, we used SB431542, a selective inhibitor of ALK4/5/7 receptor kinases. We found that Dox increased mesenchymal marker expression in both treatment and washout protocols. The SB inhibitor effectively decreased this expression, suggesting that it mitigates TGF-beta-mediated EndMT. Immunocytochemistry showed that Dox also upregulated transgelin, a smooth muscle mesenchymal marker, in endothelial cells, identified by staining for the endothelial marker CD31. This increase in transgelin expression was inhibited by SB431542.

Our future studies will examine mesenchymal protein expression in cardiac endothelial cells of mice treated with Dox. In conclusion, our results suggest that Dox promotes EndMT in HUVECs through the TGF-beta pathway, and this process can be suppressed by an ALK4/5/7 receptor kinase inhibitor.

Zhang, Zhe **Poster Location: 16** **Poster Judging Number: 416**

The effect of an NAMPT mutation on motor neuron metabolism

Zhe Zhang, Nannan Zhang, Shinghua Ding

Abstract: Nicotinamide adenine dinucleotide (NAD^+) is a required cofactor in cellular metabolism and is crucial for energy production. In mammalian cells, NAD^+ is primarily synthesized through the salvage pathway, where nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme. Previous studies from our and other labs have revealed that NAMPT is predominantly expressed in neurons while little in glia cells under normal condition. Increasing evidence suggest neuronal protective roles of NAMPT in a range of neurological diseases such as ischemic stroke, Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS). Recent studies using global and neuron-specific inducible and conditional knockout mice have displayed that NAMPT is indispensable for neuronal survival and is significant for maintaining neuronal functions. However, despite extensive investigated, no genetic diseases stemming from NAMPT variants have been recognized. Here, we report an undiagnosed neurological disease attributed to a homozygous missense mutation (c.472.C>G, p.P158A) in the coding region of NAMPT gene. In the present 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 findings demonstrated that the P158A mutation in NAMPT disrupts cellular metabolism and energy homeostasis and causes mitochondrial dysfunction. Our study reveals an undiagnosed motor neuropathy caused by NAMPT P158A mutation and confirms NAMPT as a key factor in the mediation of neuronal metabolism and functions and indicates that targeting NAMPT might be provide novel therapeutic strategy for motor neuron disease.

Wagoner, Eryn Poster Location: 17 Poster Judging Number: 317

Distinct Transcriptomic Adaptations in Coronary and Cerebral Arterioles in Female Ossabaw Swine with Cardiometabolic Heart Failure Treated with a RSK3/mAKAPβ Gene Therapy

Eryn P. Wagoner[1,3], Taylor J. Kelty[2,3], Pamela K. Thorne[1,3], Michael S. Kapiloff[4], Darla L. Tharp[1,3]

[1]Biomedical Sciences, [2]Nutrition and Exercise Physiology, [3]NextGen Precision Health, University of Missouri, [4]Ophthalmology and Medicine, Stanford Cardiovascular Institute, University of Stanford.

Cardiogenic dementia is associated with an increased risk of mortality in heart failure patients. We previously demonstrated cerebrovascular insufficiency in female Ossabaw swine with cardiometabolic heart failure (CM-HF), and showed improved cardiac function and ventricular-vascular interactions by a RSK3/mAKAPβ targeted gene therapy (RBD). The goal of this study was to examine the effects of RBD gene therapy on transcriptomic signaling in coronary and cerebral arterioles in an Ossabaw swine model of CM-HF. Female Ossabaw pigs were assigned to HF (n=4-5) and HF+RBD (n=4-7). Animals were fed a Western diet (3 mo.) and aortic banded (6 mo.) prior to terminal experiments (12 mo.). RBD biologic was infused immediately after aortic banding. Wet lung weight was decreased in HF+RBD animals compared to HF (283±19 vs. 423±64g; unpaired t-test, $p<0.05$) and was associated with improved diastolic function by a decrease in the end diastolic pressure-volume relationship (EDPVR: 0.039±0.004 vs. 0.022±0.003 mmHg/mL; unpaired t-test, $p<0.05$). RBD also increased stroke volume (40±2 vs. 50±2mL; unpaired t-test, $p<0.05$) and improved hemodynamic coupling between the left ventricle and peripheral vasculature. RBD increased endothelial-dependent dilation to bradykinin (BK) compared to HF (Two-way ANOVA, Group x Dose interaction; $p<0.05$) in isolated second order (2A) pial arterioles, which was unaltered by L-NAME and indomethacin. RNA sequencing identified 26 differentially expressed genes (DEGs) in cerebral arterioles and 11 DEGs in coronary arterioles. Ingenuity pathway analysis (IPA) of cerebral arterioles predicted inhibition of upstream regulator POR (z-score>-2), a gene associated with decreased fibrosis and organismal death, as well as increased sterol synthesis and quantity of leukocytes. Gene enrichment of coronary arterioles predicted cytokine (TNF, z-score=1.96; IL1B, z-score=1.98) and growth factor activation (TGFB1, z-score=1.99). These findings demonstrate RBD improvement in coronary and cerebral vascular function occurs through distinct transcriptomic adaptations involving pathways associated with fibrosis, leukocyte recruitment, cytokine production, and growth factor activation.

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

Gonsalves, Anna **Poster Location: 18** **Poster Judging Number: 318**

Title: β_3-adrenergic receptor agonism blunts sympathetic vasoconstriction in healthy young females

Authors: Anna M. Gonsalves[1], Natasha G. Boyes[1], Dain W. Jacob[1], Brian Shariffi[1], Vina D. Vedala[1], Brian P. Bostick [3,4], Jacqueline K. Limberg[1,2]

Affiliations: [1]Department of Nutrition and Exercise Physiology; [2]Dalton Cardiovascular Research Center; [3]Department of Medicine - Division of Cardiovascular Medicine; [4]Department of Medical Pharmacology and Physiology

Objective: Females are historically underrepresented in cardiovascular research; as a result, therapeutic approaches are derived from primarily male cohorts, leading to less effective treatments. Vascular β-adrenergic receptors (ARs) mediate blood flow and blood pressure responses to sympathetic nervous system activation. Preclinical studies show greater vascular β3AR expression and enhanced β3AR vasorelaxation in female compared to male rodents, indicating a unique potential for β3ARs to modulate blood flow in females. We hypothesized pharmacological β3AR activation would attenuate the peripheral vasoconstrictor response to acute sympathetic activation in healthy young females.

Methods: Ten young healthy female participants (27±7 yr, 25±3 kg/m^2) completed two study visits randomized and blinded to oral placebo or Vibegron (75mg; a β3AR agonist). On each visit, participants completed a 5-min rest followed by a 2-min cold pressor test (CPT) to achieve sympathetic activation. Forearm blood flow (FBF, venous occlusion plethysmography) and blood pressure (BP, finger photoplethysmography) were assessed. FBF was normalized for mean BP (forearm vascular conductance, FVC) and reported as absolute values and relative (%) changes from baseline.

Results: On the placebo visit, sympathetic activation with CPT elicited robust decreases in absolute FBF (2.0±1.1 to 1.6±0.9 mL/dL/min; p=0.003) and FVC (2.4±1.3 to 1.7±1.1 mL/dL/min/100mmHg; p=0.001), confirming vasoconstriction with increased sympathetic activity. In contrast, the FBF (3.0±1.8 to 2.8±1.7 mL/dL/min; p=0.396), but not FVC (3.6±2.1 to 3.0±1.8 mL/dL/min/100mmHg; p=0.037), response to CPT was lost following β3AR agonism. Vibegron attenuated CPT-mediated relative reductions in FBF (-21.2±16.7 to -0.3±23.0%, p=0.016) and FVC (-27.3±17.4 to -11.2±18.6%, p=0.021) compared to placebo.

Conclusion: β3AR agonism attenuates the vasoconstrictor response to acute sympathetic activation in young healthy females. These preliminary data indicate β3ARs are capable of restraining sympathetic-mediated vasoconstriction in females. Based on the known role of estrogen in βAR signaling, future work will investigate the impact of menopause and estrogen loss on this response.

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

Liu, Yixiao **Poster Location: 19** **Poster Judging Number: 419**

A cyclic stretch-operated mechnotransdunction to maintain contractile phenotype of vascular smooth muscle cells

Yixiao Liu[1,2], Janxin Yan[1,2], Xue Jiang[1], Minna Huang[1], Mahsa Saheb Farmanabadi[1], Nathan Schwartz[1], Michael J. Davis[2], Taixing Cui[1,2]

[1]Dalton Cardiovascular Research Center, [2]Department of Medical Pharmacology and Physiology, University of Missouri School of Medicine

Cyclin stretch-induced mechanotransduction plays a crucial role in vascular homeostasis and disease. However, the underlying mechanisms remain poorly understood. Herein, we established a physiological cyclin stretch which drives vascular smooth muscle cell (vSMC) phenotype switch from a dedifferentiated state to differentiated one and identified a novel signaling pathway for the physiological stretch-operated mechanotransduction. Using a mouse aortic SMC-derived cell line (MOVAS), we demonstrated that uniaxial cyclic stretch (12.9%, 1Hz) alone induced a phenotype switching from a dedifferentiated state to differentiated one, characterized by reduced growth and enhanced expression of a multitude of SMC contractile markers. Combined analysis of multi-omics data revealed an indispensable role of cyclin-dependent kinase 8/19 (CDK8/19) associated with downregulation of piezo-type mechanosensitive ion channel component 2 (Piezo2) in driving vSMC dedifferentiation, illustrating the mechanical origin of the discovered CDK8/19 signaling in vSMCs. In completely ligated mouse carotid arteries with reduced cyclic stretch, we found that the expression of CDK8/19 was dramatically upregulated in medial and neointima SMCs, underscoring a CDK8/19-operating mechanotransduction for vSMC dedifferentiation. Conversely, knockout of CDK8/19 in vSMCs and perivascular delivery of CDK8/19 inhibitors strongly suppressed neointimal formation in completely ligated carotid arteries in mice. Taken together, our results indicate that CDK8/19 are essential mediators of the mechanotransduction for vSMC dedifferentiation and may be potential therapeutic targets for occlusive vascular disease.

Chen, Victor **Poster Location: 20** **Poster Judging Number: 120**

Title: Anatomical Distribution of Cardiac Vagal Motor Neurons in Dorsal Motor Nucleus of the Vagus

Authors: Victor Q. Chen, Yoko B. Wang, Carie R. Boychuk

Associations: Dalton Cardiovascular Research Center, Department of Biomedical Sciences, College of Veterinary Medicine, University of Missouri - Columbia

Abstract: Cardiac vagal motor neurons (CVNs) innervate cardiac ganglia critical to chronotropy through the vagus nerve. Historically, CVNs in nucleus ambiguus (CVN^{NA}) were the focus of central circuit examinations for CVN control of HR. However, motor neurons in dorsal motor nucleus of the vagus (DMV), known for innervating gastrointestinal organs, can also be retrogradely traced from cardiac tissue and cause cardio-inhibition upon activation. Unfortunately, little is known about this CVN^{DMV} population. This study hypothesized CVN^{DMV} would exhibit columnar organization similar to gastrointestinal-projecting neurons.

To test this, C57/Bl6J mice (7-11 weeks) received cardiac injections of a retrograde tracer Fluorogold (1-2%) followed by transcardial perfusion 2-3 weeks later. CVN^{DMV} were identified throughout rostro-caudal extent of DMV, with significantly more in locations near caudal area postrema (AP; 11±1 CVN^{DMV} @ -7.60mm vs 4±1 @ –6.9mm; p=0.0271). CVN^{DMV} overlapped with rostro-caudal distributions of CVN^{NA} but were more prevalent in caudal brainstem (-7.86mm; 7±2 CVN^{DMV} vs 2±1 CVN^{NA}; p=0.0238). CVN^{DMV} count was bilaterally symmetrical across hemispheres (16±2 CVN^{DMV} left vs 18±3 right, p=0.1856).

However, mediolateral examination found CVN^{DMV} occupying right hemisphere were lateralized from midline; counts in lateral third of DMV were significantly higher (10±1 CVN^{DMV}) compared to medial third (3±1 CVN^{DMV}; p=0.0017), being most pronounced near caudal AP(-7.86mm, -7.60mm; medial vs lateral, p=<0.001). No lateralization was found in left hemisphere (p=0.9969).

Dual retrograde injections were conducted to examine co-labeling between CVN^{DMV} and gastric-projecting neurons (GVNs), at caudal locations (-7.86mm, -7.6mm). Despite robust labeling of CVN and GVN, only ~2.5% of total neurons were co-labeled (~7.5% of CVN^{DMV}, ~4.5% of GVN).

This indicates CVNDMV follows columnar organization but with atypical lateralization in the right hemisphere. Limited co-labeling of CVNDMV with GVN raises questions about neuronal coordination of parasympathetic function within DMV. Future study will investigate co-localization of CVNDMV with liver projecting neurons, also highly lateralized.

Funding:
R01HL157366 NIHLB to CRB

miR-146a Deficiency Ablates Thoracic Aortic Aneurysmal Rupture in Mice

Authors: Nithya Ramesh[1], Eddie Downey[1], Venkateswaran Subramanian[1].

[1] Department of Medicine, Division of Cardiovascular Medicine, University of Missouri, Columbia, MO.

Background: Thoracic aortic aneurysm (TAA) is an asymptomatic, life-threatening disease with mortality greater than 80% after rupture. The assembly of cytoskeletal structural proteins,e.g. Filamin A (FLNA) with extracellular matrix(ECM), which helps in maintaining aortic structural integrity and function, is highly disrupted in TAA. Besides surgical interventions, no effective medical therapies are available to blunt TAA progression and rupture. miR-146a, a short non-coding microRNA, is well known to regulate inflammatory and auto-immune processes under cardiovascular diseases. Increased miR-146a has been observed in plasma and dissected aortic tissue of TAA patients. Our preliminary studies, by in-situ hybridization and qPCR analyses showed that miR-146a is highly upregulated in the aortic media of mouse TAAs.To examine the effect of miR-146a deficiency on TAA rupture in mice induced by Lysyl oxidase inhibitor,β-aminopropionitrile(BAPN).

Methods: Three-week-old male and female C57BL/6J miR146a wild type (WT) or deficient (KO)[n=12-18/group] mice were administered with either vehicle or BAPN (0.5% wt/vol) in drinking water for 28 days. TAA was examined by in vivo ultrasound aortic lumen measurements and ex vivo aortic external width measurements.

Results:BAPN administration promoted TAA development equivalently in both WT and KO male(WT=67%,12/18;KO=62%,8/13) and female(WT=75%,9/12;KO=50%,8/16) mice compared to vehicle control. miR-146a deficiency significantly protected mice from TAA rupture(Male-WT=33%,6/18;KO=0%,0/13; Female WT=33%,4/12;KO=0%,0/16; $P<0.05$) and improved survival rate($P<0.05$).Histological and immunofluorescent analyses showed that BAPN-induced TAA is associated with increased elastin breaks, less ECM-collagen, and cytoskeletal disassembly as evidenced by decreased filamentous F-actin in the aortic media only in WT mice. In silico target prediction identified miR-146a binding sites in the cytoskeletal structural protein FLNA 3'UTR.Western blot and immunohistochemical analyses revealed a strong reduction of aortic FLNA in the SMC-rich aortic medial layer, whereas miR-146a deficiency prevented BAPN-induced loss of aortic FLNA protein.

Conclusion: These findings suggest that miR-146a plays a critical role in mediating TAA rupture by influencing aortic cytoskeletal-ECM structural assembly and integrity.

Saunders, Sandy **Poster Location: 22** **Poster Judging Number: 422**

Chronic stress in epilepsy induces robust increase in vagal reflex promoting SUDEP in a murine model

Sandy E. Saunders[1], Kaylie E. Dow[1], Grace E. Bostic[1], Misty M. Strain[2], Jamie L. Maguire[3], Carie R. Boychuk[1]

[1]Department of Biomedical Sciences, University of Missouri, Columbia, MO
[2]Department of Cellular and Integrative Physiology, University of Texas Health, San Antonio, TX
[3]Department of Neuroscience, Tufts University, Boston, MA

Objective: Sudden unexpected death in epilepsy (SUDEP) is the leading cause of death in patients with epilepsy and results from severe apneas and bradycardias that promote cardiorespiratory collapse. Considering a significant comorbidity with stress-related disorders and epilepsy, we hypothesized that stress exaggerates vagal reflexes that underlie severe bradycardias observed during SUDEP.

Methods: Experiments were performed using a novel mouse model where central corticotropin-releasing hormone neurons are hyperactive leading to a "stressed" phenotype. Seizures were induced in both control and "stressed" mice via microinjection of chemical convulsant, kainic acid, into the ventral hippocampus. Mice were chronically monitored with EEG and ECG using in vivo telemetry and/or underwent terminal testing of the Bezold Jarisch reflex.

Results: We found that 40% (8/20) of mice with hyperactive stress circuits spontaneously died 11.5 ± 6.6 days after seizure induction, with no deaths in epileptic controls (0/6). "Stressed" mice had a resting tachycardia (Δ57.5 ± 34.6 bpm; n=9) 14 days after seizure induction, compared to control epileptic mice (Δ19.5 ± 6.7 bpm; n=3; p=0.0286). During seizures, tachycardia consistently occurred in both "stressed" mice (Δ43.86 ± 30.61 bpm; n=6) and controls (Δ 78.66 ± 67.84 bpm; n=5; p=0.2859). Interestingly, steady state tachycardia was interrupted by transient (12.6 ± 6.29 sec) drops in heart rate that were more pronounced in "stressed" mice (Δ -179.1 ± 48.9 bpm; n=4) than controls (Δ -106.2 ± 42.0 bpm; n=5; p=0.0469). Finally, the magnitude of Bezold Jarisch reflex-induced bradycardia was significantly increased in "stressed" epileptic mice (Δ-410.1 ± 74.8 bpm; n=6) compared to control epileptic mice (Δ-172.7 ± 158.8 bpm; n=9; p=0.0080) 10 days after seizure induction.

Conclusion: Taken together, we found that epilepsy with hyperactive stress circuits, despite a resting tachycardia, promotes an exaggerated vagal reflex that is potentially activated during seizures to drive severe bradycardias resulting in SUDEP.

Funding sources: R01NS102937 NINDS to CRB/JLM

Foote, Christopher **Poster Location: 23**

Vascular remodeling and stiffening after spinal cord injury

Christopher A. Foote[1,3], Zhifeng Qi[1,3], Fateme Mericle[1,3], Francisco I. Ramirez-Perez[2], Luis Martinez-Lemus[2,4], Michael A. Hill[1,4], Shaoping Hou[1,3,5]

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

Cardiovascular dysfunction often occurs after traumatic spinal cord injuries (SCI). Patients with SCI have an approximately 4-fold increased risk of heart disease and stroke compared to able-bodied individuals. Few studies have addressed SCI-related arterial changes, including contributions from reduced vessel luminal diameter and increased arterial stiffness. However, the mechanisms underlying the development of these cardiovascular pathologies have yet to be defined. We recently reported that neuraminidase, an immune response protein important for leukocyte recruitment and adhesion, upregulates a disintegrin and metalloproteinase 17 (ADAM17) activity to mediate endothelial dysfunction by reducing nitric oxide bioavailability, leading to vascular stiffening. Herein, we hypothesize that elevation of neuraminidase activity as an inflammatory response to SCI initiates a serial signaling cascade to dampen endothelial function and cause arterial remodeling and stiffening. Adult female rats underwent a complete transection at the 4th thoracic (T4) spinal cord. Sham-injured rats, with laminectomy only, served as controls. Eight weeks later, in vivo ultrasonic imaging of aortic dynamics revealed that pulse wave velocity (PWV) was significantly reduced in SCI rats vs. the sham controls. In contrast, aortic radial strain and distension were decreased in the same animal cohort. After sacrifice, spinal cord tissue was collected for bioassays. The activity of the inflammatory mediator ADAM17, was significantly upregulated at the injury site. Using pressure myography, excised femoral and 3rd order mesenteric arteries were examined for arterial remodeling and stiffness. Notably, femoral and mesenteric arteries from SCI rats had significantly reduced luminal diameters, indicating inward arterial remodeling. Further, isolated mesenteric arteries displayed significantly larger incremental moduli of elasticity, indicative of pathological vascular stiffening. Together, these data demonstrate that both large and small arteries exhibit remodeling and stiffening in rats with SCI. The inflammatory response to the injury could potentially drive the pathological changes contributing to cardiovascular dysfunction that arises following SCI.

(Supported by: NIH/NINDS R01 NS121336, MU SCIDRP2024, and Mizzouforward Initiative)

Feeney, Grace **Poster Location: 24** **Poster Judging Number: 224**

Respiratory Responses Following H_2O_2 Injections Into the Nucleus Tractus Solitarii (nTS) of an Alzheimer's Disease (AD) Animal Model and Their Controls

Grace J. Feeney, Daniela Ostrowski, Tim D. Ostrowski

Besides memory loss, AD often displays respiratory dysfunction possibly due to oxidative damage in respiratory brain areas such as the nTS. We previously identified respiratory dysfunction and oxidative damage in the nTS of the streptozotocin (STZ)-induced AD model, leading to this study about the interplay between these findings.

AD was induced by injecting 6-week-old rats with 2.5 mg/kg of STZ into the fourth ventricle. Two weeks later, we implanted EMG electrodes into the diaphragm to assess respiratory responses to repeated glutamate (20 nL, 40 mM) injections into the caudal nTS before and after a single bolus of H_2O_2 (40 nL, 37.5 mM) into that same location.

While nTS glutamate injections increased respiratory frequency by 12-15 breaths/min in both CTL and STZ-AD, a single bolus of H_2O_2 or artificial CSF (aCSF) alone did not change respiration. However, following aCSF, CTL animals had an elevated peak response to glutamate, an effect that was absent following H_2O_2. Respiratory responses to glutamate in STZ-AD animals did not change following either treatment. Under stress conditions with 10 repetitive glutamate injections, respiratory responses of all experimental groups accommodated by ~40-60%. After aCSF, glutamate stress responses of CTL remained higher than those of STZ-AD. Following H_2O_2, response magnitudes of CTL were reduced to a level similar to STZ-AD.

The elevated respiratory response of CTL after aCSF may resemble a form of long-term facilitation with repeated and spaced glutamate injections. Inducing an oxidative environment eliminated this response elevation and CTL responses resembled those of STZ-AD. STZ-AD had no change in response following H_2O_2, which may reflect an already established oxidative environment from disease progression. Understanding respiratory changes under oxidative stress from STZ-AD may clarify mechanisms involved with respiratory dysfunction in AD patients.

NIH R15AG065927 (TDO & DO), KCOM Biomedical Graduate Program (GJF & TDO)

Qi, Zhifeng Poster Location: 25

Neuroendocrine regulation of autonomic dysreflexia following spinal cord injury

Zhifeng Qi, Fateme Mericle, Shaoping Hou

Dalton Cardiovascular Research Center, Department of Pathology and Anatomical Sciences, Department of Physical Medicine and Rehabilitation

Abstract:

Neural and hormonal regulation of cardiovascular function is out of balance following spinal cord injury (SCI). Among various hemodynamic pathologies, autonomic dysreflexia is a potentially life-threatening condition, which is characterized by an episode of sudden hypertension accompanied by baroreflex-mediated bradycardia under somatic or visceral stimulations. Though neural regulation of autonomic dysreflexia has been determined, detailed underpinnings of the development of autonomic dysreflexia are mostly unknown. Some previous investigations implicated the renin-angiotensin system (RAS) plays a role in the dysautonomia while controversies emerged in other animal and human studies. To address this discrepancy, adult female rats underwent a complete transection at the 4th thoracic (T4) spinal cord to interrupt supraspinal control. Four weeks post-SCI, an HD-S10 radio-telemetric transmitter was implanted in the abdominal cavity with a catheter sensor inserted into the descending aorta to measure blood pressure and heart rate. Using systemic pharmacological intervention, we assessed the extent of neural or RAS regulation of resting hemodynamics and colorectal distension (CRD)- induced autonomic dysreflexia. Intraperitoneal (*i.p.*) delivery of the ganglionic blocker Hexamethonium to inhibit neural control and the angiotensin-converting enzyme 1 (ACE1) inhibitor Captopril or the AT1R antagonist Losartan to suppress the ACE/Ang II/AT1R axis all significantly reduced mean arterial pressure (MAP). In contrast, injections of the MasR antagonist A779 or the ACE2 inhibitor MLN4760 to block the ACE2/Ang-(1-7)/MasR axis and the transient receptor potential vanilloid 1 (TRPV1) antagonist SB366791 to prevent sensory information of visceral pain did not change resting blood pressure and heart rate. Meanwhile, administration of Hexamethonium significantly attenuated CRD-induced hypertension and bradycardia, whereas drugs antagonizing the RAS pathway did not reduce the severity of dysreflexia. In conclusion, although neuroendocrine machinery modulates resting hemodynamics, autonomic dysreflexia is mediated by neural but not RAS regulation following SCI.

(Supported by: NIH/NINDS R01 NS121336, MU SCIDRP2024, and MizzouForward Initiative)

Mericle, Fateme **Poster Location: 26** **Poster Contest Number:426**

Inhibition of spinal D1-D2 heteromer formation suppresses bladder voiding reflex in rats with spinal cord injury

Fateme Khodadadi-Mericle, Ruweida Ben Musa, Zhifeng Qi, Shaoping Hou

Dalton Cardiovascular Research Center, Department of Pathology & Anatomical Sciences, Department of Physical Medicine and Rehabilitation School of Medicine.

Micturition dysfunction occurs after spinal cord injury (SCI). Though voluntary bladder activity is immediately ablated in response to the trauma, a segmental micturition reflex can gradually be established to facilitate involuntary voiding, which is driven by newly-formed spinal circuits due to intraspinal plasticity below the injury level. We previously disclosed that a spinal dopaminergic machinery emerges to regulate the partially recovered bladder function following SCI. Preliminary data suggests the possibility of the D1-D2 receptor heteromer being engaged in mediating the spontaneous urination. Herein, we tested this hypothesis in the micturition reflex. Adult female rats underwent a complete transection at the 10th thoracic (T10) spinal cord. After 3-4 weeks, anesthetized animals received bladder catheterization and electrode insertion into the external urethral sphincter (EUS). A lumbar laminectomy was performed, and an intrathecal (*i.t.*) catheter was inserted rostrally to the L6/S1 spinal level for drug delivery. Then, conscious rats were restrained for continuous bladder cystometrogram (CMG) and EUS electromyography (EMG) recordings. A serial of doses (4, 40, and 400 µg/kg, 10 µL) of the TAT-D1 peptide, an antagonist of D1-D2 heteromers, was i.t. administered. The dose of 400 µg/kg 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 (1 mg/kg,10 µL), a D1 receptor agonist, and Quinpirole (0.03 mg/kg,10 µL), a D2 receptor agonist, enhanced voiding volume (VV) and reducing VI, which suggested improved voiding capability. Subsequently, injection of TAT-D1 (400 µg/kg, 10 µL) eliminated these effects. The results identify the role of spinal D1-D2 heteromers in regulating the micturition reflex following SCI. Ultimately, activation of this receptor heteromer may serve as a therapeutic target for improving voiding following SCI.

(Supported by: NIH/NINDS R01 NS121336, MU SCIDRP2024, and MizzouForward Initiative)

Russell, Jacob **Poster Location: 27**

Role of cardiomyocyte-specific LARP6 overexpression on cardiac remodeling following permanent ligation of the left anterior descending coronary artery.

Jacob Russell, PhD, Tadashi Yoshida, PhD, Daniel Davis, PhD, Laurel A. Grisanti, PhD, Chastidy Bailey, MS, (Shawn B. Bender), PhD, Biomedical Sciences; (Bysani Chandrasekar), DVM, PhD, Medicine

Objective: Previous studies have shown that transgenic overexpression of the La ribonucleoprotein 6, translational regulator (LARP6) in cardiomyocytes reduces Angiotensin II-induced cardiac dysfunction and interstitial fibrosis. RNA sequencing revealed that LARP6 overexpression leads to widespread changes in the cardiac transcriptome. Ingenuity Pathway Analysis further identified altered gene networks linked to a predicted inhibition of cardiomyocyte apoptosis in LARP6-overexpressing (LARP6-OE) mice. However, the exact role of LARP6 in cardiomyocyte apoptosis remains unclear. To test the hypothesis that LARP6 overexpression protects against cardiomyocyte death, we used a model of cardiac injury, where the left anterior descending (LAD) coronary artery was permanently ligated in wild-type (WT) and LARP6-Tg littermate mice.

Methods: At 12 weeks of age, male and female WT and LARP6-OE mice were evaluated for baseline cardiac function via echocardiography. Following baseline assessment, the mice underwent anesthesia, intubation, and ventilation, followed by permanent LAD ligation. Post-surgery ultrasounds were performed on days 3, 7, 14, and 28. On day 28, the mice were euthanized, and their hearts were collected. The aorta was cannulated, and hearts were retrogradely perfused with phthalocyanine blue dye to determine the size of the occluded area. Viable myocardial tissue was stained with 2,3,5-triphenyltetrazolium chloride to measure infarct scar size.

Results: Echocardiography revealed no significant differences in baseline cardiac function/morphology between WT and LARP6-OE mice. Three days post-surgery, LAD ligation resulted in a significant reduction in systolic function in both genotypes. By day 7, LARP6-OE mice exhibited a significant increase in left ventricular mass (LVM) compared to both baseline and day 3 values, indicating enhanced hypertrophic remodeling. In contrast, the same degree of hypertrophy was not present in WT mice until day 14, suggesting an accelerated hypertrophic response in LARP6-OE mice.

Conclusion: LARP6 overexpression accelerated hypertrophic remodeling of the left ventricle in response to LAD ligation compared to WT littermates.

Foulks, Zachary **Poster Location: 28** **Poster Judging Number: 328**

Ketone Body Metabolism is not Required for Improvement of Heart Failure by Ketogenic Diet in Mice

Zachary Foulks, Carla J. Weinheimer, Attila Kovacs, Jessica Michael, Kelly D. Pyles, Thiago N. Menezes, Kevin Cho, Gary J. Patti, Kyle S. McCommis

Heart failure (HF) involves a host of metabolic alterations which are potential targets of nutritional or pharmacologic modulation. Prior studies have demonstrated a protective effect of ketone bodies in HF, potentially due to increased ketone body metabolism. However, when on a high-fat, low-carbohydrate ketogenic diet (KD) which greatly increases ketogenesis, some evidence also suggests that ketone metabolism is downregulated despite increased cardiac ketone delivery. Our objective was to determine whether ketone metabolism is necessary for improving HF with a KD. 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 before and after being randomized to 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, supplemented with gene expression and mitochondrial respiration studies. Our gene expression and isotopic tracer results confirmed that cardiac ketone metabolism decreased with KD feeding or BDH1 deletion, 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. Gene expression and mitochondrial respiration results further demonstrated that a KD enhanced fat oxidation instead. Therefore, while further experiments and current development of a cellular BDH1-/- model may reveal additional mechanisms, these findings suggest that ketone body metabolism does not significantly drive KD-mediated improvement of HF, and instead suggest that enhancement of fat oxidation is a significant component of this process.

Beltran-Ornelas, Jesus **Poster Location: 29** **Poster Judging Number: 429**

Sex-Specific Effects of Type 1 Diabetes on Arterial Vasodilation in Western Diet-Fed Mice

Jesus H. Beltran-Ornelas[1], Mariana Morales-Quinones[1], Natnicha Imkaew[1], Juan D. Gonzalez-Vallejo[1], Augustine L. Udefa[1,2], Francisco I. Ramirez-Perez[1], Jaume Padilla[1,2,4], Luis A. Martinez-Lemus[1,3,5], and Camila Manrique-Acevedo[1,4,6]

[1] NextGen Precision Health
[2] Department of Nutrition and Exercise Physiology
[3] Center for Precision Medicine, Department of Medicine
[4] Harry S. Truman Memorial Veterans' Hospital
[5] Department of Medical Pharmacology and Physiology
[6] Department of Medicine, Division of Endocrinology and Metabolism

Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of insulin-producing pancreatic beta cells. Despite advances in medical care and glycemic control, individuals with T1D still experience shorter lifespans compared to their peers without T1D, with an increased risk of cardiovascular disease (CVD). Consumption of a Western diet (WD) high in fat and sugars is highly prevalent and increases CVD risk, which may contribute to the high incidence of CVD in patients with T1D. We hypothesize that the combination of T1D and WD consumption has a more deleterious effect on vasomotor function and arterial stiffness than WD consumption alone, irrespective of sex. Eight-week-old wild-type (control) and Akita (genetic T1D model) male and female mice were fed a WD for 12 weeks to induce obesity. At the end of WD feeding, female Akitas were heavier, while male Akitas weighed less than their corresponding controls. Irrespective of sex, no significant differences were observed within genotypes in blood pressure (assessed via tail-cuff) or aortic pulse wave velocity. Vasodilatory responses to insulin, acetylcholine, and sodium nitroprusside (SNP) were assessed in the abdominal aorta and mesenteric arteries. Passive pressure-diameter curves were obtained to assess the mechanical properties of mesenteric arteries. Male Akitas exhibited impaired aortic and mesenteric artery vasodilatory responses to SNP (endothelial-independent) vs. controls. Endothelial-dependent vasodilation to insulin was also lower in Akita males compared to controls, only in the aorta. Mesenteric arteries but not aortas from female Akitas had a trend ($p=0.07$) towards a greater vasodilatory response to insulin compared with controls. Mechanical properties were not different between mesenteric arteries from Akitas and controls in either sex. These findings suggest that the combination of T1D and WD consumption has greater deleterious vascular effects in males than females. Further studies are needed to determine if these effects are related to insulinopenia.

Chynoweth, Bryn **Poster Location: 30** **Poster Judging Number: 230**

Role of SHP2 in cardiac muscle disease of Duchenne Muscular Dystrophy

Bryn Chynoweth,[1] Alphonsus Umeh-Ezenwoye, [2] Maike Krenz M.D. [1,2]
[1]Department of Medical Pharmacology & Physiology; [2]Dalton Cardiovascular Research Center
University of Missouri-Columbia

Duchenne Muscular Dystrophy (DMD) is a severe degenerative muscle disorder caused by deletion, duplication, or point mutation in the gene encoding dystrophin. As advancements in respiratory support have increased DMD patient lifespan, dilated cardiomyopathy has become a concerning source of morbidity and mortality in DMD, underscoring the need for further research in this area.

Pilot data from our lab has shown that Src homology region 2 domain-containing phosphatase-2 (SHP2) inhibition may have cardioprotective effects. SHP2 is a protein tyrosine phosphatase that dephosphorylates proteins involved in several cellular processes. We aim to investigate the beneficial effects of SHP2 inhibition through analysis of cardiac muscle function and histology in DMD mice.

To genetically reduce SHP2 activity, we generated mice that express dominant negative SHP2 (dnSHP2) in cardiac myocytes. For this project, dnSHP2 mice were crossed with DMD mice to determine the extent of the hypothesized protective effects.

To quantify fibrosis, hearts from 18+ months old mice were fixed with 4% paraformaldehyde in cardioplegic buffer and sections stained with Gomori trichrome. To compare ejection fraction, fractional shortening, and chamber dimensions between control (wildtype with/without dnSHP2) and experimental groups (DMD with/without dnSHP2), anesthetized 4–7-month-old mice underwent high-resolution echocardiography. After acclimatization, conscious echocardiography was performed on mice aged 22-24 months and the same measurements taken.

Data collected show a significant reduction in left ventricular fibrosis in dnSHP2 DMD mice. Additionally, dnSHP2 DMD mice exhibit marked improvements in ejection fraction and fractional shortening compared to DMD mice. These improvements were particularly pronounced in the aged cohort. The cardioprotective effects observed support additional investigation into SHP2 as a possible therapeutic target.

Revolutionizing Cardiology with Artificial Intelligence and Machine learning

Thomas Cartwright[1], Mohammad Alkhateeb[1]

1. Department of Medicine, University of Missouri Health Care, Columbia, Missouri, United States.

Abstract:

Background: Cardiovascular diseases are the leading cause of death worldwide, highlighting the need for innovative solutions to improve patient outcomes. Advances in technology have opened new possibilities for addressing these challenges. Among these, Artificial Intelligence (AI) and Machine Learning (ML) stand out as transformative tools with the potential to revolutionize cardiology and redefine approaches to patient care. This review explores AI and ML driven advancements in cardiology. **Methods:** A narrative review was conducted by identifying relevant studies through searches of peer-reviewed journals, review articles, and research databases using terms including "artificial intelligence", "machine learning", "cardiology", "cardiovascular", "diagnostic imaging", "electrocardiography". Studies were selected based on their clinical significance. **Results:** AI, ML and deep learning (DL) have shown unparalleled potential in medicine, particularly in cardiology. Predictive algorithms integrate clinical, imaging, and biomarker data to predict cardiovascular events such as myocardial infarction, stroke, and heart failure. In imaging, AI significantly reduces time and operator variability in image interpretation. Convolutional neural networks have automated tasks such as plaque characterization, ventricular function quantification, and myocardial tissue assessment, with diagnostic accuracy on par with expert clinicians. DL models use ECGs to detect arrhythmias and predict left ventricular dysfunction years before clinical manifestation. In electrophysiology, AI refines ablation targeting by mapping arrhythmogenic substrates with precision. Natural language processing (NLP) tools have also facilitated cardiology related research. Despite advancements, challenges such as data privacy, biases, interpretability issues, and dataset discrepancies hinder clinical adoption. Collaborative initiatives between clinicians, engineers, and policymakers are beginning to address these gaps. **Conclusion:** AI is poised to democratize cardiac care, offering high- quality diagnostics, as well as broadening access. Their integration demands multidisciplinary collaboration to address challenges to ensure successful implementation. With ongoing innovation and validation, these technologies can drive the field toward more predictive, preventive, and personalized care.

Garcia Delgado, Gabriela **Poster Location: 32** **Poster Judging Number: 332**

Clinical Hyperpolarized ^{129}Xe MRI for Assessment of Vascular Function in Pulmonary Hypertension Garcia Delgado, Gabriela M[1], Parks, Isabella[1], Shammi, Ummul Afia[2], Ruppel, Mia[1], Altes, Talissa[3], Thomen, Robert P[1, 3]

[1] Chemical and Biomedical Engineering, University of Missouri, Columbia, MO 65201
[2] Biomedical Engineering, School of Engineering, University of Illinois, Chicago, IL, USA 61801
[3] Radiology, School of Medicine, University of Missouri, Columbia, MO 65201

Introduction: Hyperpolarized Xenon MRI (HPX) of the lung is an imaging tool in which a patient inhales a bolus of xenon gas which is then directly imaged via MRI. The inhaled xenon can be separately imaged in the alveolar airspaces, lung parenchymal tissue (membrane) and red blood cells (RBCs) all within a single 10-15 second breath-hold. HPX is used to quantify the percentage of the lung with inadequate ventilation (common in obstructive disease), impaired gas transfer (common in fibrotic disease), and defective perfusion (common in pulmonary vascular disease). This information is invaluable to clinicians who desire quantitative physiologic markers of disease status, progression, and response to treatment. Pulmonary hypertension (PH) in particular is a disease in which HPX can help delineate disease etiology and improve patient management. Here we present HPX images and results in a healthy control subject, a pre-capillary PH patient, and a post-capillary PH patient.

Method: A healthy control patient (28/F) and two PH patients – one pre-capillary PH (63/M) and one post-capillary PH (52/F) – were imaged on a 3T Siemens Vida MRI scanner using a 3D-radial-DIXON sequence which allows for separate reconstruction of xenon in airspaces, lung membrane, and RBCs.

Results: Ventilation was normal in all patients, but gas-exchange was impaired in the PH cases: 5% of the lung volume had reduced RBC signal in the control compared with 39% and 31% in the pre- and post-capillary PH patients respectively. The post-capillary PH patient exhibited more extensive and complete defects concentrated in the lower lobes. In contrast, the pre-capillary PH patient showed less severe defects that were distributed across a larger lung volume.

Conclusion: Gas exchange can be utilized to evaluate pulmonary vascularity in PH and may aid in clinical management of these patients.

Ben Musa, Ruwaida **Poster Location: 33**

Repeated autonomic dysreflexia impairs renal function after spinal cord injury

Ruwaida Ben Musa[1], Olugbenga Michael[2], Zhifeng Qi[1], Fateme Khodadadi-Mericle[1], Adebowale Adebiyi[2], Shaoping Hou[1]

[1]Dalton Cardiovascular Research Center

[2]NextGen Precision Health

Cardiovascular dysfunction is a common complication in subjects with spinal cord injuries (SCI). Autonomic dysreflexia, a life-threatening condition characterized by sudden and severe hypertension often accompanied by bradycardia, occurs in response to somatic or visceral stimuli. Although it has been known that autonomic dysreflexia is harmful to multiple systems, its impact on renal function remains understudied. As the kidneys are particularly vulnerable to hypertension, reduced renal perfusion often leads to progressive renal dysfunction. Accordingly, we posit that repeated episodes of autonomic dysreflexia exacerbate renal dysfunction following SCI. To test this hypothesis, adult female rats underwent a complete transection at the 4th thoracic spinal cord level to interrupt supraspinal control. Two weeks post-SCI, colorectal distension (CRD) was performed twice daily to artificially induce autonomic dysreflexia for three weeks (n = 6), while SCI rats that did not receive CRD and sham-injured rats (laminectomy only) served as 2 controls (n = 6/7). Subsequently, noninvasive and transcutaneous measurement of glomerular filtration rate (GFR) was conducted by injecting fluorescein isothiocyanate (FITC)-sinistrin, as a biomarker, into the tail vein. Blood and urine samples were collected for bioassays to examine kidney injury. Furthermore, renal blood flow (RBF), renal vascular resistance (RVR), and mean arterial pressure (MAP) were recorded in anesthetized rats. After sacrifice, the kidneys were harvested for histological analysis, including H&E staining to evaluate morphological changes and Masson trichrome staining to assess collagen deposition. Compared to the sham group, GFR was significantly reduced in SCI rats exposed to CRD (one-way ANOVA, p = 0.025) but not in SCI rats that did not receive CRD (p = 0.19). In addition, SCI rats treated with CRD exhibited increased RVR (p = 0.026) compared to the sham group. Bioassays and histological assessments are ongoing. Collectively, the results indicate that repeated exposure to autonomic dysreflexia impairs renal function after SCI.

(Supported by: NIH/NINDS R01 NS121336, MU SCIDRP2024, and Mizzouforward Initiative)

Ramirez-Perez, Francisco **Poster Location: 34**

Neuraminidase-3 Inhibition Mitigates Vascular Endothelial Dysfunction and Reduces Arterial Stiffness in Type 2 Diabetic Mice

Francisco I. Ramirez-Perez[1], Christopher A. Foote[1,2], Marc A. Augenreich[1,3], Gavin Power[1,3], Larissa Ferreira-Santos[1], Mariana Morales-Quinones[1], Edgar E. Betancourt- Cortes[1], Thomas J. Jurrisen[1], Juan D. Gonzalez-Vallejo[1], Natnicha Imkaew[1], Camila Manrique-Acevedo[1,4,5], Jaume Padilla[1,3,4], Luis A. Martinez-Lemus[1,2,6]

[1]NextGen Precision Health, [2]Dept. of Medical Pharmacology and Physiology, [3]Dept. of Nutrition and Exercise Physiology, [4]Harry S. Truman Memorial Veterans' Hospital, [5]Div. of Endocrinology and Metabolism, Dept. of Medicine, [6]Center for Precision Medicine, Dept. of Medicine

Endothelial dysfunction and arterial stiffening are key features of type 2 diabetes (T2D) that promote cardiovascular disease. Elevated plasma neuraminidase (Neu) activity in diabetes degrades the endothelial mechanosensitive glycocalyx by removing sialic acids from glycoproteins and glycolipids. This disrupts shear stress signaling and impairs arterial flow-mediated dilation (FMD). We previously demonstrated that inhalation of the Neu inhibitor Zanamivir (10 mg-BID for 5 days) improves endothelial function in diabetic (db/db) mice, without affecting arterial stiffness. As Zanamivir has a moderate IC_{50} (6.8±3.1 μM) against Neu3, the major Neu isoform in plasma, we hypothesized that changing the route of administration and increasing the duration of treatment would further improve endothelial function and reduce arterial stiffness in diabetic mice. We further hypothesized that the novel Neu3-specific inhibitor C22601 (Neu3i, 100 μM) would also ameliorate endothelial dysfunction and reduce arterial stiffness in diabetic mice via endothelial glycocalyx restoration. We tested our hypotheses using human umbilical vein endothelial cells (HUVECs), isolated mesenteric arteries from 12-15-week-old male C57BL/6, and db/db mice. All differences reported herein are significant at $P < 0.05$. We found that subcutaneous Zanamivir infusion (671 μg/kg/day for 28 days) reduced pulse wave velocity (PWV), increased glycocalyx length, decreased arterial stiffness, and improved FMD without altering sodium nitroprusside (SNP)-induced vasodilation. Similarly, Neu3i overnight exposure improved glycocalyx integrity in HUVECs and enhanced FMD in isolated arteries. In db/db mice, Neu3i subcutaneous infusion (692 μg/kg/day for 28 days) reduced PWV, increased glycocalyx length, reduced arterial stiffness, and improved FMD without affecting SNP responses. These results indicate that Neu inhibition with Zanamivir or the novel Neu3i, C22601, improves glycocalyx structures and ameliorates endothelial dysfunction and arterial stiffening in diabetic mice, providing a potential therapeutic strategy for mitigating cardiovascular disease in individuals with T2D.

LM-L and JP are supported by the National Institutes of Health grant: R01HL153264. FIR-P and LF-S are supported by the Research Excellence Program at the University of Missouri.

Elahi, Fazle **Poster Location: 35** **Poster Judging Number: 435**

Intermittent hypoxia associated with obstructive sleep apnea disrupts microvasculature networks in the mouse retina

[1]Fazle Elahi, [1,2]Mohammad Badran, [1]Charles E. Norton

[1]Deparment of Medical Pharmacology and Physiology,
University of Missouri, Columbia, MO 65212

[2]Department of Child Health and Child Health Research Institute, School of Medicine,
University of Missouri, Columbia, MO 65212

Obstructive sleep apnea (OSA) is prevalent in obese individuals and linked to retinopathy. Intermittent hypoxia (IH), associated with OSA, and metabolic disorders, elevate levels of reactive oxygen species (ROS) which mediate retinal damage. While diet-induced eye dysfunction is well studied (i.e. diabetic retinopathy), effects of IH, and interactions between diet and IH, are less understood. We hypothesized that smooth muscle cells (SMCs) and endothelial cells (ECs) of the retinal microvascular network are modified by IH to limit the area of vascular perfusion. To test how diet and IH contribute to microvascular network remodeling, male mice were fed a high fat diet (HFD) or control diet (CD) for 26 weeks and exposed to IH (90 s cycles 21% F_{iO2}-6% F_{iO2} for 12 hr/day) or sham for the final 16 weeks of feeding. ROS production in the retina was evaluated using (DHE,10 µg/mL). Retinal ROS levels were significantly greater ($P<0.05$, n=5) following both HFD and IH individually, with no additive effect when combined. Vascular network density of SMCs (anti-SMA actin antibody; 1:500) and ECs (lectin; 1:200) was determined by evaluating the area (%) of each cell type's coverage in images from fixed retinas (4 regions/eye). Arterial diameter was quantified in arteries originating from the optic disc. Surprisingly, HFD had no significant effect on EC or SMC density, whereas IH reduced in EC and SMC coverage of both CD and HFD mice ($P<0.05$, n=5). Consistently, IH decreased arterial diameter in retinas from mice fed either diet, while HFD alone had no effect ($P<0.05$, n=5). We conclude that while both IH and HFD augment ROS production in the retina, IH has a far greater contribution to microvascular network disruption which may impair tissue perfusion and provide unique insight into the role of IH in vascular dysfunction associated with OSA-induced retinopathy.

Hairston, Amaris **Poster Location: 36** **Poster Judging Number: 336**

Title: Cerebrovascular reactivity to hypoxia and hypercapnia: role of β-adrenergic receptors

Authors: Amaris Hairston, Brian Shariffi, Megh Patel, Dain W. Jacob, Anna M. Gonsalves, Jennifer L. Harper, Brian P. Bostick, Jacqueline K. Limberg

Background: β-adrenergic receptor (β-AR) antagonists are prescribed to patients experiencing migraines. Although the mechanisms behind the efficacy of their treatment remain unclear, data suggest β-AR antagonists may exert a vascular action within the cerebral circulation. We assessed resting cerebral perfusion as well as cerebrovascular reactivity to a hypoxic and a hypercapnic stress with and without β-AR antagonism via oral propranolol. We hypothesized cerebrovascular reactivity would be reduced after propranolol administration compared to placebo.

Methods: Middle cerebral artery velocity (MCAv, transcranial Doppler ultrasound) was assessed in 21 healthy young adults (12M/9F, 27.1±7.8 yrs; 24.3±2.6 kg/m2). Individuals completed two study visits randomized and blinded to oral placebo or propranolol (1 mg/kg). At each visit, participants completed a quiet resting period followed by two study conditions separated by a 10-min washout: 1) 5-min steady-state hypoxia (10.7±0.6 FiO_2, 81.7±4.3% SpO_2), 2) 5-min hyperoxic hypercapnia (+7.9±2.4 mmHg partial pressure of end-tidal CO_2).

Results: MCAv increased in response to both steady-state hypoxia (main effect of hypoxia, $p=0.0005$) and hypercapnia (main effect of hypercapnia, $p<0.0001$). The MCAv response to hypoxia was augmented with propranolol compared to placebo (main effect of propranolol, $p=0.0279$; interaction of hypoxia and propranolol, $p=0.0371$). In contrast, the MCAv response to hypercapnia was unaffected by propranolol (main effect of propranolol, $p=0.4362$; interaction of hypercapnia and propranolol, $p=0.7949$).

Conclusion: Reductions in oxygen delivery (hypoxia) or retention of metabolic byproducts like carbon dioxide (hypercapnia) both elicit increases in cerebral perfusion in an attempt to restore homeostasis. Contrary to our hypothesis, the non-specific β-AR antagonist propranolol had no effect on hypercapnic reactivity and resulted in a paradoxical increase in the cerebrovascular response to hypoxic stress. These findings support a role for the β-AR in restraining cerebral blood flow during hypoxia, but not hypercapnia.

McDonald, Matthew **Poster Location: 37** **Poster Judging Number: 337**

Title: Impact of Resveratrol on Neurovascular Coupling Following Acute Sleep Restriction in Young Men and Women

Authors: Matthew J. McDonald, Sharon D. Fears, Sam A. Martin, Todd Lancaster, Brian Shariffi, Jacqueline K. Limberg, Jill A. Kanaley

Affiliations: Department of Nutrition and Exercise Physiology, University of Missouri, Columbia, MO.

Background: Shortened sleep is associated with increased risk of Alzheimer's disease and dementia. Data from our group show acute sleep restriction (4 hours-time in bed) impairs the link between neural activity and cerebral blood flow. Insufficient sleep increases reactive oxygen species production and reduces nitric oxide bioavailability within the cerebral vasculature. Resveratrol, a polyphenol with known antioxidant properties, reduces oxidative stress and improves vascular function. The purpose of this study was to examine the effect of resveratrol on NVC following acute sleep restriction. We hypothesized NVC following acute sleep restriction would be improved with acute oral resveratrol supplementation.

Methods: Thirteen adults (7M/6F, 28±7 y, 25±4 kg/m^2) completed two visits following a night of normal (431±57 min) or restricted sleep (240±13 min). Posterior cerebral artery velocity (PCAv, transcranial Doppler ultrasound) was measured at rest and during five trials consisting of a period of eyes closed (30-sec), followed by eyes open (40-sec) while completing a visual search paradigm. Trials were completed prior to and 45 min following oral resveratrol administration (250mg). NVC was assessed as the peak change in PCAv from baseline during the first 30-sec of visual stimulation.

Results: Peak change in PCAv during the visual stimulus was reduced following sleep restriction (normal sleep: 12±4 cm/s; restricted sleep: 9±4 cm/s; p=0.03). The PCAv peak response to visual stimulation was unaffected by resveratrol under normal sleep conditions (control: 12±4 cm/s; resveratrol: 10±4 cm/s; p=0.26), while the PCAv peak response to visual stimulation increased following resveratrol supplementation on the restricted sleep visit (control: 9±4 cm/s; resveratrol: 11±5 cm/s; p=0.01),

Conclusion: These data demonstrate that one night of restricted sleep impairs NVC of the PCA during a visual stimulus test which can be restored with acute oral resveratrol supplementation.

Gama de Barcellos Filho, Procopio **Poster Location: 38**

Corticotropin-releasing hormone (CRH) effects on the properties of GABAergic and non-GABAergic neurons within the nucleus tractus solitarii (nTS).

Procopio G. de Barcellos-Filho, Heather A. Dantzler, David D. Kline.
Department of Biomedical Sciences and Dalton Cardiovascular Research Center, University of Missouri, Columbia, USA.

Peripheral cardiorespiratory reflexes in response to various stressors are processed and integrated in the nucleus tractus solitarii (nTS). Additionally, the paraventricular nucleus of the hypothalamus (PVN) significantly contributes to the regulation of cardiorespiratory function, partly through its nTS projections that contain CRH. Within the nTS, we have demonstrated that CRH enhances overall nTS activity and affects the cardiorespiratory response to hypoxia. However, the effect of CRH on the specific phenotypes of neurons within the nTS remains unclear. We hypothesized the overall excitatory influence of CRH on nTS function is due to its excitation of non-GABAergic (glutamatergic) and/or inhibition of GABAergic nTS neurons. Male and female transgenic GAD1-EGFP mice (4-5 weeks) were used to identify GABAergic and non-GABAergic neurons. The influence of CRH on synaptic neurotransmission to monosynaptic GABAergic and non-GABAergic nTS neurons and the expression of CRH receptors was determined. Synaptic events were examined during exogenous CRH (300nM) and/or CRH receptor block (CRHR-x, Astressin 100nM). In the presence of CRH, network-driven spontaneous (s) EPSC amplitude decreased in GABAergic neurons yet not in non-GABAergic neurons. sEPSC frequency was not altered in either group. CRH also attenuated the amplitude of afferent (TS)-evoked EPSCs in GABAergic neurons yet enhanced the amplitude in non-GABAergic neurons. CRHR-x prior to application of CRH ablated the synaptic responses in both phenotypes. GABA neurons were tonically influenced by CRH, as CRHR-x increased TS-ESPC amplitude. CRH-positive fibers were adjacent to GABA and non-GABAergic nTS neurons (immunohistochemistry). While the total expression of CRHR2 mRNA was more prevalent than CRHR1 within the entire nTS, CRHR1 expression primarily occurred in GABA neurons within the intermediate nTS compared to CRHR2 (RNAScope). Altogether, our data shows that the influence on nTS activity depends on neuron phenotype. CRH projections from PVN to nTS likely increase excitation and reduce inhibition in nTS.

Funding: HL166183

Wright, Hayden **Poster Location: 39** **Poster Judging Number: 439**

The effect of sustained hypoxia on GABAergic and non-GABAergic neurons of the nucleus tractus solitarii (nTS)

Hayden Wright[1,2], Victoria Felton[1], Ethan Pull[1], Heather Dantzler[1,2], David D Kline[1,2]

[1]Dalton Cardiovascular Research Center, University of Missouri, [2]Department of Biomedical Sciences, University of Missouri

Exposure to hypoxia occurs during physiological (e.g., high altitudes) and pathophysiological conditions (e.g., obstructive pulmonary disease). The nucleus tractus solitarii (nTS) integrates sensory input from peripheral chemoreceptors and maintains cardiorespiratory homeostasis. nTS activity is determined through balanced glutamate (Glu) and gamma-aminobutyric acid (GABA) signaling, which is critical for generating proper reflexes in response to hypoxia. This study investigated how sustained hypoxia (SH) affects GABAergic neurons in the nTS, hypothesizing that reduced GABAergic inhibition increases nTS excitability and cardiorespiratory function following hypoxia.

Using male and female GAD1-EGFP mice to distinguish GABA (GAD+) and presumptive Glu (GAD-) neurons, we exposed subjects to sustained hypoxia (10% O2, 24 hours) or normoxia (21% O2, 24 hours) before experimentation. Plethysmography revealed increased tidal volume and minute ventilation during an acute hypoxia challenge (15% O2) following SH exposure. Immunohistochemistry showed SH increased c-Fos expression in GAD- neurons. Ratiometric Ca2 + imaging in isolated nTS neurons demonstrated that while GAD+ neurons maintained consistent intracellular Ca2+ responses to repeated high K+ challenges regardless of oxygen conditions, GAD- neurons from SH-exposed mice maintained Ca2+ elevation, unlike their normoxic counterparts.

Lastly, horizontal brainstem slices were generated from normoxic or SH exposed mice, and using whole-cell patch-clamp recordings, we examined synaptic neurotransmission and electrophysiological properties of GAD+ and GAD- nTS neurons. Neurons were further characterized by monosynaptic or polysynaptic connection with the tractus solitarii (TS) via TS stimulation. Under normoxic conditions, monosynaptic GAD+ neurons exhibited greater spontaneous excitatory post-synaptic current (sEPSC) amplitude than GAD- neurons. However, SH exposure increased sEPSC amplitude in monosynaptic GAD- neurons and enhanced sEPSC frequency in polysynaptic GAD+ neurons. Additionally, SH increased the paired-pulse ratio in GAD + polysynaptic neurons. Taken together, these findings suggest that SH enhances non-GABAergic nTS neuron activity via alterations in GABA influence on local NTS circuitry.

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

Chi, Jingshu **Poster Location: 40** **Poster Judging Number: 340**

Chronic Helicobacter pylori Infection Impairs Endothelial Function and Exacerbates Atherosclerosis Selectively in Male Mice

Jingshu Chi[1], Linfang Zhang[1], Xiujuan Xia[1], Feng Chen[1], Caiping Zhang[1], Aiping Lin[1], Qin Zhou[1], Hong Hao[1], Xunlei Kang[1], De-Pei Li[1], Luis A. Martinez-Lemus[1], Michael A. Hill[2], Canxia Xu[3], and Zhenguo Liu[1]

[1]Center for Precision Medicine and Division of Cardiovascular Medicine, Department of Medicine, University of Missouri School of Medicine

[2]Dalton Cardiovascular Research Center, University of Missouri

[3]Department of Gastroenterology, the Third Xiangya Hospital, Central South University, Changsha, China

Background: Helicobacter pylori (*H. pylori*) infection increases the risk of cardiovascular diseases including atherosclerosis. Endothelial dysfunction is closely associated with atherosclerosis. This study aimed to test the **hypothesis** that chronic *H. pylori* infection attenuates endothelial function and promotes atherosclerosis selectively in males.

Methods: Male and female wildtype C57BL/6 mice and LDL receptor-deficient ($LDL^{-/-}$) mice (4 to 6 weeks old) were infected with *H. pylori*. Mice were fed a regular diet and were euthanized at either 12 or 15 months after *H. pylori* infection. Aortas were collected from C57BL/6 mice to evaluate endothelial function, and aortas from $LDL^{-/-}$ mice were evaluated for atherosclerotic burden. Blood pressure, blood glucose and lipid profiles were obtained and analyzed. The presence of *H. pylori* infection was assessed at the end of the experiment using Rapid Urease Test and Giemsa staining of gastric mucosa.

Results: Over 80% of mice remained infected with *H. pylori* for up to 15 months. The aortas from male, not female, mice with either 12 or 15 months of *H. pylori* infection exhibited a significant reduction in acetylcholine-induced endothelium-dependent relaxation, while nitroglycerine-induced endothelium-independent relaxation remained intact along with increased blood pressure. Blood glucose in male, not female, wildtype mice with *H. pylori* infection for 15 months were also elevated. Additionally, aortic atherosclerotic burden was significantly increased in male, not female, $LDL^{-/-}$ mice with chronic *H. pylori* infection. Similarly, blood glucose levels, non-HDL cholesterol, total cholesterol, and LDL cholesterol were all significantly increased in $LDL^{-/-}$ male, not female, mice with *H. pylori* infection.

Conclusion: Chronic *H. pylori* infection induces significant metabolic abnormalities with increased blood glucose and lipid levels, significantly impairs endothelial function, and promotes atherosclerosis selectively in males. These findings suggest that chronic *H. pylori infection* could significantly contribute to endothelial dysfunction and cardiovascular diseases such as atherosclerosis in males, not females.

Amarante, Marlusa **Poster Location: 41**

Swallow produces autonomic changes in a modified artificially perfused rat preparation

Marlusa Karlen-Amarante[1,2], Kimberly Iceman[1,2], Clinton Greene[1,3], Teresa Pitts[1,2]
[1]Dalton Cardiovascular Research Center, University of Missouri,
[2]Department of Speech, Language, and Hearing Sciences, University of Missouri,
[3]Veterinary Medicine Surgery, University of Missouri

Text: The autonomic nervous system is composed of the sympathetic and parasympathetic nervous systems which are important for cardiovascular, respiratory and ingestive coordination. The vagus nerve (X) is the main nerve carrying parasympathetic outflow affecting digestion, heart rate (HR), and the immune system; the vagus is also a key pathway for the entire swallow reflex. The maintenance of arterial pressure at adequate levels requires tonic sympathetic drive to control the contraction and the caliber of blood vessels, however the participation of the sympathetic drive during swallow behavior and any role it might play in swallow dysfunction have not been the subject of many studies. In this study, we are characterizing the contribution of the sympathetic nerve and any corresponding parasympathetic activity in rodents to assess its coordination with swallow and breathing, and the possible changes in heart rate and blood pressure. To achieve this aim, we utilized a modified non-paralyzed *working heart-brainstem preparation* of rats (P23-P28). Rather than transecting the animal, the spinal cord was left intact to obtain electrophysiological recordings of muscle activity from the diaphragm, submental and laryngeal complexes. Swallow was stimulated by infusing 0.1mL of water into the mouth before and after application of drugs. We observed a correlation between an increase of perfusion pressure during swallow stimulation and a decrease of heart rate. Under atenolol, a β-adrenergic blocker, spontaneous and evoked swallows still occurred but with no changes in heart rate. On the other hand, scopolamine, a nonselective muscarinic acetylcholine antagonist, prevented any changes in heart rate and perfusion pressure during swallow. We suggest that swallow triggers an excitatory vasomotor sympathetic pathway which is involved in the control of upper airway motor activity.

Funding: NIH Grants: NS110169, HD110951

Alam, Perwez **Poster Location: 42**

Title: Partial Reprogramming of Adult Cardiomyocytes Enhances Cardioprotective Mechanisms in Adult Heart.

Authors: Perwez Alam*, Hunter J Bowles, Hieu Bui, Douglas K Bowles

Abstract:

Heart disease is the leading cause of death in the U.S., affecting over 28 million adults, with nearly 6.7 million living with heart failure. Traditional strategies to regenerate functional cardiomyocytes (CMs) have been insufficient. Recent research suggests partial reprogramming of adult CMs as a promising strategy to protect the failing heart by secreting rejuvenation-associated paracrine factors that improve the heart's microenvironment and enhance cardioprotection. We previously showed that inhibiting senescence-related genes Rb1 and Meis2 promotes cardiac rejuvenation, improving myocardial infarction (MI) outcomes by enhancing vascular density, reducing fibrosis, and increasing CM survival. Additionally, transcriptomic analysis identified MCM2 as a key effector, with its overexpression improving cardiac function and angiogenesis post-MI. However, the mechanisms behind MCM2-mediated reprogramming remain unexplored.

To investigate this, we performed comprehensive omics analysis to identify CM-specific mechanisms responsible for *MCM2*-mediated cardioprotection. We overexpressed *MCM2* in adult CMs in vivo using an adeno-associated viral (AAV9-cTnT-*MCM2*) vector. One month after injection, CMs were isolated and cultured for 48 hours. Cultured CMs and extracellular vesicles (EVs) from conditioned media were analyzed through transcriptomic and proteomic profiling, respectively. Data were analyzed using Ingenuity Pathway Analysis (IPA). Transcriptomic analysis revealed a set of differentially expressed genes in MCM2-overexpressing CMs compared to controls. When these genes were compared to the expression profile in MI hearts (public data), a significant reversal in gene expression was observed. This suggests that MCM2-overexpression in CMs modulates gene expression to counteract the disease state, offering potential protection against myocardial injury. Furthermore, *MCM2*-overexpression modulated key pathways involved in fibrosis, CM survival, angiogenesis, immune modulation, and hypoxia response, confirmed by EV proteomics. These findings suggest that *MCM2*-overexpression activates reprogramming associated pathways in adult CM and induces protective paracrine signaling, which may play a role in cardiac remodeling, angiogenesis, and immune modulation, ultimately contributing to cardioprotection after myocardial injury.

Shariffi, Brian **Poster Location: 43**

Title: Intranasal insulin increases cerebral vascular reactivity to carbon dioxide in young adults with obesity: a magnetic resonance imaging study

Authors: Brian Shariffi[1], Samuel A. Martin[1], Natasha G. Boyes[1], Xiaoxi Chen[2,3], Mai-Lan Ho[2], Camila Manrique-Acevedo[4,5,6], Jaume Padilla[1,4,6], Jacqueline K. Limberg[1,7]

Affiliations: [1]Department of Nutrition and Exercise Physiology, University of Missouri, Columbia, MO; [2]Department of Radiology, University of Missouri, Columbia, MO, USA; [3]Department of Radiology, Hospital of Zunyi Medical University, China; [4]NextGen Precision Missouri, Columbia, MO; [5]Division of Endocrinology and Metabolism, Department of Medicine, University of Missouri, Columbia, MO; [6]Harry S. Truman Memorial Veterans' Hospital, Columbia, MO; [7]Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO.

Objective: Obesity increases cerebrovascular disease risk, partly due to impaired cerebrovascular reactivity (CVR). Preclinical studies show reduced brain insulin levels in obesity, linking impaired CVR and insulin signaling. Notably, insulin is vasoactive and may be required for optimal cerebrovascular function. Emerging data support the use of intranasal insulin to increase brain insulin levels. Herein, we tested the hypothesis that intranasal insulin would enhance CVR to carbon dioxide (CO_2) in both adults with normal weight and obesity.

Methods: Twenty young adults [normal weight: n=10, 25±8 yrs, 23±1 kg/m^2; obesity: n=10, 30 ±7 yrs, 34±6 kg/m^2] underwent MRI (3 Tesla) with pseudo-continuous arterial spin labeling to quantify regional cerebral blood flow. Measurements were taken during a 5-min baseline and 5-min of hypercapnia (+7.5±3.4mmHg). Assessments were conducted before and 20-min after insulin administration (160IU). Region of interest analysis focused on the parietal, occipital, temporal, and insular lobes. CVR was assessed as a relative (%) change from baseline with CO_2. The effect of insulin on CVR was compared between groups (2x2 mixed methods ANOVA, Bonferroni pairwise multiple comparisons).

Results: Insulin had no effect on CVR in the temporal (p=0.163) or insular (p=0.348) regions in either group. In the parietal and occipital regions, a group-specific effect of insulin on CVR was observed (p=0.018 and p=0.002 respectively). *Post hoc* comparisons showed no effect of insulin on CVR in the parietal (p=0.363) and occipital (p=0.137) regions of normal weight adults, yet an insulin-mediated increase in adults with obesity (p=0.013 and p=0.002, respectively).

Conclusions: Intranasal insulin had no effect on CVR to CO_2 in normal weight adults but was enhanced in select brain regions of adults with obesity. The effect of insulin on CVR may reflect improved endothelial function and neurovascular coupling in obesity and will be the focus of future investigations to understand how intranasal insulin impacts cerebrovascular health.

Funding: UL1TR002345 (JKL)

Patro, Advaya **Poster Location: 44** **Poster Judging Number: 144**

Title: Mouse Lymphatic Muscle Cells Exhibit Functional Store-Operated Ca^{2+} Entry Mediated by Stim1 Protein

Authors: Advaya Patro, Sarah Broyhill, Grace Pea, Soumiya Pal, Karen Bromert, Scott Zawieja

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

Funding: MizzouForward URTG, R01HL175083 to SDZ

Lymphatic muscle cells (LMCs) are innate pacemakers which rely on oscillatory IP3R1-dependent Ca^{2+} release from the sarcoendoplasmic reticulum (SR) to regulate the frequency of lymphatic collecting vessel contractions. However, the mechanisms that sustain SR Ca^{2+} homeostasis to permit these SR Ca^{2+} oscillations in LMCs remains undefined. In non-excitable cells, SR Ca^{2+} depletion is coupled to calcium entry through a process termed store-operated Ca^{2+} entry (SOCE) where a reduction in SR Ca^{2+} induces oligomerization of the SR Ca^{2+} sensor Stim1/2. Stim1/2 then translocate to PM-SR junctions and activates the homo- and heterohexameric Orai Ca^{2+} channels and Ca^{2+} influx. We recently reported LMC expression of Stim1, Stim2, Orai1, and Orai3 using scRNASeq. Critically, we demonstrated functional SOCE activity in LMCs in response to store depletion is predominantly mediated by Orai1 channels. The aim of this study is to test the hypothesis that Stim1 is the primary SR sensor contributing to SOCE in LMCs and to test its role in regulating lymphatic collecting vessel contractility. To test this hypothesis, we performed isolated vessel isobaric myography using mouse inguinal-axillary lymphatic vessels (MIALVs) from *Stim1*$^{fl/fl}$ control mice, and *Stim1*$^{fl/fl}$ mice crossed to the inducible smooth muscle targeted Myh11CreER^{T2} to achieve *Stim1*^{ismKO}. MIALVs were challenged with a pressure step protocol, the prototypical Ca^{2+} depletion add back protocol, or treated with ryanodine to simulate elevated Ca^{2+} SR leak and depletion.

Contractile responses from MIALVs from both *Stim1*$^{fl/fl}$ mice and *Stim1*^{ismKO} mice exhibited spontaneous contractions at all pressures, with differences in contractile parameters being further assessed. Control MIALVs developed a strong and sustained constriction in response to the Ca^{2+} add back protocol, although this constriction was either transient or absent in *Stim1*^{ismKO} MIALVs. Similarly, control MIALVs developed paralytic rigor in response to 10μM ryanodine mediated SR Ca^{2+} leak, but MIALVs from *Stim1*^{ismKO} mice were protected from rigor and maintained phasic contractions. Interestingly, these protected responses varied among *Stim1*^{ismKO} mice, with multiple distinct patterns being observed. Our results suggest that LMCs display functional SOCE which is primarily mediated by Stim1, but that Stim2 may also be able to partially activate Orai1 channels. This challenges the belief that voltage-gated L-type Ca^{2+} channels are the only Ca^{2+} influx mechanism in LMCs.

Power, Gavin **Poster Location: 45** **Poster Judging Number: 345**

ADAM17 cleaves the hyaluronan receptor CD44 from the endothelial surface, leading to impaired mechanotransduction of shear stress

Gavin Power, Min Jeong Cho, Marc A. Augenreich, Natnicha Imkaew, Francisco I. Ramirez-Perez, Larissa Ferreira-Santos, Camila Manrique-Acevedo, Luis A. Martinez-Lemus, and Jaume Padilla.

Hyaluronan is a primary component of the endothelial glycocalyx and participates in mechanotransduction of shear stress, mediating vasodilation. However, extracellular hyaluronan interacts indirectly with endothelial cells via its cell surface receptor CD44, which has recently been demonstrated to be mechanosensitive. CD44 is also a substrate for *A Disintegrin and Metalloprotease-17* (ADAM17), a sheddase that is elevated in type 2 diabetes (T2D). Of note, T2D is characterized by reduced flow-mediated dilation (FMD), indicative of impaired shear stress mechanotransduction. Thus, we hypothesized that increased endothelial ADAM17 activity causes cleavage of CD44, impairing shear stress mechanotransduction. In support of this hypothesis, we show that ADAM17 overexpression in endothelial cells sheds CD44 from the cell surface and reduces the cellular responses to flow-induced shear stress. We also demonstrate the specificity of ADAM17 for CD44 using a cell-free assay in which co-incubation of recombinant human active ADAM17 (rh-ADAM17) with recombinant human CD44 (rh-CD44) results in the generation of cleaved fragments of CD44. This cleavage is prevented in the presence of TAPI-0, an ADAM17 inhibitor. Furthermore, using phorbol 12-myristate 13-acetate (PMA), activation of ADAM17 in endothelial cells cleaves CD44 and impairs mechanotransduction of shear stress. Notably, these effects of PMA are prevented by TAPI-0. To further establish the consequences of ADAM17-mediated cleavage of CD44,and likely other mechanosensors, we show that endothelial exposure to rh-ADAM17 reduces FMD in mouse isolated mesenteric arteries. Taken together, these data suggest that ADAM17-mediated cleavage of CD44 may represent a novel mechanism contributing to endothelial dysfunction in T2D.

Funding: R01HL151384, R01HL153264, and AHA23PRE1020897.

Fleury, Ava **Poster Location: 46** **Poster Judging Number: 146**

Title: Pro-arrhythmic atrial calcium handling and atrial arrhythmogenesis in hearts of GCaMP6f transgenic mice

Authors: Ava C. Fleury, Jessica Cayton, Matthew Hayden, Jorge A. Castorena-Gonzalez, and Timothy L. Domeier

Atrial tachyarrhythmias, including ectopic atrial tachycardia, atrial fibrillation (AF), and atrial flutter (AFL), affect 10.5 million adults in the United States. A recent study found that atrial cardiomyocytes of patients with AF had reduced levels of the dystrophin protein compared to those without AF. Prior ECG data also indicate the presence of AF/AFL in dystrophic $Dmd^{mdx\text{-}4Cv}$ mice following acute preload and hypokalemia challenge. Therefore, this study tested the hypothesis that calcium transients are altered in atrial cells of dystrophic mouse hearts following acute hypokalemia and preload challenge. Hearts were isolated from mid- aged (12-13 month) normal (n=3 males, n=4 females) or dystrophic mice (n=5 male $Dmd^{mdx\text{-}4Cv}$) with cardiomyocyte-specific transgenic expression of a GCaMP6f calcium indicator. Isolated hearts were cannulated via the aorta and superior vena cava and perfused with physiological saline solution. The right atrium of each heart was imaged using high-speed laser-scanning confocal fluorescence microscopy to assess atrial calcium handling before and following a combined right atrial preload (16cm H_2O) and hypokalemia (2mM K^+) challenge. FIJI was used to create average intensity projections of atrial tissue images and rectangular regions of interest (ROIs) encompassing approximately 40 cells were drawn. Custom analysis programs were used to analyze calcium transient amplitude (F/F_0) within atrial tissue ROIs. In the mid-aged dystrophic hearts, calcium transient amplitude increased ($P<0.05$) from $F/F_0=1.59$ to $F/F_0=2.06$ in response to the combined preload/hypokalemia challenge. In contrast, calcium transient amplitude was similar prior to ($F/F_0=1.63$) and following ($F/F_0=1.73$) the preload/hypokalemia challenge in mid-aged normal hearts. In conclusion, these data indicate atrial calcium handling abnormalities may underlie arrhythmogenesis in the setting of dystrophin deficiency.

Boosani, Anvitha **Poster Location: 47** **Poster Judging Number: 147**

Title: Evaluation of NIH ImageJ parameters for quantitative analysis of foam cell formation

Authors: Anvitha Boosani[1,2], Jonathan A. Green[1]

Affiliations: [1]Division of Animal Sciences, University of Missouri, Columbia, MO, USA, [2]Department of Biochemistry, University of Missouri, Columbia, MO, USA

Immune cells are a major contributor to atherosclerotic plaque growth in arteries. The transition of monocytes into macrophages, and macrophages into foam cells (large, obtrusive, lipid-laden macrophages) via oxidized LDL uptake, leads to foam cell accumulation in arterial plaques. This advances disease progression and may cause blockage of blood flow. We have previously investigated the role of the epigenetic protein TET1 (Ten-Eleven Translocation 1) in macrophages, and found that modulating its expression may lead to a decrease in oxidized LDL (oxLDL) uptake and subsequent foam cell formation.

Since drugs that inhibit foam cell formation can be tested through *in vitro* cell culture studies, evaluation of microscopically-acquired images would help in both quantification of foam cells and identification of statistical differences in drug effects. Currently, there are no effective software tools that can identify foam cells and quantitatively measure the extent of inhibition of foam cell formation in acquired images. The goal of this study is to utilize NIH ImageJ parameters for identification of foam cells in microscopic images. In the future, such parameters in the ImageJ software may be integrated in AI-based approaches to analyze large volumes of images acquired using automated microscopes.

The present study evaluated different functionalities in the NIH ImageJ software to assess foam cell formation. U937 monocytes were cultured in T25 flasks and differentiated into macrophages via treatment with phorbol 12-myristate 13-acetate (PMA). Differentiated macrophages were then treated with 33µM Bobcat339 (TET1 inhibitor) and 80µg/mL oxLDL to initiate foam cell formation and evaluate the effects of TET1 inhibition on oxLDL uptake. Images of foam cell populations in different treatment conditions were acquired using an upright light microscope. Different parameters within the NIH ImageJ software were evaluated for effective detection and quantitation of foam cells in the acquired images.

Funding: MizzouForward Undergraduate Research Training Grant (2024), MU Honors College Peggy & Andrew Cherng Summer Scholarship (2023)

Christie, Mary **Poster Location: 48** **Poster Judging Number 348**

Title: "Empowering Rural Clinics: Enhancing Clinical Research Participation through Research Readiness Training"

Context: With the growing emphasis on the importance of rural health, there is a strong need to engage rural practices in generating evidence. Existing programming to train rural practice clinicians and staff to participate in and/or lead research, including clinical trials, is insufficient and overly onerous as an introductory effort. Barriers include a lack of knowledge, anxiety about requirements, and fear of making mistakes. To provide the preparatory training needed to expand rural clinic engagement, we developed a “Research Readiness Training” program. This program is a collaborative effort designed for the rural community clinical team to enhance their ability to support participant recruitment, enrollment, informed consent process, and active partnership with academic partners conducting research. This education may offer rural learners a combined clinical and research experience, reinforcing the importance of adopting a “research embedded in practice” approach to rural healthcare delivery, and perhaps enhancing the rural primary care practice experience. Objective: The primary objective of this study is to evaluate the effectiveness of the Research Readiness Training course in increasing the interest and preparedness of rural medical practice clinicians and staff to participate in research studies. Study Design: Mixed Methods using qualitative descriptive approaches in parallel with prospective cohort approach. Dataset: Participant demographics, practice characteristics, pre- and post-training surveys, engagement metrics, and qualitative feedback data. Population Studied: Clinicians and staff from rural medical practices across Missouri, in non-urban areas, with limited access to larger healthcare facilities and research institutions. Intervention: Research Readiness Training. Outcome measures: Primary - improved knowledge of, confidence in participating in, and positive attitude toward research. Secondary - Understand barriers to engaging in clinical research. Results: Development of the Research Readiness Training was informed by a comprehensive approach involving focus groups, town halls, interviews, practical experiences, and literature review. In collaboration with university and external partners, will establish connections with rural sites across Missouri. Expected Outcomes: Participants will experience increased knowledge of clinical research and confidence to engage in clinical research activities. Additionally, we will gain a better understanding of the barriers that face rural healthcare providers interested in research, with the ultimate goal of increasing rural research collaboration with the academic medical center and inclusion of these underserved populations.

RECK Overexpression in Smooth Muscle Cells Aggravates Abdominal Aortic Aneurysm Progression

Manikandan Nagarajan[1], Neekun Sharma[1,2], Yoskaly Lazo-Fernandez[1], Edgar E. Betancourt-Cortes[1,3], Francisco I. Ramirez-Perez[1], Juan Gonzalez[1], Camila Manrique-Acevedo[1,2,4], Jaume Padilla[1,4,5], Yusuke Higashi[6], Chandrasekar Bysani[4,7], Luis A. Martinez-Lemus[1,3,8]

[1]NextGen Precision Health; [2]Division of Endocrinology and Metabolism, Department of Medicine; [3]Center for Precision Medicine, Department of Medicine; [4]Harry S. Truman Memorial Veterans' Hospital; [5]Department of Nutrition and Exercise Physiology; [6]John W. Deming Department of Medicine, Tulane University; [7]Division of Cardiovascular Medicine, Department of Medicine; [8]Department of Medical Pharmacology and Physiology

RECK (Reversion-inducing Cysteine-rich Protein with Kazal Motifs) is a known negative regulator of matrix metalloproteinases (MMPs), which play a key role in extracellular matrix (ECM) degradation and vascular remodeling. Abdominal aortic aneurysm (AAA) is a devastating vascular disease characterized by the permanent localized dilation of the abdominal aorta by $\geq$ 1.5 times its normal diameter. A common pathophysiological feature of AAA development is the ECM degradation of the aortic wall by MMPs. Therefore, we hypothesized that RECK overexpression in smooth muscle cells (SMCs) protects against AAA by suppressing MMP activity. We tested this hypothesis in constitutive ApoE knockout (KO) male mice with tamoxifen-inducible SMC-specific RECK overexpression or SMC-specific RECK KO. All mice (16-22 weeks old) were fed tamoxifen for 2 weeks (40 mg/kg/day) followed by the continuous infusion of angiotensin II (via an osmotic minipump, s.c., 1000ng/Kg/min for 28 days) and AAA development was compared to their corresponding RECK wild-type littermate controls. All differences reported herein are significant at $P<0.05$. Contrary to expectations, SMC-RECK overexpression led to increased aortic diameters and incidence of AAA with significantly elevated mortality rates. In comparison, SMC-RECK KO had no significant effects on AAA incidence or mortality. These findings suggest that, rather than being protective, RECK overexpression in SMCs exacerbates AAA progression. Further analysis is needed to determine the underlying mechanisms, including potential changes in MMP activity or alternative signaling pathways. Our study challenges the conventional role of RECK as an MMP inhibitor in AAA pathology and highlights its unexpected impact on disease progression. These preliminary data emphasize the complexity of ECM regulation in AAA and suggest that RECK may influence aneurysm development through mechanisms beyond MMP inhibition.

Keywords: Abdominal aortic aneurysm, Matrix metalloproteinase, Smooth muscle cells, Extracellular remodeling.

Lin, Aiping **Poster Location: 50**

Differential Effects of Particulate Matter Exposure on Macrophage Populations in Different Tissues in an age-dependent and sex-independent manner

Aiping Lin[1], Jing Zhang[1], Jingshu Chi[1], Feng Chen[1], Bo Peng[1], Dan Ni[1], Caiping Zhang[1], Qin Zhou[1], Yue Guan[2], Hong Hao[1], Xunlei Kang[1], De-Pei Li[1], Luis A. Martinez-Lemus[1], and Zhenguo Liu[1]

[1]Center for Precision Medicine and Division of Cardiovascular Medicine, Department of Medicine; [2]Flow Cytometry Core.

Background: Fine particulate matter (PM) triggers a systemic inflammation, and macrophages are critical to the initiation and maintenance of inflammation. Both sex and age have significant impact on inflammatory responses. The present study aimed to test the hypothesis that PM exposure differentially alter macrophage populations in different tissues in a sex- and age-dependent manner.

Methods: Young (8-10 weeks old) and aged (20-24 months old) male and age-matched female C57BL/6 mice were exposed to PM or PBS (control) via intranasal instillation every other day for six weeks. Peripheral blood was collected at baseline (before exposure), 4 weeks, and 6 weeks of PM exposure, and lung, liver, peritoneal cells, bone marrow, and spleen were harvested at the end of PM exposure. Macrophage populations (M1 and M2) were analyzed using flow cytometry.

Results: The M1/M2 ratio in peripheral blood exhibited a time-dependent increase in both young male and female mice with PM exposure at both week 4 and 6 of exposure, compared to controls. The M1/M2 ratio was also significantly elevated in the lungs in both young male and female mice with PM exposed at week 6 of exposure. However, no significant difference in macrophage populations were observed in the liver, spleen, bone marrow or peritoneal cells between the mice with PM and their controls. Interestingly, there was no significant change in the M1/M2 ratio in peripheral blood or any of the analyzed tissues in aged mice with PM exposure as compared with their controls.

Conclusion: Chronic PM exposure increases pro-inflammatory M1 macrophage population in peripheral blood and lung tissue in both young male and female mice, while no change was observed in aged mice with PM exposure. These data suggest that PM exposure led to an increase in M1 macrophages in circulation and lung in an age-dependent and sex-independent mechanism.

de la Cruz Conty, Julia Poster Location: 51 Poster Judging Number: 451

Nitric oxide and endothelin-mediated vasoregulation in the protective mechanisms of renal ischemic postconditioning in neonatal acute kidney injury

Julia E. de la Cruz[1,2], Olugbenga S. Michael[1,2], Ravi Kumar[1,2], and Adebowale Adebiyi[1,2,3,4]

[1]Department of Medical Pharmacology and Physiology, [2]University of Tennessee Health Science Center, [3]Department of Anesthesiology and Perioperative Medicine, [4]NextGen Precision Health

Ischemia reperfusion is a major cause of acute kidney injury (AKI) in infants and occurs in a variety of clinical settings, including sepsis, cardiopulmonary bypass, and kidney transplantation. Rapid ischemic postconditioning (IPostC) involves subjecting an organ to brief periods of ischemia and reperfusion after an ischemic event. Although IPostC has shown potential to alleviate IR injury, its effects on immature kidneys remain unexplored. Nitric oxide (NO) is a potent inducer of heme-oxygenase-1 (HO-1) and inhibits endothelin-1 (ET-1) function. HO-1 has been proposed to be beneficial in AKI and transplantation, whereas ET-1-induced vasoconstriction contributes to renal vascular dysfunction in AKI. We used the neonatal porcine model to investigate NO, HO-1, and ET-1 signaling in the protective mechanisms of renal IPostC in ischemic-AKI. Exposure of primary neonatal pig renal vascular endothelial cells (ECs) to a NO donor increased HO-1 but decreased ET-1 levels. In neonatal pigs, one-hour renal ischemia, followed by six hours reperfusion reduced significantly kidney NO and serum HO-1 levels, which was reversed by 3 cycles of IPostC (5 minutes of reperfusion followed by 5 minutes of ischemia before complete reperfusion). IR-induced increases in urine endothelin converting enzyme-1 (ECE-1) and ET-1 were counteracted by IPostC and the HO-1 inducer cobalt protoporphyrin (CoPP). IPostC and CoPP significantly preserved GFR and renal blood flow while reducing AKI biomarker levels and morphological kidney damage caused by IR. Similarly, the selective ETA receptor antagonist sitaxentan and the ECE- 1 inhibitor CGS-26303 mitigated IR-induced AKI. These findings suggest that: (1) Renal IR reduces NO and HO-1 while increasing ET-1 levels; (2) IPostC-induced HO-1 opposes the IR-induced rise in ET-1 biosynthesis; and (3) IPostC mitigates IR injury by stimulating HO-1 production, which counteracts ET-1 synthesis. We conclude that IPostC may lessen neonatal ischemic-AKI by stimulating NO production in the kidneys, which drives HO-1 to reduce ET-1 levels.

Funding: 25POST1368875, NIH R01DK120595-01 and R01 DK127625-01.

Dalton Cardiovascular Research Center

Committed to Collaboration In Research and Teachlna

Division of Cardiovascular Medicine
University of Missouri

NEXTGEN PRECISION HEALTH

Biomedical Sciences

Nutrition & Exercise Physiology
University of Missouri

Medical Pharmacology & Physiology

We would like to thank our sponsors for their generosity in contributing to the 32nd Annual Cardiovascular Day!

FUJIFILM
Value from Innovation

VISUALSONICS

mophys

MISSOURI PHYSIOLOGICAL SOCIETY
An independent chapter of APS