2022 Abstract Book

2022 Cardiovascular Research Day

October 28, 2022

Central Bank Center | Lexington, KY

24 CARDIOVASCULAR RESEARCH DAY

October 28, 2022 | Central Bank Center | Lexington, KY

Check In | Breakfast
8:30 am

Grand Ballrooms Prefuction

Scientific Session 1

Grand Ballroom 3

9:00 am Welcome

Gregory Graf, PhD
Associate Director
Saha Cardiovascular Research Center

9:05 am Rapid Fire Presentations

Ezekiel Rozmus
Graduate Student
Delisle Lab
College of Medicine
University of Kentucky
Determining the functional significance of KCNH2 variants of uncertain significance identified in a large patient biobank

Velmurugan Gopal Viswanathan, PhD
Staff
Hubbard Lab
College of Medicine
University of Kentucky
LRP1 knockout cells are more resilient to oxidative stress induced mitochondrial dysfunction and cellular damage: implications for neurovascular dysfunction in traumatic brain injury

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Rapid Fire Presentations, continued

Samantha Xu, MPH
Graduate Student
Shen Lab
Michael E. DeBakey Department of Surgery
Baylor College of Medicine
Mitochondrial metabolism dynamics in sporadic aortic aneurysms and acute dissections

Mikala Zelows
Graduate Student
Graf and Helsley Labs
College of Pharmacy
University of Kentucky
Carnitine palmitoyltransferase 1a modulates sexually dimorphic nafld

Daniëlle Coenen, PhD
Post Doc
Whiteheart Lab
College of Medicine
University of Kentucky
Platelet secretion in wound healing: the essence of α-granule endocytosis, biogenesis, and release kinetics

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9:40 am Assistant Professor Showcase Sponsored by the *Cardiovascular RPA*

Meredith Duncan, PhD
Biostatistics
College of Public Health
Using secondary data analysis to decrease Cardiovascular health disparities

Robin Shoemaker, PhD
Dietetics and Human Nutrition
College of Agriculture, Food and Environment
Serum profiles of the renin angiotensin aldosterone system (raas) in healthy and hypertensive pregnancies

Congqing Wu, PhD
Saha Cardiovascular Research Center
Department of Surgery
College of Medicine
Extracellular histones trigger disseminated intravascular coagulation by lytic cell death

10:20 am Gill Award for Outstanding Contributions to Cardiovascular Research

Kathryn Moore, PhD
Jean and David Blechman Professor of Cardiology
Professor, Department of Cell Biology
New York University
Cardiovascular disease: impact beyond the heart

10:55 am 90 Second Poster Pitch

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Break
11:15 am

Poster Session A — Grand Ballrooms 2

11:30 am Poster Judging
All odd posters presented to judges
with a 10-minute time limit

11:40 am Poster Presentations

Lunch — Grand Ballrooms 1
12:30 pm
Lunchtime Speaker

Matt Devalaraja, DVM, PhD
University of Kentucky Alumnus
Founder, CEO
Nipuna Therapeutics
Entrepreneurism in science: a multidisciplinary effort
to translate from bench to bedside

Break
1:25pm

Scientific Session 2 — Grand Ballroom 3

1:40 pm Trainee Presentations

Tyler W. Benson, PhD
Post Doc
Owens Lab
Heart, Lung and Vascular Disease Institute
University of Cincinnati
Glycoprotein VI Platelet Receptor Blockade
Attenuates Progression of Established
Abdominal Aortic Aneurysms in Murine Models

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1:40 pm Trainee Presentations, continued

JungHee Kang, MPH, PhD
Post Doc
Moser Lab (RICH Heart Program)
College of Nursing
University of Kentucky
Health literacy moderates the impact of a cardiovascular disease risk reduction intervention on diet quality among informal rural caregivers of people with chronic illnesses

2:10 pm Early Career Gill Award

Scott Cameron, MD, PhD
Section Head, Vascular Medicine
Cleveland Clinic
Thrombo-olfaction: curing arterial aneurysms through platelet olfactory receptors.

2:45 pm 90 Second Poster Pitch

Break
3:05

Poster Session B

Grand Ballrooms 2

3:20 pm Poster Judging
All even posters presented to judges with a 10-minute time limit.

3:30 pm Poster Presentations

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Scientific Session 3

Grand Ballroom 3

4:20 pm Alumni Speaker

Moriel Vandsburger, PhD
Associate Professor
Timothy and Karen Guertin Chair in Bioengineering
University of California at Berkeley

Networking Reception

Grand Ballroom Prefunction

5:00 pm

Dinner and Awards Ceremony

Grand Ballrooms 1

6:00 pm

Sponsors

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Gill Heart and Vascular Institute Outstanding Contributions to Cardiovascular Research Award

Kathryn Moore, PhD, FAHA

Jean and David Blechman Professor of Cardiology
Professor of Cell Biology
Director, NYU Cardiovascular Research Center
New York University Langone Health

Dr. Kathryn Moore is the Jean and David Blechman Professor of Cardiology, and Director of the Cardiovascular Research Center at New York University's Grossman School of Medicine. She is internationally recognized for her research on the molecular pathogenesis of cardiometabolic diseases, particularly the roles that non-coding RNAs and dysregulated immune responses play in those disorders. By forging new links between lipids, metabolism and innate immunity, her discoveries have revealed fundamental insights into pathways that regulate cholesterol homeostasis and vascular inflammation.

Dr. Moore received her B.Sc. and Ph.D. from McGill University in Canada. Although her early research focused on the immune response to pathogens, she became fascinated with the mechanisms of chronic inflammation, and pursued postdoctoral training at Harvard Medical School in the areas of autoimmune and atherosclerotic cardiovascular disease. She joined the faculty at Harvard Medical School and Massachusetts General Hospital (Dept of Medicine) as an Assistant Professor, before moving to New York University School of Medicine, where she is a tenured professor in the departments of Medicine and Cell Biology.

Dr. Moore is an active volunteer for the American Heart Association, and served on the Leadership Committee of the Arteriosclerosis, Thrombosis and Vascular Biology Council from 2004-18, including as Chair of the Council (2012-16). During her tenure, she helped to develop recommendations for AHA policies in the areas of science and medicine and made mentoring the next generation of scientists a top priority. Dr. Moore's contributions to the fields of innate immunity and vascular biology have been recognized by numerous awards and honors, including the NIH's Outstanding Investigator Award, Clarion's List of Most Highly Cited Researchers (top 1%, 2018-21), the ATVB Mentor of Women Award, the American Heart Association's Distinguished Scientist Award, and election to the National Academy of Sciences USA, among others.

Gill Heart and Vascular Institute Early Career Award

Scott Cameron, MD, PhD

Section Head of Vascular Medicine
Department of Cardiovascular Medicine
Cleveland Clinic Foundation

Dr. Cameron is a physician scientist with training in pharmacology from the University of Edinburgh in Scotland which is his native country. He runs a basic and translational research laboratory at the Cleveland Clinic Lerner College of Medicine that has been continuously funded by the National Institute of Health since 2016 to study platelet dysfunction in vascular disorders and arterial aneurysmal disease. Scott is an Associate Editor of Vascular Medicine Journal. Scott's graduate research, medical school, and postgraduate training in medicine was completed in New York. He is a cardiologist and a specialist in vascular medicine, serving as Section Head of vascular medicine at the Cleveland Clinic since 2020. His expertise is acute cardiac care and managing arterial and thrombotic emergencies. He is board-certified in internal medicine, vascular medicine, cardiovascular disease, and he is a registered physician in vascular interpretation.

Invited Speakers

Professional Development Presentation

Matt Develaraja, DVM, PhD

CEO of Nipuna Therapeutics

Matt Devalaraja is an Adjunct Professor at School of Medicine, University of New South Wales. He is currently the CEO of Nipuna Therapeutics, a Precision Immunology company based in Boston. Prior to this, he founded and headed up R&D at Corvidia Therapeutics, a precision cardiovascular company. He spun this company out of AstraZeneca by building the basic science. Corvidia was recently acquired by Novo Nordisk for $2.1B. An inflammation biologist by training (Ph.D. 1997, University of Kentucky, Lexington, KY, USA), he had been working in the pharmaceutical and biotech industry for the last 22 years. He was Head of Biologics Discovery at Pfizer Ann Arbor and as Group head at Human Genome Sciences. He has discovered and developed multiple clinical candidates and was instrumental in getting the first lupus drug, Benlysta and first biodefense drug, anti-anthrax Ab approved by FDA. He is an Adjunct Professor at University of New South Wales, Sydney.

Invited Speakers

Distinguished Alumni Presentation

Moriel Vandsburger, PhD

Associate Professor
Timothy and Karen Guertin Chair in Bioengineering
University of California at Berkeley

Moriel Vandsburger is an assistant professor of bioengineering at the University of California, Berkeley. The focus of his lab is on developing molecular MRI techniques for targeted cardiac and cellular imaging. He completed his Ph.D. in biomedical engineering at the University of Virginia with a focus on developing MRI methods for assessment of left ventricular remodeling in mice following myocardial infarction (MI). Specifically, his work focused on developing MRI methods to assess myocardial blood flow in the healthy and diseased heart using a technique called arterial spin labeling. In addition, his work focused on elucidating the in vivo roles of nitric oxide synthase isoforms in calcium cycling using manganese-enhanced MRI. He spent ~3 years as a Whitaker International Postdoctoral Scholar at the Weizmann Institute of Science in Rehovot, Israel. There he branched out into MRI reporter gene and cell tracking research, as well as molecular cardiac MRI.

Selected Abstracts

Congratulations to these trainees whose abstracts were selected for podium presentations.

Ezekiel Rozmus
Graduate Student
Delisle Lab
College of Medicine
University of Kentucky

Velmurugan Gopal Viswanathan, PhD
Staff
Hubbard Lab
College of Medicine
University of Kentucky

Samantha Xu, MPH
Graduate Student
Shen Lab
Michael E. DeBakey Department of Surgery
Baylor College of Medicine

Mikala Zelows
Graduate Student
Graf and Helsley Lab
College of Pharmacy
University of Kentucky

Daniëlle Coenen, PhD
Post Doc
Whiteheart Lab
College of Medicine
University of Kentucky

Tyler Benson, PhD
Post Doc
Owens Lab
Heart, Lung and Vascular Disease Institute
University of Cincinnati

JungHee Kang, MPH, PhD
Post Doc
Moser Lab (IRCH Heart Program)
College of Nursing
University of Kentucky

Poster Pitch Participants

Naofumi Amioka
PostDoc
University of Kentucky

Gertrude Arthur
Graduate Student
University of Kentucky

Isha Chauhan
Undergraduate
University of Kentucky

Daniëlle Coenen
PostDoc
University of Kentucky

Nick Demas
Medical Student
University of Kentucky

Gokul Anugrah Gopakumar
Graduate Student
University of Kentucky

David Graf
Undergraduate
Mississippi State University

Sohei Ito
PostDoc
University of Kentucky

Zoe Leuthner
Medical Student
Alabama College of Osteopathic Medicine

Alex Pettey
Graduate Student
University of Kentucky

Kimberly Rebello
PostDoc
Baylor College of Medicine

Diksha Satish
Undergraduate
University of Kentucky

Alexis Smith
Graduate Student
University of Kentucky

Holly Sucharski
Graduate Student
The Ohio State University

Meghan Turner
Graduate Student
University of Kentucky

Sathya Velmurugan
Staff
University of Kentucky

Shayan Mohmadmoradi
Graduate Student
University of Kentucky

Poster Participants

Title: Endothelial Mineralocorticoid Receptor Mediates Aldosterone plus Salt-Induced Abdominal Aortic Aneurysm

Author and Affiliation: 1 Shu Liu, 2 Xufang Mu, 1 Yu Zhong, 1 Ming C. Gong and 2, 3 Zhenheng Guo

1 Department of Physiology; 2 Department of Pharmacology and Nutritional Sciences; University of Kentucky; 3 Research and Development, Lexington Veterans Affairs Medical Center; Lexington, KY 40536

Staff

Objective—We reported that administration of mice with aldosterone (Aldo) or deoxycorticosterone acetate (DOCA) plus salt induces AAA via mineralocorticoid receptor (MR). The current study defines the specific roles of endothelial MR in Aldo-salt induced AAA.

Approach and Results—A tamoxifen inducible endothelial cell (EC)-specific MR knockout mouse model (iECMRKO) knockout mouse model were developed. The iECMRKO mice were protected from Aldo-salt-induced AAA. Mechanistically, EC-specific MR deletion suppressed aortic elastin degradation, matrix metalloproteinase-2 (MMP-2) and MMP-9 upregulation, macrophage and neutrophil infiltration. Surprisingly, flow cytometry analysis showed that neutrophils, but not macrophages were decreased in iECMRKO mice in the aorta 1 week after Aldo-salt administration. Treatment of C57BL/6 mice with an anti-PMN antibody selectively suppressed Aldo-salt-induced circulating neutrophils, and protected mice from Aldo-salt-induced AAA. In cell cultures, Aldo-induced endothelial adhesion molecules (E-selectin, P-selectin, and ICAM-1, but not VCAM-1) mRNA expressions were abolished in MR-deficient ECs. Importantly, Aldo-salt-induced ICAM-1 upregulation was abolished in aortas from iECMRKO mice.

Conclusions—Endothelial MR plays an important role in Aldo-salt-induced AAA. Moreover, ICAM-1, but not VCAM-1, and neutrophils, but not macrophages, mediate the early processes of Aldo-salt-induced and endothelial MR-mediated AAA development.

Computational Fluid Dynamics Studies of Patients with Aortic Dissection.

G. Anugrah[1], J. Wenk[1], C. Brehm[2],
[1]University of Kentucky, [2]University of Maryland.

Graduate Student

Computational fluid dynamics (CFD) has helped in understanding the hemodynamics of several cardiovascular diseases. The current project deals with one such disease known as aortic dissection (AD) in which the inner walls of the aorta (a large artery branching off the heart) tear and blood surges into a secondary channel. This is a serious condition that has both long-term and short-term consequences for a patient's health and for this reason, proper diagnosis and management of this disease are inevitable. Even though researchers had been using CFD simulations to predict and diagnose AD for at least a decade, there are several inherent difficulties associated with these simulations. The non- Newtonian nature of blood, elastic behavior of aortic walls, and patient-specific aortic geometry and associated boundary condition requirements are a few such challenges. An accurate prediction of flow physics demands prescribing of proper boundary conditions and consideration of the interaction of aortic walls with the blood flow. A common practice among researchers is to prescribe a pulsating volume flow rate (obtained from velocity waveforms of the patient's medical data) at the inlet and a pressure boundary condition at the outlet. The pressure at the outlet is approximated from the volume flow rate using different Windkessel models. This study aims at developing the above-mentioned capabilities within an in-house incompressible flow solver and validating the results with in-vivo data obtained from CT scans and other medical imaging techniques. Preliminary studies were conducted with geometries of the aorta from different patients and the results are promising.

Acknowledgments: The authors would like to thank the Computational Sciences Department of University of Kentucky for providing the computing resources through Lipscomb Computing Cluster (LCC) and several people in Cardio Vascular Institute of UK Healthcare for providing valuable advice and patient data for this study.

Different Administration Times of GLP-1 Receptor Agonist, Exenatide, Results in Different Glucose Regulation

Karolina Kopyonkina, Aaron Chacon, Shu Liu, Wen Su, Zhenheng Guo, Ming Gong

Undergraduate

Objective:

GLP-1 receptor agonists (RAs) are widely prescribed for the management of type 2 diabetes. Since focus has been shifted to long-acting GLP-1 RAs, little attention has been given to the GLP-1 receptor's time-of-day effects, and whether differences arise. Different time of administration of the short-acting GLP-1 RA, exenatide, was used to explore the possibility of differing glucose outcomes.

Approach & Results:

Diabetic *db/db* mice have a point mutation, resulting in a nonfunctional leptin receptor. As such, *db/db* mice consume food irregularly and in excess. The *db/db* mice were injected (IP) with the exenatide daily at either ZT0 (onset of light phase) or ZT12 (onset of dark phase) with vehicle or exenatide. The mice were singly housed in BioDAQ (Research Diet, New Brunswick, NJ), which allows for continuous and accurate monitoring of food intake. ZT0 administration of exenatide significantly improves food intake rhythm (reduction in light phase intake) while abolishing food intake rhythm when administered at ZT12. To establish if there were benefits to different time point injections and insulin sensitivity, an Intraperitoneal Insulin Tolerance Test (ipTTT) was performed after 30 days of exenatide treatment. Mice were not treated with exenatide and were fasted for four hours prior. Basal blood glucose levels were measured by a tail cut via StatStrip Xepress^{TM} glucometer (NOVA biomedical; Waltham, MA, USA). Humalin R (0.75 U/kg of total body weight) was injected ip. Blood glucose measured after insulin injection at 15, 30, 45, 60, 90, and 120 minutes. Mice treated at ZT0 showed a trend towards lower blood glucose compared with ZT12 administration or saline-treated mice. After 90 days of treatment at either ZT0 or ZT12, hemoglobin A1c (HbA1c) and pancreatic islets, stained for insulin, were quantified. ZT0-treated mice showed a >1% reduction in HbA1c%, with more intense pancreatic insulin staining.

Conclusion:

Between the two treatment groups, ZT0 treated mice show a significant improvement in their light to dark phase food intake whereas ZT12 see a worsening. There is a trend towards greater glucose control in the ZT0 treated mice.

Abhilash Prabhat*, Isabel Stumpf, Tanya Seward, Elizabeth A. Schroder, Brian P. Delisle#

Department of Physiology, University of Kentucky
#Corresponding author
*Presenting author

A Food-Entrainable Oscillator Contributes to the Daily Rhythms in Heart Rate and Temperature in Male and Female Mice

Postdoc

Background: The daily rhythm in heart rate is classically considered to be regulated by the circadian clock in the suprachiasmatic nucleus (SCN). The circadian clock in the SCN is entrained in the light-dark cycle. We found that restricting the time of feeding to the daytime shifts the daily rhythm in the heart rate of mice to align with food availability and not the light-dark cycle. We tested the hypothesis that a food-entrainable oscillator contributes to the daily rhythm in heart rate.
Methodology: We determined the effect that daytime time-restricted feeding (TRF) has on heart rate and core body temperature in wild-type (WT) male and female mice under thermoneutral conditions (TN). Mice were implanted with a telemetry device and housed under 12 h light: 12 h dark cycle (LD) with food *ad libitum* (ALF) at 25 °C. After acclimation mice were transferred to the thermoneutral temperature 30°C for several days. The mice were subjected to daytime TRF for 5 days in LD, followed by constant dark condition (DD) under TRF, and then returned to ALF in DD.

Results: Male and female mice housed in TN had slower heart rates as compared to 25 ^{o}C without affecting the core body temperature. In both sexes, daytime TRF dramatically slowed the mean heart rate; decreased core temperature; and caused a large shift in the phases of the daily rhythm in heart rate and core temperature. Mice under TRF showed food anticipatory increase (FAI) in the heart rate and core body temperature 2 hours prior to the onset of food introduction. FAI in heart rate and the core temperature continued under TRF in DD and even persisted after the mice were returned to ALF in DD condition.

Conclusion: These data provide the first evidence that an intrinsic food-entrainable oscillator contributes to the daily rhythm in heart rate and core temperature. These findings identify a novel role for feeding behavior in the regulation of cardiac function.

Sustained Inhibition of High Mobility Group Box 1 by Antisense Oligonucleotide

Shayan Mohammadmoradi[1,2], Hisashi Sawada[1, 3], Sohei Ito[1], Adam E. Mullick[4], Deborah A. Howatt[1], Michael K. Franklin[1], Hong S. Lu[1,2,3], Alan Daugherty[1,2,3]

[1] Saha Cardiovascular Research Center
[2] Department of Pharmacology and Nutritional Sciences
[3] Department of Physiology
University of Kentucky, Lexington, KY

[4] Ionis Pharmaceuticals, *Inc*, Carlsbad, CA

Graduate Student

Background: High-mobility group box 1 (HMGB1), a highly conserved nonhistone DNA-binding nuclear protein, may contribute to vascular diseases including atherosclerosis, pulmonary hypertension, and abdominal aortic aneurysm. Since whole-body genetic deletion of HMGB1 is embryonic lethal, pharmacological approaches, such as neutralizing antibodies and functional inhibitors, have been used to manipulate HMGB1 in mice. However, it remains desirable to genetically manipulate HMGB1 for further understanding its role. In the current study, we assessed the efficacy of a novel antisense oligonucleotides (ASOs) approach to deplete HMGB1 in mice.

Methods and Results:
First, abundance of HMGB1 mRNA was assessed by qPCR in major organs and tissues of male C57BL/6J mice (N=6). *Hmgb1* mRNA was expressed ubiquitously, but it was most highly abundant in the lung, while least abundant in the testes. Next, we examined the efficacy of ASOs to reduce HMGB1 protein abundance at selected intervals. Either ASOs (25 mg/kg/day) or phosphate-buffered saline (PBS) were injected intraperitoneally into male C57BL/6J mice (8-10-week-old) at day 0 and 3 in the initial week and then once a week during the remainder of the study. Mice were terminated at either 2, 6, or 12 weeks after the initial injection of ASOs (N=5/group). Subsequently, mRNA and protein abundance of HMGB1 were determined in the lungs. Since previous studies have shown that systemic administration of ASOs mainly targets kidney and liver, HMGB1 mRNA or protein abundance was also examined in these organs. We also assessed HMGB1 protein abundance in the heart. In lungs, kidney, and liver, ASO administration resulted in a consistent >90% decrease of *Hmgb1* mRNA abundance at 2 weeks of ASO injection (P>0.001). The decreased *Hmbg1* mRNA abundance was also consistent at both weeks 6 and 12 (P>0.001). ASOs decreased HMGB1 protein abundance significantly at 6- and 12-week time intervals. Heart tissue also indicated a consistent *Hmgb1* mRNA decrease in response to ASO administration.

Conclusion: ASO approach decreased HMGB1 at mRNA and protein abundance for 12 weeks in the liver, kidney, lungs, and heart. This mode may provide more clear insights into understanding the biological functions of HMGB1.

Vivek Kumar Pandey, Sathya Velmurugan, Sanda Despa
Department of Pharmacology and Nutritional Sciences, College of Medicine, University of Kentucky

Nedd4-2 inactivation enhances SGLT1 stability in diabetic hearts

PostDoc

Rationale: Heart failure is a major complication of type-2 diabetes (T2D). While the underlying mechanisms are poorly understood, recent evidence suggests that upregulation of the Na^+/ glucose cotransporter 1 (SGLT1) plays an important role, as it causes myocyte Na^+ overload.

Objective: To identify the mechanisms involved in promoting SGLT1 protein levels in diabetic hearts.

Methods/Results: We compared failing hearts from patients with and without T2D and hearts from rats with late-onset T2D caused by overexpression of human amylin in pancreatic β-cells (HIP) versus hearts from WT littermates. SGLT1 protein level was significantly higher in the hearts of both humans and rats with T2D. In contrast, the SGLT1 mRNA levels were comparable in hearts from T2D and non-diabetic patients and rats. This result suggests that the increase in cardiac SGLT1 protein in diabetic hearts is controlled post-transcriptionally, i.e., is the result of either accelerated protein synthesis or impaired protein degradation. SGLT1 is degraded by ubiquitination, a process mediated by Nedd4-2, a ubiquitin E3 ligase. Nedd4-2 is inactivated upon phosphorylation by the serum and glucocorticoid-regulated kinase-1 (SGK1) at Ser342 residue. Using western blot, we found higher SGK1 levels in diabetic hearts. Moreover, Nedd4-2 phosphorylation was significantly increased in the hearts of diabetic humans and rats.

Conclusion/New Hypothesis: The increase in SGLT1 protein levels in the diabetic heart is governed post-transcriptionally by an impairment in SGK1/Nedd4-2 dependent ubiquitination and degradation of SGLT1.

Targeting The GLP-1 Receptor As New Chronotherapy Against Nondipping Blood Pressure In Diabetes

Aaron Chacon, Wen Su, Tianfei Hou, Ming Gong, Zhenheng Guo
Pharmacology and Nutritional Sciences, Physiology
University of Kentuckyy

Graduate Student

Objective:

It is well established that a regular blood pressure (BP) rhythm is critical for maintaining cardiovascular health. Nondipping BP (<10% reduction during rest period) is prevalent in type 2 diabetes (T2DM). Exenatide, a short half-life GLP-1 receptor agonist used to treat hyperglycemia in T2DM, has also shown to lower BP and inhibit food intake. However, whether timing of its administration has an effect on nondipping BP in T2DM has not yet been explored.

Approach & Results:

13-week old diabetic *db/db* mice, who exhibit nondipping BP and poor food intake rhythm, were injected i.p. with exenatide at either onset of light (ZT0 Ex) or dark (ZT12) phases. BP was recorded via radiotelemetry, allowing for fully conscious, free-moving data collection. Food intake was collected via BioDAQ (Research Diet, New Brunswick, NJ) allowing for continuous food intake recording. BP dipping was achieved following daily ZT0 administration and worsened to "reversed dipping" (increase in BP over dark phase levels) from daily ZT12 administration; 10.5 and -4.5% in mean arterial pressure (MAP), respectively. MAP rhythms were evaluated by cosinor analysis revealing significant changes in robustness (%, strength of rhythm) of +37.4% (ZT0 Ex) and -3.2% (ZT12 Ex) over basal levels. Amplitude (difference between peak or trough and mean value) increased signficantly in ZT0 Ex but not in ZT12 Ex. Acrophase (, the time at which sine-fitting curve amplitude is maximal) shifted 10.5 hours, peaking in the middle of the light phase for ZT12 Ex with no significant difference in ZT0 Ex. Both treatment groups saw a significant reduction in MESOR (rhythm-adjusted mean) of 10.86 (ZT0 Ex) and 10.26 (ZT12 Ex) mmHg, demonstrating equivalent BP-reducing efficacy. Correlating with BP changes, light/rest phase food intake was significantly reduced by 10.5% (ZT0 Ex) and increased 20.3% (ZT12 Ex) over basal levels. 24-h food intake was not significantly affected in either treatment group.

Conclusion:

While treatment groups saw equivalent reductions in BP, their rhythms were flipped. This reveals an important, previously unexplored role of exenatide in the treatment of nondipping BP in T2DM, potentially mediated by the improvement in food intake rhythm, independent of caloric intake.

Potential Contribution of X Chromosome Inactivation Escaped Gene to Sex Differences in Aortic Diseases---- revealed by single cell RNA-seq analysis

Yanming Li[1], PhD, Yang Li[1], PhD, Chen Zhang[1], MD, Hernan G. Vasquez[1], PhD, Abhijit Chakraborty[1], PhD, Kimberly Rebello[1], MD, Lin Zhang[1], BS, Hong S. Lu[3, 4], MD, PhD, Lisa A. Cassis[5], PhD, Joseph S. Coselli[1, 2], MD, Alan Daugherty[3,4], PhD, Ying H. Shen[1,2], MD, PhD,* Scott A. LeMaire[1,2], MD*

1. Division of Cardiothoracic Surgery, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, TX
2. Department of Cardiovascular Surgery, Texas Heart Institute, Houston, TX
3. Saha Cardiovascular Research Center, University of Kentucky, Lexington, KY
4. Department of Physiology, University of Kentucky, Lexington, KY
5. Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY

PostDoc

Background: Ascending thoracic aortic aneurysms (ATAA) can progression to dissection (ATAD) and leading to aortic rupture and other life-threatening complications. Males and females show significant difference in aneurysm formation and progression. While the overall incidence of aortic diseases is lower in females than in males, the diseases progress faster in females. The underlying mechanisms remain poorly understood. We hypothesized that genes that escape from X chromosome inactivation (XCI) in females may contribute to sex-based differences in aortic disease formation or progression.

Methods: We performed single cell RNA sequencing (scRNA-seq) on human aortic tissues from control (3 women, 5 men), ATAA (10 women, 16 men), and ATAD (3 women, 6 men), in which non-dissected/ pre-dissection area and dissected/dissection area were collected separately. Differentially expressed genes (DEGs) between females and males, as well as normal females and diseased females were identified. Genes that escaped from XCI were identified by overlapping DEGs biased in females in our sc-RNAseq data with Genotype-Tissue Expression (GTEx) identified XCI escaped genes. DDX3X interacting proteins in DDX3X overexpressed HeLa cells were identified by co-IP and Tandem Affinity Purification and Mass Spectrometry (TAP-MS). DDX3X functions were further examined in smooth muscle cells (SMCs) that were treated with double strand DNA (dsDNA).

Results: The principal component analysis showed sex-based difference in gene expression profiles in SMCs, fibroblasts and endothelial cells in pre-dissection tissues or dissected tissues. Gene ontology analysis of DEGs suggested higher proteotoxic stress in SMCs in pre-dissection tissues in females compared with males. DDX3X, a X-coded gene involved in proteotoxic stress, was identified as escaped gene as it was higher in females in SMCs across all conditions and was defined as escaped gene in aorta by GTEx. However, MYC, a DDX3X partner in inducing proteotoxic stress was only upregulated in ATADs. TAP-MS analysis suggested that DDX3X interacted with proteins in ribosome function and translation. In cultured SMCs, dsDNA induced senescence and DDX3X containing stress granules formation, which were prevented by knocking down DDX3X.

Conclusion: We have identified DDX3X as a potential XCI escaped gene that contribute to sex differences in aortic diseases. Our data suggested that, in dissection tissues with MYC highly activated, escaped DDX3X may facilitate aortic dissection progression by promoting proteotoxic stress in females.

PD-1 pathway upregulation by orchiectomy attenuates the aldsoterone and high salt induced aortic aneurysms in male mice

Xufang Mu, Shu Liu, Zhuoran Wang, Wen Su, Timothy McClintock, Arnold Stromberg, Alejandro Tezanos, Ming Gong and Zhenheng Guo

University of Kentucky

Graduate Student

- **Objective** - Male sex is a well-established risk factor for abdominal aortic aneurysms (AAA) but the underlying mechanisms remain to be fully understood. Using an aldosterone and high salt (Aldo/salt) induced AAA mouse model, we have demonstrated that androgen and its receptor mediate the high susceptibility to Aldo/salt induced AAA. The current study further investigates the mechanisms downstream of androgen.

- **Approaches and Results** - To dissect the mechanisms connecting androgen and AAA, aortas were collected for RNA sequencing from 3 groups of 10-month-old wild-type mice #1 intact mice; #2 orchiectomized mice; and #3 orchiectomized mice plus DHT. All mice were given Aldo/salt for 7 days. Differentially expressed genes were analyzed using DESeq2 in R. We filtered genes that were upregulated in group #2 compared to group #1 and the up-regulation was reversed in group #3 by the DHT, or vice versa (fold change >1.5 and padj <0.05). Selected genes were run for gene ontology analysis in Enrichr (database Bioplanet 2019). Many pathways related to T cell activity were significantly enriched, particularly PD-1 signaling was one of the top pathways upregulated in orchiectomy group #2. PD-1 is known for its role as an immune checkpoint, and inflammation is a major hallmark for AAA development. Therefore to explore the role of PD-1, we first confirmed the PD-1 mRNA changes in aorta by qPCR. Secondly, IHC staining also showed PD-1 protein was significantly increased in the spleen of orchiectomized mice compared to intact controls. Finally, to investigate the potential causal role of PD-1 in the androgen-mediated aortic aneurysms formation, we injected αPD-1 antibody or control IgG antibody to orchiectomized mice 3 days before and during the 8 weeks of Aldo/salt administration. Results showed that 5 out of 12 αPD-1 mice, while none of the 8 control mice developed aortic aneurysms ($p<0.05$).

- **Conclusions** - PD-1 pathway is involved in the androgen associated high susceptibility of Aldo/salt induced aortic aneurysms in mice.

Extracellular Ca2+ Balances Cell Repair and Lysis in Pyroptosis

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PostDoc

ABSTRACT

Pyroptosis is a critical cellular defense against microbe infection as a result of inflammasome activation. Plasma membrane rupture is the hallmark of pyroptosis. Against common belief, it has been shown that plasma membrane rupture is not a passive but rather an active process mediated by NINJ1. Here we show that extracellular calcium is required for NINJ1 activation. In the absence of extracellular calcium, NINJ1 oligomerization was blocked, and so was the rupture of plasma membrane. During pyroptosis, cell repair mechanisms are activated. For example, pyroptotic cells remove membrane GSDMD pore in the form of microvesicles to prevent plasma membrane rupture. We observe extracellular calcium inhibits the release of GSDMD positive extracellular vesicles and promotes plasma membrane rupture. Taken together, our data reveal an important role of extracellular calcium in cell repair and lysis in pyroptosis.

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Identifying Novel Biomarkers for Congenital Arrhythmia Phenotypes using Transgenic Mouse Models

Undergraduate

Background: Electrocardiographic (ECG) telemetry in mice can be used to identify novel mechanisms for cardiac arrhythmias. Long QT syndrome
(LQTS) is a deadly congenital arrhythmia syndrome that is diagnosed using the heart rate corrected QT (QTc) interval.

Purpose: Type 3 LQTS (LQT3) is caused by a gain of function mutations in the cardiac Na^+ channel. We wanted to determine the range of the QTc interval in a large cohort of people with LQT3 and the transgenic mouse model of LQT3 to identify new biomarkers for the LQT3-related phenotype.

Methods: We obtained QTc data for people with LQT3 mutations from the international LQTS registry. We measured electrocardiograms (ECG) in wild type (WT) or a transgenic mouse model of LQT3 using telemetry or the

ECGenie. Studies were done in mice housed in 12h light and dark cycles at 22^oC or 30^oC.
Results: People with LQT3 mutations have QTc intervals that show a large amount of overlap with people who do not have LQTS. To identify new biomarkers for LQT3 we studied WT and LQT3 mice housed at 22^oC using ECG telemetry. However, both WT and LQT3 mice had very fast basal heart rates (WT = 520 ± 11 bpm; LQT3 = 506 ± 18 bpm; n = 6/group). Injecting WT mice with medications that inhibit the autonomic signaling to the heart significantly slowed the heart rate to 467 ± 54 bpm (n = 4/group). Housing WT and LQT3 mice at thermoneutral temperatures (30^oC) slowed basal heart rates below values measured after injecting mice with medications that inhibit autonomic signaling to the heart (WT = 419 ± 50 bpm; LQT3 = 407 ± 42 bpm; n = 6/group).

Conclusion: Mice have very high resting heart rates driven by a high basal sympathetic tone at 22^oC. Results from thermoneutral temperatures suggest mice have basal heart rates driven by parasympathetic tone (similar to humans). In working to identify a new biomarker for the LQT3 phenotype in mice, we will study mice housed at thermoneutral temperature.

Single-cell RNA Sequencing Reveals Novel Function of Thrombospondin-1 in Murine Venous Thrombosis

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Staff

Background: Deep vein thrombosis (DVT) is a common clinical problem, but its cellular and molecular mechanisms, particularly the contribution of the vein wall, still remain incompletely understood.

Methods and Results: We utilized single-cell RNA sequencing to determine the cellular and molecular changes within the vein wall during the acute phase (24 h) of DVT, using mouse inferior vena cava (IVC) ligation model. Unbiased clustering identified 9 cell types composed of multiple subpopulations in both DVT and sham conditions. Percentages of each population and their gene expression patterns were compared between DVT-bearing and sham groups, revealing early transcriptomic changes across vascular and immune cells. Immunostaining confirmed the shift of cell type composition and the existence of sub-populations. Notable transcriptome changes induced by DVT included a marked inflammatory response, elevated cell death and hypoxia pathways, and globally reduced myogenesis. Moreover, cell-cell communication analysis uncovered that signaling sent from monocytes/macrophages to vascular cells was significantly enhanced by DVT. One of the top 3 elevated pathways was Thrombospondin signaling, and the increased expression of *Thbs1* (encodes Thrombospondin-1, TSP1) was responsible for the signaling alteration. To examine the role of TSP1 in DVT, we performed IVC ligation in *Thbs1* global deficient mice and myeloid-specific *Thbs1* knockout mice. Unexpectedly, in global *Thbs1* knockouts, both male and female mice developed larger thrombi than controls, while myeloid-specific *Thbs1* knockout increased DVT formation only in male mice.

Conclusion and Discussion: The vein wall responds to DVT induction with complex cellular and transcriptomic changes. At the acute phase, inflammation and cell death and loss of myogenesis are prominent. Despite of the established function of TSP1 in platelets, global *Thbs1* gene deficiency increased thrombus formation. The anti-thrombotic function of TSP1 is likely to be mediated through myeloid cells in male mice. Future studies will focus on delineation of the roles of TSP1 in myeloid cells, and more importantly investigating whether the sex-dependent functions of TSP1 affect human DVT formation and treatments.

Western Diet-induced Hepatic Steatosis is Ameliorated by Hepatocyte-specific Angiotensinogen Deletion with Suppression of Complement Component C4

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Graduate Student

Background:

Steatosis is a common cause of chronic liver disease. We have shown consistently that hepatocyte-specific deficiency of angiotensinogen (AGT), the unique substrate of the renin-angiotensin system, alleviates Western Diet (WD)-induced steatosis in mice. However, the molecular mechanism by which AGT contributes to WD-induced steatosis is unknown. This study aims to determine the contribution of AGT to WD-induced transcriptomic changes in the liver.

Methods and Results:

We performed sequential bulk RNA sequencing to determine the impact of WD on the hepatic transcriptome. Livers from low-density lipoprotein receptor $(LDLR)^{-/-}$ mice fed WD (42% of calories from fat) for 5, 14, or 42 days were evaluated against mice fed normal diet. Principle component analysis of unfiltered genes and z-scored heatmaps of differentially expressed genes (DEGs) revealed transcriptomic alteration at 14 and 42 days of WD. Gene ontology analysis identified positive regulation of cytokine production as the top upregulated pathway for both intervals. To evaluate the contribution of AGT to WD-induced steatosis, liver transcriptomes from hepatocyte-specific AGT deficient ($hepAGT^{-/-}$) mice and wild type ($hepAGT^{+/+}$) littermates were compared at 14 and 42 days of WD. There were 132 and 27 DEGs at 14 and 42 days of WD, respectively. Six DEGs were overlapped from both comparisons. After excluding pseudogenes and low-expression genes, complement component 4A (*C4a*) was identified as a potential target. *C4a* is a pro-inflammatory zymogen critical in all three complement pathways. Quantitative polymerase chain reaction (qPCR) of *C4a* mRNA showed 2.7 times higher expression in $hepAGT^{+/+}$ versus $hepAGT^{-/-}$ mice at 42 days of WD ($p=0.008$ by Rank Sum Test).

Conclusions:

Western Diet altered inflammatory genes as early as 14 days in the liver. Hepatocyte-specific AGT deficiency suppressed *C4a* mRNA. Future studies will determine which complement pathways are activated by WD and characterize the spatial distribution of *C4a* and its proteolytic derivatives.

The estrous cycle coordinates the daily eating behavior rhythm in mice

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Graduate Student

The estrous cycle regulates daily rhythms of locomotor activity, body temperature, and circadian gene expression. In mice and rats, wheel-running activity is greatest on the night of proestrus into estrus, when elevated estrogens cause ovulation. High activity level at the time of ovulation could increase the likelihood of finding a mate and thus increasing fitness. We previously found that exogenous estrogen regulates the daily rhythm of eating behavior in female mice fed high-fat diet. Ovariectomized females that lacked cycling estrogens had low amplitude eating behavior rhythms that were phase advanced compared to females treated with exogenous estradiol. However, it is not known whether endogenous, cycling estrogens regulate eating behavior rhythms. The goal of this study was to determine whether daily eating behavior rhythms change systematically across the estrous cycle. Twelve-week-old female C57BL/6J mice were housed in 12L:12D with running wheels and fed standard chow diet. Estrous cycle stages were determined by daily vaginal cytology. Eating behavior, which was measured with infrared video cameras, and wheel revolutions were continuously measured before and after ovariectomy. The mice had regular 4- or 5-day estrous cycles that were abolished after ovariectomy. Consistent with prior studies, the magnitude of daily wheel-running activity fluctuated with a 4- or 5-day cycle. The greatest number of wheel revolutions occurred on the night of proestrus into estrus, when estrogens peak to stimulate ovulation. We found that the amplitude, or robustness, of the eating behavior rhythm also fluctuated with a 4- or 5-day cycle. Like wheel-running activity, peak amplitude of the eating behavior rhythm occurred primarily during proestrus or estrus. The phases of eating behavior rhythms fluctuated, but not with consistent 4- or 5- day rhythms, and they did not correlate with specific stages of the estrous cycle. After removal of cycling hormones with ovariectomy, the amplitude of the eating behavior rhythm peaked at irregular intervals. Together, these data suggest that fluctuations of ovarian hormones across the estrous cycle temporally organize daily rhythms of eating behavior.

Abstract Title: Characterization of the modulators, α-Synuclein and Cysteine String Protein-α, in Platelet Exocytosis

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Background: Platelets use SNARE-mediated exocytosis to control vascular microenvironments via the release of cargo from the three types of granules: dense, α, and lysosomes. In addition to affecting vascular homeostasis, these secretion events also affect thrombosis and hemostasis. To better understand how the process of exocytosis is regulated, we have probed for new SNARE regulators and in this study focus on α-synuclein, a potential v/R-SNARE chaperone, and its binding partner, Cysteine String Protein-α (CSPα). These abundant proteins are the only detectible members of their respective families present in platelets.

Objectives: To determine the role(s) of α-Synuclein and Cysteine String Protein-α in SNARE-mediate platelet secretion**.**

Methods: We have performed detailed phenotyping of both α-synuclein$^{-/-}$ and CSPα$^{-/-}$ mice, specifically examining their platelet function. The levels of platelet secretory machinery were assessed by quantitative western blotting. Secretion from each granule type was measured using secretion kinetic experiments. Platelet aggregation and ADP release were examined using a Lumi-aggregometer. Platelet activation and morphology were examined using cytometry and microscopy. Localization of proteins in platelets was also assessed by Super-Res Immunofluorescence microscopy. Protein-binding partners were examined by pull-down experiments using GST-tagged proteins. An *ex-vivo* arterial microfluidics model was used to examine thrombus formation at low arterial shear rates. Hemostatic defects were evaluated in different injury contexts using tail-bleeding, $FeCl_3$ carotid injury, and jugular puncture models.

Results: Secretion measurements showed that α-synuclein$^{-/-}$ platelets have slightly defective serotonin and β-hexosaminidase release, but Platelet Factor 4 (PF4) secretion was similar to wild-type. Tail bleeding times for α-synuclein$^{-/-}$ mice were slightly increased compared to wild-type mice. Occlusion times in the $FeCl_3$ carotid injury model and cessation of bleeding in the jugular puncture model were similar between α-synuclein$^{-/-}$ and wild-type mice. The v/R-SNARE and t/Q-SNARE levels were unchanged in α-synuclein$^{-/-}$ platelets. Localization studies indicate that α-synuclein is localized to a granule population in platelets with some co-localization with the lysosomal marker, LAMP-1. CSPα also appears to have some co-localization with the α-granule marker, P-selectin, but there is a cytoplasmic pool as well. Preliminary pull-down experiment data suggest that α-Synuclein does not interact with the v/R-SNARE proteins, but CSPα does. Tail bleeding times for the CSPα$^{-/-}$ mice were significantly prolonged. CSPα$^{-/-}$ mice show a significant reduction in platelet activation and P-Selectin and LAMP-1 exposure compared to wild-type and heterozygous littermates. Preliminary data from the ex-*vivo* arterial microfluidics model show that CSPα$^{-/-}$ mice have defective thrombus formation at low arterial-shear rates compared to their wild-type and heterozygous littermates. Preliminary data examining v/R-SNARE and t/Q-SNARE levels in CSPα mice suggest that there may be a reduction in α-synuclein and SNAP-23 levels in the CSPα$^{-/-}$ mice. Further experimentation is underway to determine how α-synuclein and CSPα interact with the secretory machinery proteins to affect hemostasis.

Conclusion: These experiments demonstrate a role for α-synuclein and CSPα in platelet exocytosis and hemostasis. These data fill gaps in our knowledge of the physiological functions of α-synuclein and CSPα and our understanding of how platelet exocytosis is regulated.

This work is supported by grants from the NIH, NHLBI, VA Merit Award, and an NSF KY-WV LSAMP BD Fellowship (HL56652, HL138179, HL150818, NSF HRD 2004710) to S.W.W. and A.N.S.

Factor XIII has Differential Effects in Mouse Models of Abdominal Aortic Aneurysm

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Undergraduate

Background: The pathogenesis of abdominal aortic aneurysm (AAA) involves progressive dilation of the abdominal aorta with concomitant deposition and growth of a metabolically active intraluminal thrombus (ILT). The activated form of coagulation factor XIII (FXIII-A_2B_2), FXIII-A*, is a hemostatic enzyme essential for inhibiting fibrinolysis by irreversibly crosslinking fibrin and antifibrinolytic proteins. While FXIII polymorphisms have been linked to increases in human AAA incidence, the role of this transglutaminase has not been definitively established in aneurysm pathophysiology. The objective of this study was to determine the role of FXIII depletion and deletion in mouse models of AAA.

Methods and results: Male *C57BL/6J* mice (10 – 12 weeks old) were given a specific siRNA sequence, designed to knock down FXIII-B mRNA in mice (siFXIIIB) or against luciferase as a placebo control (siLuc), via encapsulated lipid nanoparticles (LNPs) containing an ionizable cationic lipid administered to mice with a single IV injection at 1 mg siRNA/kilogram body weight. One week after injections, siFXIIIB (n = 10) and siLuc (n = 10) mice underwent laparotomy and topical elastase application (5µl 10 mg/mL porcine pancreatic elastase for 5 minutes). Mice underwent a second injection two weeks after topical elastase and were then sacrificed four weeks after elastase. The concentration of plasma FXIII-A protein was reduced by 97% ± 2% in the siFXIIIB mice at the end of the experiment compared to siLuc mice. While we observed increases in the abdominal aortic diameter (siLuc: 1.68 ± 0.13 mm – vs – siFXIIIB: 1.86 ± 0.14 mm), we observed Type I > Type III collagen deposition and decreased CD68 macrophage infiltration in siFXIIIB treated mice. Utilizing *Ldlr*$^{-/-}$*/FXIIIb*$^{-/-}$ mice, we demonstrated similar effects of genetic *FXIII* ablation in the angiotensin II model compared with the elastase model of aneurysm.

Conclusions: Our results demonstrate an unexpected disconnect between aneurysm diameter versus macrophage and collagen accumulation with siRNA attenuated or genetically deleted *FXIII* mice. These results suggest the effects of FXIII are disconnected from fibrinogen and may play a role in macrophage inflammation.

Mechanism of iGC regulation of host responses in pediatric sepsis

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Graduate Student

Background: Glucocorticoid (GC) therapy had been strongly recommended for pediatric sepsis (grade 1A). However, the recommendation was changed to grade 2C in 2020. Thus, there is an urgent need to determine if GC therapy is beneficial in pediatric sepsis. Relative adrenal insufficiency (RAI), characterized by impaired inducible GC (iGC) production in response to stress, develops in 33.3% of pediatric septic patients. But little is known about the pathogenesis of RAI in pediatric sepsis. Understanding the mechanisms of how iGC functions in sepsis may improve the efficacy of GC therapy for septic patients.

Methods and Results: Using 21-day-old adrenal SR-BI-specific knockout mice (SF1CreSRBIfl/fl) as an RAI pediatric mice model, we elucidated the mechanism of iGC regulation of host responses in pediatric sepsis. We performed RNA-seq analysis in the liver at 6h of CLP-induced sepsis to identify the genome-wide expression profiling in RAI mice. Analysis revealed the dysregulated inflammatory signaling in mice with RAI, including dysregulated GR signaling pathway in which NF-$\boldsymbol{\kappa}$ B and AP-1 serve as the major upstream transcriptional regulators. As a result, excessive inflammatory cytokines are produced and thus further augment inflammatory responses. Of note, major prognosis risk biomarkers in pediatric septic patients are conserved in mice with RAI, demonstrating the link between prognostic biomarkers to poor outcomes of pediatric sepsis displayed in the RAI mice model.

Conclusions: Using the unique adrenal insufficiency mice model, we demonstrated that iGC regulates host responses in pediatric sepsis mainly through transcriptional regulations of AP-1 and NF-κ B in GR signaling pathway, and cross-talk between inflammatory cytokines. The link between prognostic biomarkers to poor outcomes of pediatric sepsis is displayed in the RAI mice model. These findings suggested that lack of iGC production is a risk factor in pediatric sepsis.

Role of Glycogen Mobilization and Mitochondrial Bioenergetics in platelet function, hemostasis, and thrombosis

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Graduate Student

Platelets are one of the most metabolically active cells in the bloodstream. They are known to show metabolic flexibility, switching between glycolysis and OxPhos depending on oxygen tension and availability of substrates. Platelets also have metabolizable glycogen granules that contribute to the energy pool upon activation. The goal of this study is to investigate platelet energy metabolism under normal physiological conditions to better understand the relative roles of the major ATP-generating processes (glycolysis, OxPhos, and glycogenolysis).

To address the role of glycogen, we used 2 glycogen phosphorylase inhibitors and tested their effects on standard platelet functions *e.g.,* secretion, aggregation, clot contraction, and thrombus formation under shear. We also measured the glycogen levels in resting and activated platelets and in the presence of inhibitors. Consistent with previous studies, glycogen was utilized when platelets are activated with a 25% decrease upon thrombin-activation. Both inhibitors blocked the use of glycogen and led to a 3-fold increase in glycogen compared to controls. This data suggest the active turnover of glycogen in resting platelets. Our inhibitor studies strongly indicated that glycogen is dispensable for low-energy processes like aggregation but contributes to secretion, clot contraction, and thrombus formation under shear. The inhibition was rescued by either increasing external glucose or bypassing glycolysis with added pyruvate. This study shows that the glycogen granules are metabolically active and contribute to the energy pool upon activation; this pool is important for high-energy platelet functions like clot contraction.

Deletion of platelet glucose transporters (GLUT1 and GLUT3) revealed an essential role in platelet function and hemostasis and related glucose metabolism to hemostasis *in vivo*. Glucose can power platelets via glycolysis alone or in combination with OxPhos (in the mitochondria). We sought to probe the importance of platelet mitochondrial bioenergetics in hemostasis and thrombosis. Previously used mitochondrial inhibitors (antimycin, oligomycin) are toxic and cannot be used for *in vivo* studies, thus we developed two novel mouse models with altered mitochondrial function using a platelet-specific deletion of TFAM and QPC. TFAM (Transcription Factor A Mitochondrial), is essential for the maintenance, transcription, and translation of mitochondrial DNA. Its deletion is expected to disrupt platelet mitochondrial DNA, which encodes 13 subunits of OxPhos. QPC is a subunit of ubiquinol-cytochrome c reductase complex III. Its deletion is expected to disrupt Complex III, which is part of the platelet mitochondrial respiratory chain.

We confirmed TFAM KO using Western blot analysis. Using Seahorse Analyzer, we confirmed that deleting TFAM disrupted platelet mitochondrial bioenergetics in both resting and thrombin-stimulated platelets as seen as a 40% decrease in Oxygen Consumption Rate (OCR). We also observed a significant decrease in ATP production indicating the contribution of OxPhos to ATP generation. Aggregation was not affected but *in vitro* clot contraction and thrombus formation under shear were defective (58% increase in lag time, 30% increase in AUC, and 60% decrease in surface area coverage in KO). Both the KO animals showed an increase in tail-bleeding time in the KO, an increase in occlusion time in the $FeCl_3$ carotid injury model, and delayed hemostasis in a jugular puncture injury model with significantly higher rebleeding indicating that mitochondrial bioenergetics is important for clot stability. Using two novel mouse models with dysfunctional mitochondrial bioenergetics, we show that OxPhos is dispensable for low-energy demanding platelet functions such as aggregation but is important for secretion, clot contraction, hemostasis, and thrombosis.
Platelets show considerable plasticity in energy metabolism using both glycolysis and oxidative phosphorylation. Using mouse models and inhibitors, we show the relative importance of these energy-producing processes (table) and the fuels and metabolic pathway choices made by platelets at different functional stages. Improving our understanding of platelet metabolism and the relative contributions of each pathway in the future could lead to a better understanding of how metabolic disorders can affect thrombotic risk in diabetic and obese patients.

This work is supported by grants from the NIH and NHLBI (HL56652, HL138179, and HL150818), and a VA Merit Award

Functional Assay	**CP-31/CP-91 (glycogen Phosphorylase inhibitors)**	**TFAM (Platelet Mitochondrial Dysfunction)**	**QPC (Platelet Mitochondrial Dysfunction)**
Clot contraction	Defective	Defective at low thrombin	Defective at low thrombin
Secretion	Defective	Normal	Defective at low thrombin
Aggregation	No effect	No effect	No effect
Thrombus formation under shear	Defective	Defective	No effect (preliminary data)
Tail bleeding (hemostasis)	Not applicable	Defective	Defective
Jugular puncture (venous thrombosis)	Not applicable	Increased bleeding time	Slight increase in bleeding time
Ferric Chloride Injury (arterial thrombosis)	Not applicable	Increased occlusion time	Increased occlusion time

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Endosome Trafficking and Glycogen Metabolism in Platelet Function

Undergraduate

Endosome Trafficking:

Platelets are critical cell fragments most known for their role in clot formation to stop bleeding.However, they can also endocytose various substances including viruses, bacterial fragments,polystyrene beads, and even soot particles from diesel exhaust. Can these substances activatesaid platelets? Through endocytosis, materials can be trafficked to acidic environments wherethey can then be degraded by hydrolytic enzymes. Pathogens may manipulate this systemcausing lysosomal membrane permeabilization (LMP) then lysosomal cell death (LCD). To testwhether LMP occurs in platelets and thus affect their function, we assayed the effects of L-leucyl-L-leucine methyl ester (LLOMe), an acid-dependent polymer/pore forming compound.It was found that increasing concentrations of LLOMe led to a dose-dependent aggregation ofwash mouse platelets, indicating that the pore-forming drug can cause platelet activation.

Glycogen Metabolism:

Glycogen is a branched glucose polymer that is a major store of metabolic energy. Glycogen isused in several tissues but its role in platelet function is largely inferred and has not been directlytested.To probe the role of glycogen mobilization in platelets, pharmacologicalinhibitors ofglycogen phosphorylase CP-316819 and CP-91149 were tested in several assays that measureplatelet function.The results concluded that there was varying energy demand dependent on thebiomechanical process. It was found that when glycogenolysis is blocked, there is a dosedependent inhibition in clot contraction as well as an inhibition in secretion and thrombusformation but not aggregation.

Treatment of Monocyte-Derived-Macrophages with Plasma to Determine Age and *APOE* Effects on Lipid Droplet Accumulation

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Undergraduate

Rationale: Apolipoprotein E, or ApoE, is a lipid transport protein made in the periphery by the liver. There are three polymorphic alleles for *APOE*: *ε2, ε3, ε4*, each corresponding to different isoforms of the protein. The E2 and E4 isoforms differ in conformation and ability to bind and transport lipids. As a result, those with the ApoE2 isoform are predisposed to familial type III hyperlipoproteinemia, which often leads to atherosclerosis, a disease characterized by lipid-droplet-rich macrophages, or "foamy macrophages". Lipid droplets are organelles that store neural lipids such as cholesteryl ester and triacylglycerol and the ApoE protein can be found on the surface of these droplets. Lipid-laden foam cells in E2 carriers can aggregate in plaques and contribute to disease pathogenesis. Alternatively, the ApoE4 isoform has a conformational change that results in its reduced ability to transport low-density lipoproteins (LDL) and cholesterol, leading to a predisposition to hypercholesterolemia. The *APOE ε3* allele, which occurs in 77% of the population, is relatively less susceptible to cardiovascular disease. optimizes cell viability in culture to later determine the effects of APOE genotype, sex, and age on lipid droplets accumulation in monocyte-derived macrophages. freeze, and thaw protocol that

Methods: Peripheral blood mononuclear cells (PBMCs) isolated from whole blood samples using density-grade centrifugation in SepMate™ tubes were either cultured for immediate use. Or frozen for culture at a later date with DMSO in RPMI media. Varying concentrations of DMSO (5%, 10%, 15%) were tested to determine which would sustain the highest cell viability after thawing. Macrophage cell cultures were treated with a plasma: media solution in a 1:10 ratio. Plasma samples were treated with Calcium chloride to derive serum in order to prevent media coagulation due to clotting factors present in the plasma samples. Since the plasma samples had varying levels of lipids, triglyceride and cholesterol assays were used to quantify the amount of fatty acid in each sample to determine if there was a direct correlation to lipid droplet accumulation in the cell culture. Additionally, since lipid-droplet accumulation is of interest for this project, the FBS concentrations added to the DMEM:F12 media used to culture the monocyte-derived macrophages were varied in order to determine how strongly they affected lipid droplet area per cell to observe any possible ceiling effect.

Results: 10% DMSO freezing media was determined to be optimal for cell recovery after a freeze-thaw cycle. There was no significant correlation between the total cholesterol/triglyceride concentration in a plasma sample and the lipid droplet accumulation of macrophages. 5% FBS in DMEM:F12 media was the minimum concentration necessary to maintain the cell culture without cell death.

Conclusion/Future Directions: We currently have a database of almost 200 *APOE* genotyped individuals to age and sex match for venipuncture and subsequent experimentation. Using the above-mentioned optimized protocols, we will begin culturing and treating cells from human subjects in order to understand how lipid droplet accumulation is affected by *APOE* genotype, sex, and age.

The effect of testosterone on mouse blood pressure and food intake circadian rhythm and clock genes expression

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Postdoc

Objective - Men are more prone to hypertension than women, especially below the age of 50. Recently, it is appreciated that not only the level but also the normal circadian rhythm of blood pressure (BP) are essential. Disruption of BP circadian rhythm is emerging as an index for detrimental cardiovascular outcomes. However, it is mostly unknown whether, and if so, via what mechanism, male sex steroids regulate the circadian rhythm of BP. The current study investigates the role of male steroids on BP circadian rhythm and clock gene per2 oscillations.

Approach and Results - We measured BP, heart rate (HR), and locomotor activity in 36-weeks-old male mice at baseline, and 1-, 2-, 3- and 4-weeks after orchiectomy by radiotelemetry. In addition, we also measured food intake at baseline and 2 weeks after orchiectomy in BioDAQ cages. We found that the amplitude and robustness of circadian rhythms in BP, food intake HR, and locomotor activity were significantly reduced 2 weeks after the orchiectomy. A reduction in the active dark-phase mostly accounted for the decrease in the BP and locomotor activity circadian rhythms. In contrast, a reduction in the resting light-phase accounted for the decrease in HR circadian rhythm. The 24-hour average BP and locomotor activity were lowered from 111.5 mmHg to 105.2 mmHg and from 4.5 counts/min to 3.1 counts/min by orchiectomy ($p<0.05$, $n=8$). In contrast, the 24-hour average HR was not significantly altered by orchiectomy (533.0 beats/min vs. 546.2 beats/min). To investigate the potential role of clock gene in the orchiectomy-induced changes in the circadian rhythms, we used mPer2Luc mice, in which clock gene Per2 is fused with a luciferase reporter thus allow real-time monitoring of the clock gene Per2 oscillations. We investigated the Per2 oscillation ex vivo by LumiCycle. The preliminary results of the ex vivo monitoring found that the peripheral oscillators in aorta and white adipose tissue were phase advanced to various extents by orchiectomy.

Conclusions - Our findings suggest that male sex steroids play an essential role in maintaining normal circadian rhythms in BP, food intake, HR, and locomotor activity. In addition, male sex steroids modulate clock gene Per2 oscillations in various peripheral tissues to different extents.

Title: Depressive Symptoms and Health Activation in Family Caregivers of Patients with Chronic Illnesses

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Postdoc

Background: Family caregivers of patients with chronic illnesses are at higher risk of developing cardiovascular disease (CVD) than non-caregivers because of caregiver burden, lack of support, long-term caregiving responsibilities, caregiving-related distress, and higher rates of depression. Rurality is a major social determinant of health, and caregivers' problems are more acute in rural than non-rural caregivers and thus the risk of CVD is higher. To reduce CVD risk, health activation is critical among caregivers so that they engage in their own healthcare. However, little is known about the association of depressive symptoms with health activation in rural family caregivers.

Purpose: The purpose of this study was to examine the relationship between depressive symptoms and health activation in rural family caregivers of patients with chronic illnesses.

Methods: We used baseline data from a randomized, prospective, intervention study designed to reduce cardiac disease risk factors among family caregivers from rural locations. Health activation was measured using the Patient Activation Measure-10 (PAM-10). Depressive symptoms were measured using the Patient Health Questionnaire-9. We analyzed data using multiple linear regression and controlled for relevant covariates including age, gender, years of education, health status, and caregiving burden.

Results: Among the 217 caregivers with a mean age of 54 ± 14 years, 78% were female, 74% were married or cohabiting, 48% were caring for their spouse, and 66% had more than 12 years of education. About 44% perceived having good health and 25% perceived having very good and excellent health. Depressive symptoms were a significant predictor of activation after controlling for covariates (unstandardized $B = -0.227$, $p = 0.001$). Higher levels of depressive symptoms were significantly associated with lower activation.

Conclusions: Screening for mental health and offering mental consultation is important to promote health activation in family caregivers of patients with chronic illnesses.

Characterization of Platelets from A Newly Developed Obese Mouse Model: MS-NASH

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Graduate Student

Obesity is a multifactorial disease with many co-morbidities leading to multi-organ dysfunction. Current studies point towards dysregulation of platelet signaling as a key driver for increased risk of death due to cardiovascular disease and stroke. However, the exact mechanism remains elusive and warrant the need to further develop our understanding of platelets in metabolic disease. Recently, a newly developed obese mouse model, MS-NASH, proves to be clinically translatable with its ability to respond to anti-diabetic drugs and mimics the multifaceted aspects of the human metabolic syndrome without high fat diet, and disease severity correlates with body weight among littermates. More importantly, there are currently no studies investigating the hemostasis phenotype in these mice.

MS-NASH mice had higher overall weight, fat, lean, and total water mass. MS-NASH mice showed increased platelet GPVI protein, correlating with current studies in human obese patients. MS-NASH males showed significant increase in red blood cells ($p=0.0444$) and mean platelet volume ($p=0.0038$) without significant change in platelet count. This suggests a younger platelet population is present and potentially an increase in platelet clearance. Megakaryocytes levels were slightly elevated in the MS-NASH mice compare to C57BL/6 mice. Rebleeding incidents was increased in the MS-NASH mice compared to the C57BL/6 mice ($p<0.0001$). Interestingly, bleeding time was positively correlated with body weight in the MS-NASH mice. Microfluidics showed a decrease in thrombus formation on collagen from the MS-NASH males, corresponding with the tail bleeding assay. Based on this preliminary study, there might be a dysfunction in platelet biogenesis and clot stability in the MS-NASH mice.

Title: High fat diet blunts the increases in blood pressure due to renal collecting duct-derived human sPRR in female mice

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Graduate Student

Several studies have shown that soluble prorenin receptor (sPRR) plays an important role in blood pressure regulation. Elevated circulating sPRR has been identified as a biomarker for obesity and hypertension, and preeclampsia in women. In rodent models of hypertension, sPRR mediates blood pressure elevation through both Ang II-dependent and independent mechanisms. However, there is a gap in knowledge concerning the functional role of human sPRR, particularly derived from the kidney, in blood pressure regulation. Therefore, we investigated the role of renal-derived human sPRR in blood pressure control in mice fed a regular chow and a high fat diet.

Human sPRR-Myc-tag transgenic mice were bred with mice expressing Hoxb7/Cre to selectively express human sPRR in the collecting duct (RHsPRR). RHsPRR and control (CTL) female mice were either fed 2 months a standard diet (SD) (CTL=8; RHsPRR=10) or a high fat diet (HFD) (CTL=9; RHsPRR=17). Body weight was examined weekly and systolic blood pressure (SBP) was measured by radiotelemetry.

Renal-derived human sPRR did not change body weight females in either diet (SD: CTL: 28±1, RHsPRR: 30±1 g; HFD: CTL: 34±3, RHsPRR: 40±2 g). SBP increased significantly in SD fed female RHsPRR mice (SD: CTL: 118.7±2, RHsPRR: 127.2±3 mmHg, $P<0.05$). In HFD fed females, SBP was increased further only in control mice (HFD: CTL: 126.0±2, RHsPRR: 126.1±1 mmHg). HFD reduced baroreflex sensitivity similarly in both groups (SD: CTL: 4.5±0.5, RHsPRR: 3.5±10.3 mms/mmHg; HFD: CTL: 3.1±0.5, RHsPRR: 2.7±0.5 mms/mmHg, $P<0.05$). Male mice showed similar blood pressure between groups.

In RHsPRR females fed a regular chow, renal AngII levels were similar in male and female HsPRR compared with controls. However, renal AT1R gene expression (1.7±0.5 and 2.6±0.4 $2^{-\Delta CT}$, $P<0.05$) and ERK1/2 activation (0.3±0.0 and 0.6±0.1 AU, $P<0.05$), along with renal ETA (1.2±0.3 and 3.8±1.0 $2^{-\Delta CT}$, $P<0.05$), ETB (1.2±0.2 and 5.6±1.6 $2^{-\Delta CT}$, $P<0.05$) and ET1 (1.1±0.3 and 2.9±0.5 $2^{-\Delta CT}$, $P<0.05$) mRNA expression were significantly increased in females but not male RHsPRR mice.

Taken together, this data indicates that CD-derived human sPRR induces increases in blood pressure in female mice that are associated with the activation of the AngII signaling potentially stimulating the Endothelin-1 system. In addition, CD-derived s pre ents increases in blood pressure during obesity. uture studies will determine the intrarenal AS and T 1 components.

Title: Characterization of Sitosterolemia-causing mutations in the cytosolic domains of ABCG5 and ABCG8

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Undergraduate

Background: Sitosterolemia is a rare, recessive form of familial hypercholesterolemia and is caused by mutations in *ABCG5* and *ABCG8*. ABCG5 and ABCG8 are glycoproteins that form an obligate heterodimer (G5G8) within the endoplasmic reticulum prior to trafficking to the cell surface where they that promote biliary secretion of sterols. Missense mutations in clinically confirmed cases of Sitosterolemiā are of pārticular interest for their potential to reveal structure-function relationships in ABCG5, ABCG8, and other ABC transporters. Mutants may then be classified based on the underlying molecular defect resulting in Sitosterolemia.

Methods & Results: 316 subjects were enrolled in a study to determine the utility of whole genome sequencing at Weill Cornell Medical Center. Inclusion criteria included pre-diabetes and type 2 diabetes (A1C>6.5%), high risk for or presence of fatty liver disease (BMI ≥ 25kg/m2, ALT>2IU/L in females, ALT>33IU/L in males), glucose tolerance (during 75-g oral glucose tolerance test levels between 140 and 199 mg/dL), and the presence of dyslipidemia (TC>200, LDL>130, HDL<40, TG>250 mg/dL including on pharmacological agents with intent to decrease LDL and HDL cholesterol,<40mg/dL in males and <50mg/dL in females). Excluded

groups were individuals under 18 years, compensated and decompensated cirrhosis, and evidence or presence of hepatocellular carcinomas. genome sequencing was performed through the New York Genome Center using CLIA approved methods. A panel of metabolites and hormones related to inflammation, glycemic control, and blood lipids including phytosterols were measured at Boston Heart Diagnostics. Based on elevated plasma phytosterols, three subjects were found to have Sitosterolemia (β-sitosterol > 10 mg/L). Analysis of the coding region for *ABCG5* and *ABCG8* revealed three variants classified as Likely Pathogenic. Variants (ABCG8_G216D, ABCG5_R446Q, and ABCG5_Q392P) were generated by long-range PCR site-directed mutagenesis and confirmed by Sanger sequencing. Plasmids encoding native and variant ABCG5 and ABCG8 were co-transfected into human Huh7 hepatocytes and cell lysates analyzed by SDS-PAGE and immunoblot analysis. Maturation of G5G8 was assessed by electrophoretic mobility of ABCG5 ABCG8. Each variant supported maturation of G5G8. Two mutants in ABCG5 enhanced maturation G5G8 (R446Q, Q392P). The subcellular distribution of each variant was then assessed by indirect immunofluorescence microscopy. Whereas native G5G8 was localized to the cell surface, ABCG5_R446Q and ABCG8_G216D was distributed within an intracellular compartment that is yet to be determined.

Conclusions: Novel, likely pathogenic alleles associated with the clinical presentation of Sitosterolemia do not interfere and may enhance maturation of G5G8. However, two appear to disrupt trafficking of the G5G8 transporter to the cell surface.

Studies to investigate the impact of suppressing SAA on the progression of established AAAs in three mouse models

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Staff

Objectives: Obesity increases the risk for abdominal aortic aneurysms (AAA) in humans and enhances angiotensin II (AngII)-induced AAA formation in mice. Obesity is also associated with increases in serum amyloid A (SAA). We previously reported that deficiency of SAA significantly reduces AngII-induced inflammation and AAA in obese C57BL/6 and hyperlipidemic apoE-deficient (apoE$^{-/-}$) mice. In this study, we investigated whether SAA plays a role in the progression of an established AAA in mice.

Approach: AngII was infused (1000 ng/kg/min) for 12 weeks in three different mouse models. For Model 1, C57BL/6 mice were fed a high fat diet (HFD; 60% kcal as fat) for 16 weeks prior and throughout AngII infusion for a total of 28 weeks. For Model 2, apoE$^{-/-}$ mice were fed standard lab diet throughout the study. In both models, mice with at least a 20% increase in the luminal diameter of the abdominal aorta as assessed by *in vivo* ultrasound (US) before and after 28 days of AngII infusion were randomized to receive 5 mg/kg/wk of either a control antisense oligonucleotide (ASO) or ASO to suppress SAA expression (SAA-ASO). For Model 3, C57BL/6 mice lacking SAA1.1, SAA2.1, and SAA3 (TKO) but harboring a transgene that provided doxycycline-inducible, adipose-specific SAA expression (TKO-Tg^{fat}) were used. In this third model, all mice were fed HFD for 28 weeks, as in Model 1. After 16 weeks of diet feeding, the mice received doxycycline water (to stimulate SAA expression), infused with AngII for 28 days, and then randomized to continued doxycycline water (ongoing SAA expression) or normal water (removal of SAA expression) based on luminal diameter as in model 1 and 2. *In vivo* US was also done at the end of the study in all the three models.

Results: The suppression of SAA expression did not impact body weight and blood pressure in any of the three mouse models. Plasma SAA levels at the end of the experiment in Model 1 were 89.2±25.1 mg/L in the control ASO group and 18.6±0.2 mg/L in the SAA-ASO group (p=0.008). In Model 2 were 271.2 ±82.0 mg/L in the control ASO group, and 35.65±1.9 mg/L in the SAA-ASO group (p=0.007). For Model 3, plasma SAA levels were 262.8±21.1 mg/L in the doxycycline continued group and 19.35±2.0 mg/L in the doxycycline withdrawn group (p<0.0001). After the first 4 weeks of AngII infusion, the average luminal diameter was 1.86±0.1 mm in obese C57BL/6 mice (Model 1), 2.31±0.1 mm in apoE$^{-/-}$ mice (Model 2) and 1.83±0.1 mm in the transgenic mice (Model 3). In the obese C57BL/6 mice (Model 1), the average luminal diameters were 2.07±0.1 mm in the control ASO group and 1.64±0.1 mm in the SAA-ASO group (p=0.0015), indicating that suppression of SAA limited progression of AAA and may even have contributed to regression of luminal dilation. However, in apoE$^{-/-}$ mice (Model 2), the average luminal diameters were 2.84±0.3 mm in the control ASO group and 2.39±0.2 mm in the SAA-ASO group, p=NS, indicating no effect of SAA suppression on AAA progression in this model. In the transgenic model, the average luminal diameters were 1.92±0.2 mm in the doxycycline continued group and 2.08±0.2 mm in the doxycycline withdrawn group, p=NS, indicating no effect of removing SAA expression on AAA progression.

Conclusions: We demonstrate that suppressing SAA reduces the progression of an established AAA in obese C57BL/6 mice, but there was no effect of lowering SAA in hyperlipidemic mice or in transgenic mice expressing SAA only in fat. Thus, our findings indicate that the efficacy of suppressing SAA may be influenced by undefined factors that contribute to disease progression. Further studies are needed to identify the multi-factorial processes contributing to the development and progression of AAA.

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Clinical Signatures that Predict Outcome in COVID+ Patients Undergoing ECMO

Graduate Student

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causes symptoms that range from mild to life threatening. COVID-19 is accompanied by a thromboinflammatory state, and disease severity has a close association with thrombotic complications. Critically ill patients with COVID-19 may require advanced support, including extracorporeal membrane oxygenation (ECMO). This investigation is a retrospective study designed to uncover clinical indication of thrombotic events and/or outcomes in COVID positive patients that are undergoing ECMO. Our population consisted of COVID+ patients at the University of Kentucky that underwent ECMO treatment between February 23, 2020 and February 23, 2021. A total of 50 patients qualified for the study and data is being collected from their clinical admission. At discharge, 25 patients had expired, 14 were discharged to long-term acute care facility, 7 were discharged to rehabilitation or another hospital, and 4 were discharged home. There were 8 recorded thrombotic events within the population (5 DVTS, 1 pulmonary embolism, 1 stroke, and 1 incident of clotting within the ECMO device. Strangely, 7 of the 8 thrombotic events occurred in patients that survived to discharge. This is perhaps due to the shorter length of stay compared to those patients that expired. In order to investigate potential clinical differences between survivors and non-survivors, a variety of routine labs and demographics were compared within the population. Interestingly, there was a significant difference in platelet count following ECMO initiation with survivors maintaining higher platelet counts than the non-survivors. Similar observations in platelet count have previously been reported in patients with sepsis. No significant differences were observed in WBC, age, gender, or BMI. To date, no clinical indication has been uncovered that would predict thrombosis in COVID+ patients undergoing ECMO. The study will continue to investigate by considering ECMO parameters, past medical history, onset of COVID symptoms, and length of stay in the hospital.

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Deficiency of Serum Amyloid A Exacerbates Sepsis-induced Acute Lung Injury in Mice

Staff

Objectives: Serum Amyloid A (SAA) is a family of proteins whose plasma levels increase > 1000-fold in acute inflammatory states such as sepsis. Here, we investigated the role of SAA in sepsis using mice deficient in all three acute-phase SAA isoforms (TKO).
Approach and results: SAA deficiency significantly increased mortality rates in two experimental sepsis models. Sepsis was induced by cecal ligation and puncture (CLP) in the 8 weeks old wild-type (WT) and TKO mice. The cecum was ligated tightly at the 2/3 position and the cecum was punctured through with a 23 G sterile needle, and gentle pressure was applied to extrude a small amount (~1 mm) of feces. The bowel was returned to the peritoneal cavity, and the abdominal incision was closed. The 7 days survival rates in TKO and WT mice were: 25% and 55% after CLP ($p=0.02$; n=10 each strain/gender); In the polymicrobial sepsis model, 8 weeks old WT and TKO mice were injected with 500 µL of cecal slurry (CS) (derived from 50 mg cecal contents) intraperitoneally (*i.p.*) to induce polymicrobial sepsis. Nonsepsis control mice were injected with the same volume of 10% glycerol. Survival was monitored for 14 days and the survival rates in TKO and WT mice were 0% and 45% after CS injection ($p<0.0001$; n=9 each strain). 24-hours after CLP, there were no apparent differences in liver, heart or kidney histology between genotypes. However, TKO mice had exacerbated lung pathology, including consolidation of lung tissues and atelectasis, compared to WT mice. RNAseq analysis of lungs excised 24-hours after CLP identified 664 genes differentially expressed (404 upregulated and 260 downregulated) in TKO compared to WT ($p<0.05$). Some of the genes that showed profound induction in the lungs of TKO compared to the WT were PROZ, DBP1, CXCL1, CXCL2, ARG1 and ACKR1. Gene ontology analysis revealed a significant enrichment of differentially expressed genes associated with chemokine production, chemokine and cytokine-mediated signaling, neutrophil chemotaxis and neutrophil migration in TKO lung tissues compared to WT tissues ($p<0.01$). There was a significantly increased number of CD3+ T-cell infiltration into the lungs of TKO mice compared to the lungs from the WT mice.
Conclusions: SAA protects mice against sepsis-induced mortality, potentially by protecting the lung from tissue damage.

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Deficiency of Serum Amyloid A impairs hepatic lipid metabolism during acute inflammation in mice

Staff

Objective: Hepatic expression and plasma levels of Serum Amyloid A (SAA) increase more than a 1000-folds in acute inflammation. However, the purpose of this dramatic and transient increase is unclear. Here, we investigated the role of SAA in hepatic lipid metabolism during acute inflammation using mice deficient in all three acute-phase isoforms of SAA (TKO).

Approach and results: Acute inflammation was induced in mice by injection of lipopolysaccharide (LPS). Wild-type (WT) and TKO mice (n=20/strain; 8-12 weeks old) were injected with LPS (*i.p.*; 7.0 mg/kg) and survival of the mice were monitored for 7 days. SAA deficiency significantly increased LPS-induced mortality in mice compared to control WT mice (Survival rates in TKO and wild-type (WT) mice were 55% and 90% respectively; $p<0.0001$; n=10 each strain/gender). There were no significant differences in endotoxin levels and plasma or hepatic inflammatory cytokines levels between the two strains of mice. However, TKO mice exhibited impaired lipid homeostasis compared to the WT mice. Plasma cholesterol levels were significantly lower for TKO mice compared to WT mice (137.7±34.9 mg/dL for WT and 103.0±17.8 mg/dL for TKO; $p=0.016$). Hepatic triglyceride levels were significantly higher in TKO mice compared to WT mice (30.55±7.8 and 40.8±6.8 mg/g tissue for WT and TKO

$p=0.007$). Consistently, histological analysis of oil Red-O-stained liver sections showed significantly increased lipid accumulation ($p=0.017$) with increased number of large lipid droplets (>20 μm^2; $p=0.003$) in TKO mice compared to WT mice. Expression of genes involved in β-oxidation of fatty acids and cholesterol metabolism were significantly lower in the livers of TKO mice compared to WT.
Conclusions: SAA protects mice against acute inflammation induced mortality without regulating endotoxin clearance or innate immune response. SAA is possibly involved in metabolic adaptation to acute inflammation by regulating metabolism of triglycerides and cholesterol.

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RAAS Triple-ATM Analysis in Hypertension Profiling: Assay Performance Characteristics and LC-MS/MS System Compatibility

Faculty

RAAS Triple-A testing is based on a clinical high-throughput mass-spectrometry assay for simultaneous quantification of Angiotensin I, Angiotensin II and Aldosterone in standard serum by renin-angiotensin - aldosterone system (RAAS) equilibrium analysis. Equilibrium angiotensin and aldosterone levels are used to calculate markers for renin-activity (PRA-S), angiotensin-converting-enzyme activity (ACE-S), and adrenal function (AA2-Ratio). In this study we, evaluated the analytical performance of the recently developed RAAS Triple-A^{TM} CE-IVD kit for clinical mass spectrometry. Fifty serum samples were obtained from hypertensive patients on standard therapy. Three frozen single use aliquots of 500 µl serum were analyzed on 3 different LC-MS/MS systems (Waters Xevo-TQ/S, AB Sciex 6500, and Thermo ALTIS+) using RAAS Triple-A LC-MS/MS kits (Attoquant Diagnostics, Vienna). Samples were processed according to the manufacturer's instructions and target analytes were quantified. Results were compared between different laboratories and kit stability and assay robustness were assessed following 3 months of kit storage.

De Novo Synthesis of Elastic Fibers in Dissected ortas of -aminopropionitrile-administered Mice

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Postdoc

Introduction
Elastin, a major component of extracellular matrix, is vital for aortic development and maintenance of integrity. It assumed that elastin synthesis does not occur in aortas of adults, as elastin fibers are stable molecule that may have a half-life as long as ~70 years. However, there is no direct evidence to verify this speculation. In addition, it remains unclear whether and how age-dependent alteration of elastin synthesis impacts the integrity of aortic wall.

Methods and Results
First, elastin mRNA abundance was assessed in the aorta of male C57BL/6J mice at different ages (0, 2, 4, 10, 20 and 40-week-old, n=6/group). qPCR revealed that elastin mRNA was more abundant in the aorta at 2 weeks than 0 week of age, and decreased drastically at 4 weeks of age. Of note, elastin mRNA was barely detectable after 10 weeks of age. Lysyl oxidase, a key regulator in crosslinking of elastic fibers during aortic development, were also examined. Consistent with the temporal changes in elastin mRNA abundance, both molecules peaked at 2 weeks of age, and were much less abundant after 10 weeks of age. These results indicate that elastin synthesis and crosslinking were highly activated in the juvenile phase, but diminished in the adult phase. We next investigated the impact of age-dependent deletion of elastin synthesis and crosslin ing on aortic integrity. -aminopropionitrile (BAPN, 0.5% wt/vol) was administered for 12 weeks to induce aortic aneurysms and dissections in juvenile and adult male C57BL/6J mice (4, 24 weeks of age, n=15, 37/group). Corresponding to elastin mRNA abundance, development of aortic aneurysms and dissections were observed only in mice with BAPN started at juvenile, but not adult, phase. It is noteworthy that multiple laminas of fragmented elastic fibers were detected adjacent to the false lumen of dissected aortas. Since our time course study revealed that elastin synthesis was diminished after 10 weeks of age, elastic fibers observed around the false lumen was considered to be synthesized in response to aortopathy formation.

Conclusion
Elastin mRNA synthesis is suppressed in the aorta of adult mice. However, its synthesis is reactivated in dissected aortas of BAPN-administered mice.

Unfolded Von Willebrand Factor Interacts with Protein S and Limits Its Anticoagulant Activity

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Graduate Student

Background: The critical plasma anticoagulant protein S (PS) circulates in two pools: free (anticoagulant) or bound to complement component 4-binding protein (C4BP) (antiinflammatory). Acquired free PS deficiency occurs in patients with severe viral infections, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and is associated with increased risk of thrombosis. von Willebrand Factor (VWF) is a large multimeric protein , which unfolds under shear, exposing protein binding sites. Elevation of VWF antigen and activity are associated with infection-induced vascular endothelial activation, including during SARS-CoV-2 infection. Here, we identified a shear-dependent association between VWF and PS, and assessed the effect of VWF on PS anticoagulant activity.

Methods: This study was approved by the University of Kentucky Institutional Review Board. Citrated plasma was collected from consenting adults, including 28 SARS-CoV-2+ inpatients, 49 SARS-CoV-2+ outpatients, and 31 healthy controls.

Results: Initial studies in our SARS-CoV-2+ patient cohort revealed that free PS, a measure of the anticoagulant pool, was similarly reduced in both inpatients (p=0.037) and outpatients (p=0.008) compared to controls, while total PS (p=0.964) was unchanged. This indicated an increase in a plasma PS-binding protein. However, known abundant PS-binding proteins C4BPβ and protein C, an anticoagulant that functions with PS, were unchanged (p=0.647 and p=0.185, respectively), and Mer, a macrophage receptor that binds PS, was only mildly increased (p=0.042).

Mass spectrometric and immunoblotting analyses revealed that PS interacts with VWF in pooled donor plasmas and that this interaction is enhanced >10,000,000-fold by shear-induced unfolding of VWF. Plasma VWF was elevated 3.8-fold in inpatients ($p<0.0001$) compared to outpatients and controls, suggesting endothelial activation, though no change in multimer distribution was apparent (n=4). We hypothesize that shear-induced unfolding of VWF exposes a binding site, which interacts either directly or indirectly with PS and contributes to acquired free PS deficiency. Consistent with this hypothesis, sheared-VWF dose-dependently inhibited free, but not total, PS antigen measurements, when measuring either purified proteins or plasma.

We next assessed the functional consequences of the VWF/PS interaction using purified proteins. Sheared-VWF dose dependently reversed FXa inhibition by the PS/Tissue Factor Pathway Inhibitor-α complex, in a FXa activity assay. Conversely, Activated Protein C (APC) cofactor function was unaffected, either on phospholipid vesicles or on the surface of activated platelets, as measured either by the cleavage of factor Va heavy chain by western blot or by the rate of thrombin activation. Finally, to assess the PS function in patients, we measured plasma thrombin generation. Despite anticoagulation, plasma thrombin generation in inpatient samples was comparable to controls (Endogenous Thrombin Potential (ETP): $p=0.257$; Lag Time: $p=0.095$), suggesting a significant hypercoagulability, possibly exacerbated by PS deficiency. Consistent with this, free PS negatively correlated with plasma thrombin generation parameters measured with thrombomodulin (TM) supplementation, which is required for sensitivity for APC/PS pathway (free PS with ETP: $p=0.0008$, $r=-0.443$); ETP ratio (ETP with TM/ ETP without TM) in inpatients were higher compared to controls indicating less APC/PS activity ($p=0.009$); and these patients had profoundly elevated D-dimer ($p<0.0001$).

Conclusion: We propose a novel mechanism of acquired free PS deficiency, by which pathological shear forces unfold VWF, which interacts with PS, and at least partially blocks its anticoagulant function. It is possible that unfolded VWF further limits PS anticoagulant activity *in vivo* by sequestering it from APC, factor IXa, or other interacting partners. We anticipate that this mechanism also contributes to acquired free PS deficiency in other inflammatory conditions in which VWF is similarly unfolded.

A comprehensive and optimized protocol for the production of the EcoHIV-1 and essential quality controls

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Advances in science and HIV1 awareness made HIV1 infection a controllable chronic health issue with increased survival. At the end of 2020, 38 million individuals were living with HIV1, a ~24% increase relative to 2010. To keep HIV1 under control, these patients must adhere to their combined Antiretroviral Therapy (cART) for the rest of their lives as there is no cure. Naive and cART treated HIV1 infected individuals have a higher risk of developing cardiovascular diseases. So, regardless of the cART therapy, HIV1 patients are at risk of developing neurodegenerative and cardiovascular complications at advanced stages of the disease. Therefore, there is an urgent need to identify the main biological players in HIV-1 infection. Currently, there are several models to study HIV-1 and among them is the EcoHIV-1 system. In this system the coding region of gp120 of HIV-1/NL4-3 and HIV-1/NDK was replaced with the gp80 coding sequence of the ectotropic Murine Leukemia Virus (MLV). This modification makes this virus safe to work with as it is not capable any more to infect humans, but capable of infecting rodents only. The EcoHIV-1 model was first described in 2005 and has been widely used to safely investigate HIV-1 vaccines, therapy and HIV-1 related pathogenesis. The current caveat in the EcoHIV-1 model is the lack of standard protocol for viral production and the abscess of quality controls to calculate the viral titer and check the infectivity of the viral preparations. Hence, we aimed to optimize a comprehensive protocol for the production of the EcoHIV-1 and what are essential quality controls that should be used to calculate the virus titer and how infective the virus preparations are. We have successfully demonstrated that we can make infectious EcoHIV-1 in our lab and inject it into different mice strain. In addition, we have successfully detected the virus RNA in the spleen and liver of the infected mice.

In-Utero Morphine Exposure Increases Cardiovascular Disease Risk Factors in Adult Offspring in association with Endogenous Opioid Peptides Dysregulation

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Abstract

Chronic opioid exposure and opioid use disorder are associated with nearly a 2-fold increased risk of CVD and 16% increase in coronary artery disease. Opioid abuse during pregnancy increases the incidence of neonatal opioid withdrawal syndrome (NOWS) 5 fold and is associated with low birth weight and premature delivery; however, the effect of OUD among pregnant women on their offspring health is understudied. We hypothesized that offspring exposed to morphine (MOR) in utero would show increased CVD risk factors associated with the dysregulation of the endogenous opioid peptides in cardiovascular tissues. Sprague Dawley dams were treated with saline (VEH, n=8) or escalating doses of MOR (5-20 mg/kg/day, s.c, n=8) during gestation. Pups were weaned on a standard diet. Maternal weight gain during pregnancy, litter size, and birth weight were not different post-MOR exposure. However, female and male MOR-exposed offspring showed reduced body length ($P<0.05$) and body weight from weeks 1-3 of life ($P<0.05$), followed by a catch-up growth effect. By week 16, female and male MOR-exposed rats showed reduced tibia length ($P<0.05$) and body weight. Lipid profile was assessed as a risk factor for coronary artery disease using FPLC. MOR-exposed offspring showed increased LDL (F-MOR vs. VEH ~2 fold; M-MOR vs. VEH ~1.5 fold mg/dL; n=6-8, $p<0.05$). Fasting blood glucose, normalized to lean body mass (LBM), was increased in MOR-exposed offspring (F-MOR 1.38±0.1 vs. F-VEH 1.11±0.06 mg/dL/g of LBM; M-MOR 1.26±0.1 vs. M-VEH 0.93±0.02 mg/dL/g of LBM, n=7-8). Proteinuria was not different in MOR-exposed rats vs. VEH (F-MOR 4.6±0.8 vs. F-VEH 5.5±1mg/ml; M-MOR 29.1±1.4 vs M-VEH 37.5±2.95 mg/mL; n=7-8), while ACR was increased in both male and female MOR-exposed males ($p<0.05$, n=7-8). Although circulating levels of angiotensin peptides were similar between groups, MOR increased mean arterial pressure ($p<0.05$), eliciting a greater depressor response to mecamylamine (F-MOR -46.25±6 vs F-VEH -18.3±1.3, M-MOR -50.5±0.28 vs M-VEH -25±5.1 delta mmHg, n=2-3, $p<0.05$). However, MOR exposure exacerbated maximal AngII-induced vasoconstriction in the isolated aorta in males ($P<0.05$) while induced endothelial dysfunction in females ($P<0.05$). Proenkephalin, an endogenous opioid peptide exerting a negative modulation of pro-constrictive G-protein coupled receptor (GPCR) signaling, showed reduced mRNA expression in the heart, kidney, and aorta from MOR vs. VEH ($p<0.05$). Taken together, dysregulation of endogenous opioid peptides in cardiovascular tissues secondary to prenatal MOR exposure is associated with neurogenic hypertension and vascular dysfunction.

Cardiomyocyte-Restricted Deletion of RAD Improves Heart Failure

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Background: Heart failure (HF) is the second leading cause of hospitalization, represents a third of cardiovascular disease deaths, and is projected to increase in prevalence by 46%. Dilated cardiomyopathy (DCM) is the most common cardiomyopathy resulting in systolic heart failure with reduced ejection fraction (HFrEF). Current therapies fail to address a principal issue: loss of contractile force. Targeting dysfunctional Ca^{2+} handling proteins involved in excitation-contraction, such as $Ca_v1.2$ may offer a means to improve quality of life and attenuate adverse cardiac remodeling. Cardiomyocyte-restricted deletion of RAD, a $Ca_v1.2$ regulatory unit that mediates beta-adrenergic receptor (beta-AR) signaling stably improves cardiac function in healthy mice and human heart slices.

Hypothesis: RAD ablation—after heart failure development—improves systolic cardiac function by increasing trigger Ca^{2+}.

Methods: The muscle lim protein knockout mouse (MLPKO) is model of human DCM, and was used to test if tamoxifen-inducible, cardiomyocyte restricted RAD deletion in 10-week-old mice improved function and attenuated pathology relative to MLPKO mice with RAD still present. Longitudinal echocardiography for *in vivo* assessment was performed, bulk RNAseq of hearts, isolated live cell ventricular cardiomyocyte Ca^{2+} imaging and sarcomere function in addition to whole-cell patch clamp recordings of $Ca_v1.2$ current. Beta-AR agonist was used to test cellular beta-AR responsiveness in isolated cells.

Results: Tamoxifen-induced cRADKO of MLPKO mice significantly increased ejection fraction versus single-knockout MLPKO mice with RAD present after 1 month in males and females. Left ventricle dilatation was also reversed significantly in cRADKO MLPKO mice. Transcripts associated with HF (*Nppb, Acta1, Myh7, Xirp2*) were downregulated significantly in cRADKO MLPKO. Electrophysiological whole-cell patch clamp recordings of cardiac $Ca_v1.2$ demonstrated increased current in cRADKO MLPKO. *Ex vivo* live cell experiments showed significant improvements in Ca^{2+} transients, notably in amplitude and decay kinetics. Sarcomeres showed significantly improved fractional shortening, integrals, and rates of contraction and relaxation.

Conclusions: This ongoing study implicates Rad as a new therapeutic target for HFrEF and DCM.

Manipulating platelet secretion to affect hemostasis

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Background:
Platelet granule secretion is crucial for maintaining vascular microenvironments. The members of soluble N ethylmaleimide sensitive Attachment Receptor family (SNARE) are critical to platelet secretion. Vesicle SNAREs/ Vesicle- Associated Membrane Proteins (VAMPs)/ v- SNAREs located on granules and t- SNAREs located on the plasma membrane mediate granule/plasma membrane fusion and subsequent cargo release. Despite significant progress, how platelet SNAREs are used and what role(s) platelet secretion plays in hemostasis are still unclear. To address this, we genetically manipulated four platelet isoforms of VAMPs (VAMP-2, -3, -7, and -8), either alone or in combinations. Platelets from these animals showed distinct secretion phenotypes and significant differences in hemostasis in three types of injury models.

Methods:
We generated global V-$7^{-/-}8^{-/-}$, V-$3^{-/-}7^{-/-}$ and platelet-specific V-$2^{\Delta/\Delta}3^{\Delta/\Delta}7^{-/-}8^{-/-}$ mice. Western blotting was used to confirm the absence of these proteins. We used *ex vivo* assays to monitor the kinetics and activation-dependency of the release of serotonin from dense, PF4 from α, and β-hexosaminidase from lysosomal granules. FACS assays were also used to monitor secretion of α (P-selectin) and Lysosomal (LAMP1) granule release. Electron microscopy and ImageJ were used to study platelet and granule morphology. In-vivo assays ($FeCl_3$-mediated arterial injury, venous puncture injury and tail bleeding assay) were used to monitor hemostasis in each mouse strain.

Results:
The strains were healthy without any gross abnormalities, although, V-$7^{-/-}8^{-/-}$ animals were smaller. Microscopic images of platelets showed no differences in α-granule number or size except for V-$7^{-/-}8^{-/-}$ platelets which were larger. Secretion analysis demonstrated that V-$3^{-/-}$ and V-$7^{-/-}$ platelets had normal secretion, however, V-$3^{-/-}7^{-/-}$ platelets showed decreased lysosomal release suggesting that they may have overlapping functions in lysosomal release. The deletion of VAMP-7 and VAMP-8, in combination, had no further defect on secretion beyond that of V-$8^{-/-}$ platelets. Interestingly, the levels of VAMP-2 were increased in the double-deletion platelets. Platelets from V-$2^{\Delta/\Delta}3^{\Delta/\Delta}7^{-/-}8^{-/-}$ mice exhibited the most dramatic decrease in the secretion, although secretion was not completely abolished. Analysis of hemostasis in these strains showed context-specific defects. V-$7^{-/-}8^{-/-}$ and V-$2^{\Delta/\Delta}3^{\Delta/\Delta}7^{-/-}8^{-/-}$ mice had the most robust bleeding in all three models. However, loss of VAMP-8 alone did affect occlusion in the $FeCl_3$ injury model. By

comparing the phenotypes in these strains, we can better define how much secretion is required for hemostasis in our injury models.

Conclusions:

VAMP-8 is the major platelet VAMP isoform. If it is present, deletion of other VAMPs had no effect on secretion. However once VAMP-8 is deleted, VAMP-2 appears to partially compensate. VAMP-3 and -7 are not significant contributors to platelet secretion though they are needed for lysosome release. The hemostatic phenotypes of our strains show that by manipulating the types and levels of VAMP-isoforms in platelets, we can modulate bleeding in three different injury models. Additionally, the bleeding phenotypes in the various strains suggest that secretion may be contextually required for some injury but not others. These models will be instrumental in probing physiological processes in which platelet contribution is critical such as wound healing, angiogenesis, and inflammation.

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Does neonatal estradiol treatment masculinize daily eating behavior rhythms in female mice?

Undergraduate

Circadian rhythms are behaviors and bodily functions, such as eating and sleep, that fluctuate every 24 hours. Disruption of circadian rhythms increases obesity and diabetes. Previous studies from our lab showed that estrogens protect female mice from weight gain and circadian disruption. Ovariectomized female mice that have no circulating estrogens and were fed high-fat diet had dampened rhythms of eating behavior and ate across the day and night. Treating ovariectomized female mice with estradiol restored eating behavior rhythms. In contrast, adult male mice treated with estradiol and fed high-fat diet had disrupted eating behavior rhythms. These data suggest that hormones are required during development to masculinize or feminize the rhythm of eating behavior. Testosterone in males can be aromatized to estradiol to masculinize the brain and behaviors. In this study, we tested the hypothesis that neonatal estradiol treatment of female mice masculinizes their daily rhythm of eating. We injected female mice subcutaneously with 17-β estradiol (E2) or oil as a vehicle for 5 days after birth. At 6 weeks old, mice were ovariectomized and implanted with Silastic tubing containing E2 or oil. Mice were single housed in cages in 12L:12D in light-tight boxes and activity and eating behavior rhythms were measured. Mice were fed 10% kcal low-fat diet for 1 week and then 45% kcal fat high-fat diet for 1 week. We found mice treated with oil as neonates and as adults, and thus approximating feminized, ovariectomized females, had greater weight, adiposity, and fasting blood glucose than the other groups. These feminized, ovariectomized (oil-oil) females also consumed more food and had disrupted eating behavior rhythms during high-fat feeding. In contrast, females treated with oil as neonates and E2 as adults, which approximates gonadally-intact females, had lower body weights and relatively robust eating behavior rhythm amplitudes during high-fat feeding. Our preliminary data suggest that high-fat feeding of females treated with E2 as neonates decreased the amplitude of the eating behavior rhythm. These data suggest that neonatal E2 treatment may alter the responsiveness of the eating behavior rhythm to high-fat diet in female mice.

HDL concentration and function are associated with adhesion molecule suppression during acute surgical stress

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Background: Atherosclerosis is the leading cause of vascular disease worldwide, and frequently requires surgical treatment. Every year in the United States, over 900,000 patients undergo cardiac surgery and over 500,000 patients undergo major vascular surgery. Postoperative acute kidney injury (AKI) is common after these surgical procedures and is associated with an increased risk of chronic kidney disease development/advancement and death. A higher preoperative high-density lipoprotein (HDL) concentration is associated with a reduced risk of AKI after cardiac and vascular surgery. In animals, one intravenous dose of HDL directly before renal ischemia reduces renal intercellular adhesion molecule-1 (ICAM-1) expression, renal neutrophil infiltration, renal oxidative damage, and AKI. In vitro studies confirm that HDL reduced endothelial ICAM-1 expression in a dose-dependent manner. Further, in vivo administration of antibodies again ICAM-1 can reduce ischemic AKI, supporting the role of ICAM-1 in AKI. We hypothesized that if higher preoperative HDL concentrations are associated with less AKI due to downregulation of endothelial ICAM-1 expression during surgery, a higher preoperative HDL concentration would be associated with a lower plasma concentration of sICAM-1 after surgery.

Methods: After obtaining IRB approval, we prospectively recruited 80 adult patients undergoing major, elective surgery on the heart and/or large blood vessels. To enrich our cohort for our outcome of interest, AKI, we recruited patients with chronic kidney disease. Plasma samples were collected at induction of anesthesia and immediately after surgery. HDL cholesterol concentration (HDL-C) was determined using a selective enzymatic hydrolysis method. Because patients with chronic kidney disease are known to have impaired HDL function, we also measured HDL cholesterol efflux capacity (CEC) as a marker of global HDL function. HDL CEC was measured in J774 macrophages loaded with ^{3}H-cholesterol in acetylated-LDL and then incubated with ApoB-depleted patient serum for 24 hours and expressed as percent efflux, normalized to a control sample to account for inter-assay variability. A commercially available ELISA assay was used to quantify plasma sICAM-1 concentration. Multivariable linear regression was used to estimate the association between HDL-C, HDL CEC, and plasma sICAM-1 concentrations.

Results and Discussion: The median HDL concentration in this cohort was 38 mg/dL (10^{th} and 90^{th} percentile (27, 55)). In this cohort, the median preoperative plasma sICAM-1 concentration was 260 pg/mL (162, 471) and the median postoperative sICAM-1 concentration was 153 pg/mL (97, 260). HDL-C was correlated with HDL CEC (Pearson's R=0.34, p=0.002). After adjustment for age, sex, diabetes mellitus, and exposure to cardiopulmonary bypass, known AKI risk factors, a higher preoperative HDL concentration was independently associated with a lower plasma sICAM-1 concentration immediately after surgery (p=0.03). Every 10 mg/dL increase in HDL concentration was associated with a 19 ng/mL decrease in postoperative sICAM-1. Similarly, a higher preoperative HDL CEC was independently associated with a lower plasma sICAM-1 concentration immediately after surgery (p=0.01), such that every 10% increase in CEC was associated with a 24 ng/mL decrease in postoperative sICAM-1.

Conclusion: Higher preoperative HDL-C and CEC were found to be independently associated with a lower plasma concentration of sICAM-1 after surgery. A previously published clinical trial demonstrated that in healthy humans with low HDL, eight weeks of fenofibrate treatment increased HDL cholesterol concentration by roughly 8 mg/dL and reduced plasma sICAM-1 concentration by 40 ng/mL, demonstrating that pharmacological treatments that raise HDL in humans can reduce sICAM-1 concentrations. To further these investigations, we will quantify each patient's HDL capacity to suppress endothelial cell expression of ICAM-1 in vitro and analyze the association between this specific HDL function and risk of postoperative AKI.

Determining the functional significance of KCNH2 variants of uncertain significance identified in a large patient biobank

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Objective: Long QT type 2 syndrome (LQT2) is a Mendelian arrhythmogenic syndrome caused by loss of function mutations in the cardiac K^+ channel *KCNH2* (Kv11.1). This study aims to determine whether we can identify candidate LQT2-causing *KCNH2* variants from a large patient biobank.
Methods: 15 rare *KCNH2* missense variants were identified from a large patient biobank (MyCode), VarSome, and ClinVar databases. Where some variants were classified as "pathogenic", "likely pathogenic", most were variants of uncertain significance (VUS). We used allele frequency, in silico prediction programs, and functional testing in Human Embryonic Kidney 293 cells to determine if rare *KCNH2* variants associated with phenotypic markers of LQT2 in Electronic Health Records (EHR).
Results: All 15 *KCNH2* variants had an allele frequency of <0.02% in the Genome Aggregate Database. In silico prediction programs FTHMM, MetaSVM, and Primate AI were used to assess variant impact on Kv11.1 channel protein. At least one of these programs predicted that all 15 variants were damaging, two programs predicted 8 of the variants were damaging, and all of these programs predicted that four variants were damaging. Functional analysis showed five variants disrupted Kv11.1 channel gating compared to wild type Kv11.1 channels. Two out of 15 variants reduced macroscopic K^+ current by disrupting Kv11.1 channel protein trafficking. Among 109 patients who harbored one of the 9 variants classified as pathogenic, damaging by all three in silico programs, or disrupted Kv11.1 channel function, none had clinical EHR data to support the presence of LQTS (e.g., QTc interval prolongation or LQTS diagnosis).
Conclusion: Although LQT2 is a Mendelian disorder, classification and functional analyses that delineate *KCNH2* VUS as pathogenic do not reliably identify people with LQTS-related phenotypes in EHR. These findings suggest the *KCNH2* VUS with phenotypic markers of LQT2 may not be associated with a LQTS diagnosis.

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Creating a pipeline to assist clinicians in the diagnosis and prognosis of type B aortic dissection

Undergraduate

Background: Type B aortic dissection is a catastrophic disease that affects 3 in every 100,000 persons. Aortic dissection occurs when a layer or several layers of the artery wall begin tearing away from the true wall, splitting the aorta into a true and false lumen. and forming a flap. There are two types of aortic dissection: Type A and Type B. For type A entry tearing occurs in the ascending aorta and Type B where tearing begins in the descending. These dissections result from elevated amounts of wall shear stress repeatedly being applied to the artery wall as the heart beats. The development of this pipeline is designed to give clinicians accurate data as to better understand the condition their patient is in.

Pipeline Flow: Diagnosis is done with 1 of 2 methods. Typically done in an emergency room setting, CT or Ultrasound are tools for aortic dissection visualization and understanding. Using the combination of the two we can reconstruct a full 3D geometry of the dissection and run a computational fluid dynamic model over this geometry. This gives us an accurate depiction of the hemodynamic state of the dissection at any moment in time. Using this visualization technique, we can analyze areas of high wall shear stress. These areas are known to lead to the growth of the dissection and possible rupture of the aorta. Understanding shear stresses on the aortic wall will allow clinicians to get a better understanding of where treatment might be more urgent than others.

Deletion of *Period* genes exacerbates diet-induced obesity in female, but not male, mice

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Staff

Obesity differs in men and women. Our previous studies demonstrate that sex differences in obesity in mice are mediated in part by differential responses of circadian behaviors to high-fat feeding in males and females. Other studies also showed circadian *Period* genes regulate diet-induced obesity in mice. In this study, we sought to investigate the role of the *Period* genes in regulating sex differences in diet-induced obesity. *Period1* and *Period2* have primary roles in the molecular circadian timekeeping mechanism, while *Period3* regulates rhythms in peripheral tissues. Male and female C57BL/6J wild type (WT), *Per1/2* KO, and *Per1/2/3* KO mice were singly housed in 12L:12D and fed high-fat diet (45% kcal fat) for 12 weeks. We measured daily rhythms of eating behavior and locomotor activity as well as adiposity, food intake, and glucose tolerance. We found a striking sex difference in obesity such that disabling the *Period* genes exacerbated adiposity in female, but not male, mice. Adiposity was doubled in *Per1/2* KO females and tripled in *Per1/2/3* KO females compared to WT females. In contrast, there was no effect of disabling the *Period* genes on adiposity in males. Increased adiposity in female *Period* KO mice was not due to increased energy intake since they ate fewer calories during the experiment than WT females. We next examined whether daily rhythms were differentially affected in *Period* KO mice. Both male and female *Per1/2* KO and *Per1/2/3* KO mice had advanced phases and reduced amplitudes of locomotor activity compared to WT mice. Since locomotor activity rhythms were similarly disrupted in male and female *Period* KO mice, activity rhythm alterations may not account for their sex difference in obesity. We previously found that during high-fat feeding, WT female mice maintain high-amplitude daily rhythms of eating behavior which protect them from diet-induced obesity. Our preliminary results suggest that *Per1/2/3* KO females had disrupted eating rhythms on high-fat diet because they had more daytime eating behavior than WT females. Together these results reveal a sex difference in *Period* regulation of diet-induced obesity. Moreover, this study demonstrates that sex is a critical factor when studying the interplay between circadian rhythms and metabolic risk.

This study was funded by National Institutes of Health grants R01DK124774, R03DK098321, P30GM127211, and the Diabetes Research Center at Washington University in St. Louis under award number P30DK020579, as well as NSF IOS-2045267, the Gertrude F. Ribble Trust, and the University of Kentucky.

Role of RIPK3-Mediated Smooth Muscle Cell Death in Sporadic Aortic Aneurysm and Dissection Development

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Background
Progressive loss of aortic smooth muscle cells (SMC) contributes to aortic aneurysm and dissection (AAD) formation through multiple cell death pathways. Two forms of lytic cell death are necroptosis, regulated by receptor-interacting protein 3 kinase (RIPK3) and downstream factors, such as mixed lineage kinase domain like pseudokinase (MLKL); and pyroptosis, initiated via gasdermin D (GSDMD). We tested the hypotheses that (1) RIPK3-mediated necroptosis contributes to aortic degeneration and AAD formation; and (2) RIPK3 activates GSDMD to initiate the pyroptosis pathway.

Methods
Activation of RIPK3 was compared in aortic tissues of 20 patients with sporadic ascending thoracic aortic aneurysms and acute dissections (ATAAD) and 8 organ donor controls. The relation of RIPK3 to GSDMD-mediated pyroptosis was studied in human aortic SMCs. In a sporadic AAD mouse model induced by challenging mice with a high fat diet for 8 weeks and angiotensin II infusion (2000ng/kg) for 4 weeks, AAD development was compared in wild type (WT; n=60) and *Ripk3* knockout (*Ripk3*$^{-/-}$; n=40) mice.

Results
The levels of phosphorylated RIPK3 and MLKL in aortic tissues were significantly higher in ATAAD patients than in controls ($p<0.05$). Challenged *Ripk3*$^{-/-}$ mice had reduced incidence and severity of AAD compared to challenged WT mice ($p<0.001$); the reduction in AAD was observed in both males ($p<0.001$) and females ($p=0.004$). In human SMCs, H_2O_2 treatment induced the expression of RIPK3 which was found to directly interact with the N-terminal of GSDMD.

Conclusions
RIPK3 and MLKL are activated in human ATAAD tissues. RIPK3 deficiency significantly reduces aneurysm formation in a murine model of AAD. RIPK3 directly interacts with GSDMD. Further studies will be required to examine the role RIPK3 in activating GSDMD-mediated pyroptosis in SMCs, and the role of SMC-specific RIPK3 in SMC injury and ATAAD formation.

Micro-CT Visualizes Extensive Vascular Pathologies in Smooth Muscle Cell-specific LRP1 Deficient Mice

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Objective:

Low-density lipoprotein receptor-related protein 1 (LRP1) is a multifunctional protein that is abundant in vascular smooth muscle cells (SMCs). SMC-specific deletion of LRP1 leads to vascular pathologies in the aorta and superior mesenteric artery (SMA) of mice. Although other vascular beds have not been explored. Micro-computed tomography (CT) enables scanning in a high spatial resolution (~20 μm). To determine the regional effects of SMC-specific LRP1 deficiency in mice, we performed detailed morphometric analyses of vascular pathologies using micro-CT.

Approach and Results:

Male and female wild-type and SMC-specific LRP1 deficient mice were monitored for aortic and SMA dilatations from 6 weeks through 40 weeks of age using ultrasonography. Progressive luminal dilatations of the ascending aorta were detected in both male and female SMC-specific LRP1 deficient mice, compared to their wild type littermates. Ultrasound was only able to detect the superior mesenteric artery within ~2 mm of the branch from the abdominal aorta. Therefore, ultrasonography is not optimal for monitoring the SMA. At the age of 40 weeks, mice were euthanized, perfused with saline and injected with Microfil silicone rubber through the left ventricle. Subsequently, each mouse was scanned by a Bruker SkyScan 1276 high-resolution desktop micro-CT and 3 dimensional images were generated. Aortic CT images showed profound dilatations of the entire aorta and its main branches, with more pronounced changes in the ascending thoracic and infrarenal aortic regions. Micro-CT also detected longer and tortuous aortic structure, compared to their wild type littermates. The most striking pathologies, including both dilation and tortuosity, were observed in the SMA and its branches with more depth and detailed insights, compared to ultrasonography.

Conclusions:

The ability to visualize the entire vasculature of mice using micro-CT technology demonstrated that SMC-specific deficiency of LRP1 has marked heterogeneity in development of vascular pathologies.

ABSTRACT TITLE: Packaging of platelet factor 4 by megakaryocytes is dependent on the intracellular proteoglycan, serglycin, and influences the bone marrow microenvironment.

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Graduate Student

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Background: Megakaryocytes (MKs) are polyploid cells resident to bone marrow that produce platelets. In our previous work, we described the role of an intracellular proteoglycan, serglycin, in the packaging of alpha-granule proteins in platelets. Serglycin-/- platelets had significant decreases in the amounts of basic cytokines, particularly the abundant platelet factor 4. The goal of this study is to examine the role of serglycin at the megakaryocyte stage of alpha-granule biogenesis in order to determine where alpha-granule proteins are being aberrantly trafficked in the absence of serglycin. Many of the cytokines stored in the alpha-granules of platelets have documented effects on the bone marrow microenvironment, such as platelet factor 4 affecting hematopoietic stem cell and MK development, and TGF-beta driving fibrosis. This led us to hypothesize that potential changes in the sorting of these molecules in the tightly regulated microenvironment of the bone marrow may affect marrow development.

Methods: To investigate this relationship, we have utilized primary MKs from Serglycin$^{-/-}$ and NBEAL2$^{-/-}$ mice. Whole bone marrow was isolated from euthanized mice, mature cells were removed by magnetic separation and immature cells were then cultured with thrombopoietin. Samples for western blot and ELISA were taken daily. PF4 levels were then assessed by western blot and ELISA (R&D systems). To address *in vivo* levels of PF4 we isolated bone marrow plasma by centrifugation. The *in vivo* changes to MK number/size as well as distribution of alpha-granule proteins were evaluated by confocal microscopy of marrow in femurs. To assess the maturation of MKs within the bone marrow, we analyzed DNA content by PI staining.

Results: The results of these experiments showed that PF4 is secreted by MKs normally during their development as shown in WT mice. However, the deletion of Serglycin led to increased secretion of PF4 during earlier stages of MK development, and a near complete depletion of internal PF4 by day 5. Comparison to NBEAL2$^{-/-}$ MKs showed a lack of internal PF4 and very low levels of secreted PF4. These results are supported by the *in vivo* measurements of PF4 in bone marrow plasma, which showed much higher levels of extramedullary PF4 in Serglycin$^{-/-}$ compared to WT, and almost no detectable PF4 in NBEAL2$^{-/-}$ bone marrow plasma. Analysis of DNA content of marrow cells indicated a shift toward the 2n population and a decrease in the number of higher ploidy cells in both Serglycin$^{-/-}$ and NBEAL2$^{-/-}$ mice. Consistently, examination of marrow from Serglycin$^{-/-}$ and NBEAL2$^{-/-}$ mice showed decreased MK size and a diffuse pattern of PF4 staining, comparted to WT.

Conclusions: These results taken together indicate that: Serglycin and NBEAL2 are necessary for proper PF4 storage and secretion in MKs; that Serglycin is needed to retain PF4 inside the MK, and that without Serglycin, PF4 secretes or "leaks" from α-granules and MKs into the extracellular space; MKs derived from Serglycin$^{-/-}$ and NBEAL2$^{-/-}$ mice are not matured to the same extent as those from WT mice, possibly due to alterations in the levels of PF4 in the microenvironment.

Micro-CT Imaging and 3D Reconstruction of Mouse Aorta and Major Branches

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Undergraduate

Objective:

Aortic aneurysm is the disease in which the pathology is mostly defined as permanent dilation of aortic walls. Therefore, accuracy in quantification of aortic dilation in mice is crucial to research of the disease. Traditional technique of measuring aortic dilations, such as *in situ, ex vivo,* and ultrasonography, all exhibit certain shortcomings. Micro-CT scan provides imaging with very high resolution (up to ~20 m), which can be used to reconstruct 3D models of mouse aortas using an open-source program *3D Slicer*. We adopted these two techniques combined to determine volume of aortas and how this model provide better quantification of aortic dilation in mice.

Approach and Results:

Male and female wild-type and SMC-specific LRP1 deficient mice were euthanized at the age of 40 weeks. Then, they were perfused with saline and injected with Microfil silicone rubber through the left ventricle. Subsequently, each mouse was scanned by a Bruker SkyScan 1276 high-resolution desktop micro-CT. The micro-CT images were processed to reconstruct 3D models and take volume measurements of aortas and superior mesenteric arteries (SMAs) by *3D Slicer*. The 3D models showed dilatations along the aorta, with most severe pathology on the ascending aorta, aortic arch, and infrarenal region. There is significant dilation of SMAs in the knock-out mice with aneurysm as well. Moreover, measurements of aortic lengths indicated the aneurysmal aortas had longer average length than their wild-type counterparts.

Conclusion:

The micro-CT imaging and 3D reconstruction technique provides an important method to visualize and quantify aortic dilations with high accuracy. Meanwhile, small branches on the aorta like SMA, which was not detectable by ultrasonography, can be clearly measured using micro-CT.

Title: Effects of human angiotensinogen and human renin in proximal tubule cells on development of atherosclerosis in hypercholesterolemic mice

Authors:
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PostDoc

Objective: This study determined whether angiotensinogen (AGT) interacted with renin in renal proximal tubule cells (PTCs) to promote atherosclerosis.

Approach and Results: We first determined whether hepatocyte-derived AGT interacts with renin in PTCs to promote atherosclerosis. Transgenic mice expressing human renin in PTCs driven by a kidney androgen-related protein promoter (KAP-hREN) in an LDLR -/- background were used. To induce the synthesis of human AGT in hepatocytes, AAV containing human AGT with a liver-specific promoter was injected intraperitoneally. Three groups of male littermates were administered testosterone to activate human renin expression in PTCs: (1) wild type mice administered null AAV, (2) KAP-hREN transgenic mice administered null AAV, and (3) KAP-hREN transgenic mice administered AAV containing human AGT. Two weeks after administration of testosterone and AAVs, mice were fed a Western diet for 6 weeks. Induction of human AGT in liver and human renin in PTCs did not increase atherosclerosis, comparing to the other two groups. Next, to evaluate the direct interaction of AGT and renin in PTCs for promoting atherosclerosis, KAP-human AGT (KAP-hAGT) and KAP-hREN double transgenic mice were fed a Western diet for 12 weeks. Although immunostaining confirmed the presence of human AGT and human renin in PTCs, double transgenic mice did not have increased percent atherosclerotic lesion area, comparing to either wild type or single transgenic littermate groups.

Conclusions: The presence of human AGT in liver or PTCs with the combination of human renin in PTCs did not augment Western diet-induced atherosclerosis in mice.

Popliteal Artery Aneurysm Repair – A Single Center Experience

Nicholas Demas MSII
Research Mentor: Sibu P. Saha, MD, MBA
University of Kentucky

Medical Student

Objective:

Popliteal artery aneurysm (PAA) is a relatively rare disease diagnosed clinically or with imaging modalities. These aneurysms may present with lower extremity ischemia but can be asymptomatic at the time of discovery. The two current repair methods are endovascular and open repair. Our aim is to review the experience at University of Kentucky Medical Center and compare endovascular versus open repair and their outcomes from January 2010 to December 2019.

Clinical Materials:

I have reviewed a total of 79 charts with IRB approval. These charts included 4 females and 75 males ranging in age from 22 to 89 years (mean 63.5). Seventy cases presented with symptoms of lower limb ischemia (88.61%), and nine cases were asymptomatic at the time of aneurysm discovery (11.39%). Endovascular repair was done in 38 cases (48.10%), and open repair was done in 41 cases (51.90%). Four open repair cases used a GoreTex graft, and the remaining 37 open repair cases used vein grafts. Thirteen patients underwent bilateral popliteal artery aneurysm repair (16.46%). Eight cases were diagnosed as popliteal artery pseudoaneurysms (10.13%). Seven of the eight pseudoaneurysm diagnoses received open repair, and the remaining 1 case received endovascular repair. Five pseudoaneurysm cases were due to an infection, one case was from pseudoaneurysms forming after reintervention of a femoral graft, one case was due to trauma from a crush injury, and one case was a focal giant cell reaction with fibrotic vessel wall elements.

Results:

Thirty-four patients were followed for more than 2 years (43.04%). Twelve total cases required surgical reoperation within 30 days of the initial repair (15.19%). 5 endovascular repairs required

reoperation, and 7 open repairs required reoperation. Regarding the 5 endovascular repair reoperation cases, one was to fix a thrombosed endovascular stent with open repair, one was a fasciotomy for compartment syndrome, one was debridement of necrotic tissue and fasciotomy for compartment syndrome, one was a balloon angioplasty for a partially occluded stent, and one was debridement of necrotic tissue and a balloon angioplasty. Regarding the 7 open repair reoperation cases, one was evacuation of a leg hematoma, one was evacuation of a leg hematoma and bypass repair for an area of active extract, one was open evacuation for a surgical incision site hematoma, one was for ligation of groin lymphatics and wound vac placement for incision site drainage, one was a balloon angioplasty, one was debridement of necrotic tissue, and one was a fasciotomy with debridement of necrotic tissue. Fourteen total amputations were performed after the initial aneurysm repair (17.72%), with ten involving the knee, one involving the forefoot, and four involving the toes. Eight amputations were performed within 30 days of aneurysm repair, with 7 being above-the-knee (AKA) and 1 being a forefoot amputation. Four amputations were performed within 90 days of aneurysm repair, with 3 being above-the-knee and 1 amputation of four toes. Two amputations were performed after 90 days of aneurysm repair, where one was a transphalangeal amputation and the other a transmetarsal amputation. Three patients died within 30 days of their procedure during their hospital stay (3.80%). One patient died from acute respiratory failure due to cardiac failure; one patient died from a non-ST elevation myocardial infarction; one patient died from respiratory failure.

Conclusion:

Popliteal artery aneurysms are a relatively rare disease, and the current methods of repair include endovascular repair or open repair using either a synthetic or vein graft. In our experience, endovascular repair and open repair had a similar number of reoperations within 30 days. Open repair reoperations were done more often for debridement of necrotic tissues and related to the surgical incision site and hematomas. Endovascular reoperations were done more to fix thrombosed grafts. Endovascular repair had a higher number of amputations within 30 days of aneurysm repair, with 6 amputations compared to the 2 amputations for open repair cases. Endovascular repair is appealing for its shorter hospital stay, especially for asymptomatic cases and when a minimally invasive approach is preferred, but it does have an increased risk of stent thrombosis and amputation from our study findings.

Cardiac fibroblast deactivation: the Holy Grail or another obstacle to overcome?

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PostDoc

Objective: Cardiac fibrosis is a major component of heart disease and is a hallmark of decreased cardiac function. Additionally, 1 in 4 people who suffer from a heart attack will have another, leading to a need to determine if there are consequences of repeated fibroblast activation. Recently generated mouse models have allowed for our lab to perform the first study of cardiac fibroblasts during *in vivo* cardiac fibrosis resolution.

Methods and Results: Our preliminary data demonstrates that the activated fibroblast lineage is maintained in the heart after fibrosis resolution, and we have interrogated the fate and altered function of these previously activated cardiac fibroblasts during deactivation and healing. Interestingly, transcriptome analysis on these retained cardiac fibroblasts showed many fibroblast-specific genes had returned to quiescent levels. However, these deactivating fibroblasts also acquired a unique gene expression profile with an up-regulation in genes involved in extracellular matrix degradation, proliferation, and myofibroblast dedifferentiation leading us to a variety of potential therapeutic targets. Of particular interest is Runx1, a transcription factor with a well-defined role during development, which is not expressed in mature cardiac fibroblasts until after injury. As reported in the literature, *Runx1* is turned on in activated fibroblasts, but never returns to baseline levels. To further investigate the role of *Runx1* in the fibroblast activation continuum, we have generated fibroblast specific *Runx1* conditional knockout mice to study the role of this transcription factor during activation, deactivation, and reactivation.

Conclusion(s): These genetic changes in previously activated fibroblasts, including a constant expression of *Runx1*, indicate that deactivated fibroblasts are altered from their previous quiescent state and our preliminary data further demonstrates that these altered fibroblasts become hyperactive upon subsequent insult and these changes result in severe pathological consequences.

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Protease activated receptor 2 is critical for vascular smooth muscle cell transition to a macrophage-like state

Graduate Student

Introduction: Studies have demonstrated that in atherosclerosis, vascular smooth muscle cells (VSMCs) migrate from the media to the intima and undergo dedifferentiation into macrophage-like cells (MLCs). It is estimated that dedifferentiated VSMCs contribute to 50-70% of the population of macrophages present in atherosclerotic lesions. Protease activated receptor 2 (PAR2) is a receptor found on the surface of VSMCs and is upregulated in atherosclerosis. The objective of this study is to determine the role of PAR2 in VSMC dedifferentiation in atherosclerosis.

Methods and Results: To determine the role of PAR2 in atherosclerosis, *LDLr* $^{-/-}$ mice that were either *Par2*$^{+/+}$ or *Par2*$^{-/-}$ were fed a high fat/cholesterol diet for 12 weeks, which then were sacrificed to quantify atherosclerotic burden. *Par2*$^{-/-}$ showed an attenuation in atherosclerotic lesion area in the aortic sinus and the aortic arch compared to *Par2*$^{+/+}$ mice. The proposed pathway of this attenuation is PAR2 mRNA in VSMCs is bound and stabilized by the RNA binding protein human antigen R (HuR), which leads to an upregulation in PAR2 expression. PAR2 may also upregulate Krüppel-like factor 4 (KLF4), which plays a role in the regulation of VSMCs dedifferentiation. *Par2*$^{+/+}$ and *Par2*$^{-/-}$ VSMCs were treated with water soluble cholesterol for 72 hours or were left untreated for control. qPCR was performed to measure VSMC markers (α-actin and myosin heavy chain) and macrophage markers (CD68 and Mac-2). After cholesterol treatment, *Par2*$^{+/+}$ VSMCs showed a downregulation of VSMCs markers and upregulation of macrophage markers when compared to *Par2*$^{-/-}$ VSMCs, suggesting *Par2*$^{+/+}$ VMSCs transition to a MLC. In a hybrid mouse diversity panel conducted on 101 strains of mice with induced atherosclerosis at the University of California at Los Angeles, PAR2 was found to be significantly correlated with KLF4 (top 50 gene; P = 2.77 x 10- 17). In *in vitro Par2*$^{+/+}$ and *Par2*$^{-/-}$ VSMCs, KLF4 mRNA expression was lower in *Par2*$^{-/-}$ VSMCs versus *Par2*$^{+/+}$ VSMCs at baseline. Finally, *Par2*$^{+/+}$ and *Par2*$^{-/-}$ VSMCs were analyzed for HuR mRNA expression using qPCR. *Par2*$^{-/-}$ VSMCs had less HuR mRNA expression compared to *Par2*$^{+/+}$ VSMCs. To further investigate PAR2 and HuR, VSMCs were treated with actinomycin D and then were treated with a HuR inhibitor for 3 hours or left untreated as control. HuR inhibitor treated cells had less PAR2 mRNA expression than the control cells, suggesting HuR binds and stabilized PAR2 mRNA. Preliminary data using an SM22 Cre mouse line bred to a HuR flox mouse line is demonstrating a trend towards more atherosclerotic burden in the Cre negative mice versus their Cre positive littermates.

Conclusions: These results suggest that VSMC PAR2 activation mediates the dedifferentiation of VSMCs via the upregulation of KLF4 and the subsequent binding by HuR, which stabilizes PAR2 mRNA and leads to further upregulation of PAR2. Future studies will continue to study the proposed VSMC dedifferentiation pathway regulated by PAR2 using various *in vitro* experiments as well as several mouse models.

INHIBITION OF SODIUM GLUCOSE COTRANSPORTER 1 (SGLT1) O-GLCNACYLATION REDUCES Na^+ INFLUX AND THE OCCURRENCE OF PRO-ARRHYTHMOGENIC Ca^{2+} SPARKS IN DIABETIC RAT CARDIOMYOCYTES

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Staff

Rationale: Type-2 diabetes (T2D) is associated with a higher risk for arrhythmias, but the underlying mechanisms are incompletely elucidated. The activity of Na^+-glucose cotransporter 1 (SGLT1) is increased in T2D hearts, resulting in myocyte Na^+ overload, which promotes the occurrence of pro-arrhythmogenic Ca^{2+} sparks. Addition of *O*-lin ed β-*N*-acetylglucosamine (O-GlcNAc) to Ser/Thr residues is a post-translational modification that is exacerbated in diabetes and was previously shown to affect the activity of several proteins involved in cardiac ion transport.
Methods/Results: Hearts from patients with T2D showed higher levels of global protein O- and GlcNAcylation compared to hearts from lean patients without T2D. Using rats that express human amylin in the pancreatic β-cells (HIP rats) as a model of late-onset T2D and their wild-type (WT) littermates as controls, we found that a larger fraction of SGLT1 co-immunoprecipitates with O-GlcNAc in T2D hearts. Thus, cardiac SGLT1 undergoes O-GlcNAcylation and this modification is enhanced in T2D. Inhibition of O-GlcNAcylation with 6-diazo-5-oxo-L-norleucine crystalline (DON) resulted in reduced Na^+ influx in HIP myocytes. This effect was prevented in myocytes where SGLT1 was inhibited with phlorizin. In reverse experiments, enhancing O-GlcNAcylation with Thiamet-G in myocytes from WT rats resulted in larger Na^+ influx. These results suggest that O-GlcNAcylation affects myocyte Na^+ regulation by activating SGLT1. Inhibition of O-GlcNAcylation also reduced Ca^{2+} spark frequency in HIP rat myocytes and this effect was significantly less pronounced with SGLT1 blocked.
Conclusion: O-GlcNAcylation increases SGLT1 activity in T2D hearts; Inhibition of O-GlcNAcylation reduces Na^+ influx and the frequency of Ca^{2+} sparks in T2D rat cardiomyocytes.

Time Restricted Feeding Modifies Autonomic Regulation of the Heart in Young and Older Mice

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Undergraduate

Background: Studies show restricting the timing of food intake to the wrong time of day in mice can have large effects on cardiac electrophysiology, including slower heart rates and a shift in the 24-hour rhythm of the heart rate to align with timing of feeding. Our hypothesis is time restricted feeding to the wrong time of day impacts heart rate by altering autonomic regulation of the heart.

Methods: Wild type male mice aged 4-6 (younger) and 16-18 months (older) were housed in a 12-h:12-h light-dark cycle with ad libitum access to food and water or with food access restricted to a 7-hour window during the light cycle. Mice were implanted with telemetry transmitter units and in vivo telemetry was used to measure electrocardiograms. We measured heart rate (HR) and heart rate variability (HRV) using the RR interval. Both HR and HRV were continuously measured with ad libitum access to food (ALF) or following 2-weeks of time restricted feeding (TRF).

Results: We identified a robust correlation between the fluctuations in the RR interval and the power of the high frequency component in HRV (P_{hf}) in young mice during ALF and TRF. The amplitude of the P_{hf} increased with TRF. Compared to younger mice, the strength of the correlation between fluctuations in the RR and P_{hf} during ALF was weaker in older mice. However, TRF in older mice strengthened the correlation and increased the amplitude of P_{hf} similar to younger mice.

Conclusion: Our data suggest that TRF increases autonomic regulation of the heart to slow HR and align the 24-hour rhythm in HR with the timing of feeding. TRF in older animals modifies the autonomic regulation of the heart similar to younger animals. These data suggest feeding behavior is a key driver in the autonomic regulation of the heart in young and older animals.

Platelet-derived Transforming Growth Factor-β Ameliorates AAA in a Murine Model

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Graduate Student

Background: Platelets are rapidly recruited to the forming intraluminal thrombus (ILT) in abdominal aortic aneurysm (AAA), stabilizing the aortic wall and preventing rupture. Adhesion and activation of platelets releases a diverse mix of inflammatory mediators such as transforming growth factor β (TGFβ). TGFβ is a pleiotropic cytokine that influences processes ranging from cell proliferation to regulation of inflammatory signaling. While initial studies found TGFβ signaling was increased and correlated with increased risk for AAA, repeated TGFβ neutralization studies have found contradictory augmented aneurysm and ruptures in AAA and Marfan Syndrome models. The source of this protective TGFβ signaling remains unknown. The purpose of this study was to determine if platelet-derived TGFβ augments aortic growth in the elastase AAA model.

Methods and Results: *Tgfβ1* and *Tgfβ* receptor 2 (*TgfβR2*) floxed mice were obtained from Jackson Labs and bred with the platelet-specific Cre line platelet factor 4 (*Pf4*). Male *Tgfβ1-Pf4*$^{Cre+}$ (n = 2) and *Tgfβ1-Pf4*$^{Cre-}$ (n = 4) were subjected to laparotomy and topical elastase application (5μl 10 mg/mL porcine pancreatic elastase for 5 minutes). Aortic diameter was measured weekly via in vivo ultrasound (VisualSonics Vevo 2100) and mice were sacrificed at 28 days. *Tgfβ1-Pf4*$^{Cre+}$ mice had augmented abdominal aortic diameters compared to *Pf4*$^{Cre-}$ mice (*β1-Pf4*$^{Cre-}$: 1.53 ± 0.21 mm; *β1-Pf4*$^{Cre+}$: 2.20 ± 0.04 mm). A combination of male and female mice with *TgfβR2-Pf4*$^{Cre-}$ (n = 4) and *TgfβR2-Pf4*$^{Cre+}$ (n=9) underwent the same experimental process. *TgfβR2-Pf4*$^{Cre+}$ mice had significantly augmented abdominal diameters compared to *Cre*- mice (*TgfβR2-Pf4*$^{Cre-}$: 1.42 ± 0.08 mm; *TgfβR2-Pf4*$^{Cre+}$: 1.72 ± 0.09 mm; $P < 0.05$).

Conclusion: Our results further support the view that TGFβ signaling has a protective role in AAA and that platelet-derived TGFβ, in particular, is important for AAA mitigation. The mechanism by which TGFβR2 signaling on the platelet requires further research as well as investigation of other platelet-derived TGFβ (β2 and β3) in other mouse models of AAA.

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Adipose tissue expression of HuR modulates cardiac pathology via adipose tissue-derived extracellular vesicles

Staff

Adipose tissue serves a broad role as an endocrine organ and has been shown to have a multitude of effects on cardiac physiology depending on metabolic state, adipose depot location, and primary cell type. We have previously shown that adipocyte-specific deletion of HuR (Adipo-$HuR^{-/-}$) in mice impairs acute thermogenesis, a canonically brown adipose tissue (BAT)-driven function. Interestingly, we also show that loss of HuR expression in adipose tissue is sufficient to induce the spontaneous development of cardiac pathology marked by hypertrophy and fibrosis.

The goal of this work is to identify the adipose depot(s) responsible for the cardiac endocrine effects observed in our model and begin to decipher the underlying mechanisms. Here, we show that mice with HuR deletion specifically in brown and beige adipocytes (BAT-$HuR^{-/-}$ using a UCP1-driven cre) maintain normal cardiac function compared to Adipo-$HuR^{-/-}$ mice. This result suggests a white adipose tissue (WAT)-specific mechanism, consistent with our previously published bioinformatic analysis suggesting HuR-dependent adipose tissue-derived extracellular vesicles (Ad-EVs) from subcutaneous WAT (scWAT) as the mediator of cardiac pathology. Accordingly, our results show that EVs isolated from scWAT of Adipo-$HuR^{-/-}$ mice, but not wild-type littermate controls, induce a significant increase in hypertrophic gene expression in cultured cardiomyocytes.

In conclusion, our results demonstrate that loss of HuR expression in adipose tissue is sufficient to induce cardiac hypertrophy and fibrosis, in part through endocrine-mediated Ad-EV signaling.

Nicotine Exposure Dysregulates RAS components in 3T3-L1 preadipocyte cells

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Undergraduate

Over 36.5% of the United States population is affected by obesity, which is a major contributing factor to the rise of hypertension and cardiovascular disease. Nicotine consumption causes an increase in blood pressure and a decrease in adipogenesis. The prorenin receptor (PRR) and its soluble form (sPRR) are newly discovered components of the renin angiotensin system (RAS), a blood pressure regulatory pathway. Circulating levels of PRR and sPRR are increased in obese subjects. Therefore, this study tested the hypothesis that nicotine promotes the PRR and sPRR production in 3T3-L1 preadipocyte cells.

3T3-L1 cells were grown to confluence and differentiated over 8 days. On day 8, the cells were treated with nicotine dose response treatments vehicle and nicotine doses (0.01-10 μM). After 24-hour treatment, cells were collected to measure protein levels of PRR and sPRR via western blot. Additionally, PRR, renin, 18S, furin, and S1P mRNA expression were measured in all treatments.

Nicotine significantly decreased PRR protein expression at 1μM compared to vehicle treatment (1.00±0.37 vs. 0.46±0.09 au; $p < 0.05$). In addition, nicotine significantly decreased sPRR protein expression in a dose -dependent manner compared to vehicle treatment (1.00±0.42 vs. 0.47±0.18 vs. 0.32±0.12 au vs. Vehicle; $p < 0.05$). Nicotine did not change the expression of S1P, and furin, two enzymes involved in PRR cleavage. Angiotensinogen (AGT) and renin increased significantly in differentiated adipocytes treated with nicotine compared to vehicle treatment ($p<0.05$).

In addition, mice were treated via inhalation chamber to either control cigarette smoke, or electronic vape daily for one hour, four days a week, for 1 month. The mice adipose tissue and plasma were then collected for analysis. Protein levels of PRR and sPRR in adipose tissues showed no changes in response to both smoke and electronic cigarette; however, male mice showed greater expression compared to female mice. Plasma AngII and sPRR were similar between group and were not affected by nicotine.

Together our data indicated that nicotine influences adipose tissue RAS expression by up-regulating the angiotensin II precursors, renin and AGT, and down-regulating PRR and sPRR expression. Together, these data suggest that nicotine could activate RAS components favoring pro-constrictive effects. In addition, reductions in PRR/sPRR could be a compensatory mechanism to counterbalance the RAS activation only in vitro conditions.

Female mice exposed to early life stress show MR-dependent increases in circulating IL17a and impaired endothelial function in response to an obesogenic diet

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Graduate Student

Studies have shown that interleukin 17 (IL-17) from Th17 lymphocytes may be an important factor in the pathophysiology of hypertension. IL-17 plasma concentration is increased in hypertensive type 2 diabetes (T2D) patients compared to non-hypertensive diabetes patients. IL-17 secretion is modulated by Th17 downstream of mineralocorticoid receptor (MR) activation on dendritic cells (DC). Th17 lymphocytes demonstrate tissue-specific infiltration and remodeling in models of obesity. Previously, we have shown that female mice exposed to maternal separation and early weaning (MSEW), a model of early life stress, display exacerbated obesogenic response to high fat diet feeding (HF), metabolic syndrome-like phenotype, and hypertension that can be attenuated by the chronic treatment with the MR antagonist spironolactone. Thus, the aim of this study was to test if there is an MR-dependent increase in IL-17a in female MSEW mice, and whether perivascular adipose tissue (PVAT) may contribute to modulation of endothelial function by producing higher amounts of this cytokine.

MSEW and control (C) mice were weaned onto HF (60 % Kcal from fat) for 20 weeks. Mice were randomized to receive either vehicle (50% Ora swift in drinking water) or spironolactone (100 mg/kg/day in vehicle) treatment for 2 weeks. Plasma was collected at the end of the study to measure cytokines using miliplex technique (n=5 per group). Also, thoracic aortas were isolated and cleaned for vascular reactivity studies, and perivascular adipose tissue (PVAT) was collected in DMEM (2% BSA, 2-hour incubation, 37C). Cumulative concentration response (CCR) curves were performed for acetylcholine (Ach, 10^{-5} to 10^{-9} M) and sodium nitroprusside (SNP, $1x10^{-6}$ to $1x10^{-14}$ M) after pre-constriction with serotonin (2×10^{-3} M, 5 ul).

Vascular relaxation (VR) was similar between groups in isolated rings, however, preincubation with PVAT media explant impaired vascular relaxation only in MSEW mice. Compared to controls, obese MSEW mice showed increased levels of circulating INFg (14.5 ±5.9 vs. 35.2 ± 7.7, $p<0.05$, respectively), TNFa (29.6 ± 8.2 vs. 118.2±29.1 vs, $p<0.05$, respectively) and IL-17a compared to controls (65.8 ±16.8 vs. 181.9 ± 39.4, $p<0.05$, respectively), which was blunted by spironolactone (94.1±23.4, $p<0.05$). Interestingly, levels of IL-17a in PVAT showed similar pattern to plasma concentrations, suggesting that IL-17a may also be produced in adipose tissue by infiltrating cells. Further investigation is needed to pinpoint the role of the Th17/Treg axis and DC in fat and other tissues comprising the immune system contributing to vascular endothelial dysfunction in obese female mice exposed to early life stress.

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Glycoprotein VI Platelet Receptor Blockade Attenuates Progression of Established Abdominal Aortic Aneurysms in Murine Models

Postdoc

Objective: Abdominal aortic aneurysms (AAA) frequently feature the formation of a nonocclusive intraluminal thrombus (ILT) along the site of aortic dilation. Platelets are known to maintain hemostasis and propagate thrombosis through several redundant activation mechanisms, yet the role of platelet activation in the pathogenesis of AAA associated ILT is still poorly understand. Thus, we sought to investigate how platelet activation via the glycoprotein VI receptor pathway impacts the pathogenesis of AAA.

Methods and Results: RNA sequencing was performed on age-matched control human aortic tissue, AAA aortic tissue, and AAA ILT. Platelet transcripts comprised 25% of significantly enriched genes in the comparison of AAA thrombus to AAA tissue and control aortic wall, with GPVI increased by 9.1 Log2-FC ($P = 3.21E^{-08}$). Circulating GPVI was significantly elevated in platelets isolated from AAA patients versus age-matched controls. Soluble GPVI (sGPVI–indicator of platelet activation), was significantly increased in the plasma of patients with fast-growing AAAs compared to slow-growing AAAs and healthy control subjects. To determine if GPVI blockade was protective from AAA progression, mice underwent either the angiotensin II (AngII) infusion or the topical elastase models of aneurysm formation. Following model initiation, aortic diameter was monitored by weekly ultrasounds. After two weeks, mice which developed an aneurysm (defined as an aortic diameter of >1.2mm) were randomized into control (IgG–50μg) or treatment (JAQ1–50μg, monoclonal GPVI antibody) groups. As expected, platelets isolated from JAQ1 treated mice did not activate in response to the GPVI specific agonist convulxin. Furthermore, JAQ1 treatment attenuated aneurysm progression, increased type I collagen deposition, attenuated macrophage accumulation, and increased survival in both murine AAA models versus IgG control treated mice.

Conclusions: Platelets play a critical role in AAA pathophysiology and specific blockade of the GPVI-mediated activation pathway attenuates progression of established aneurysms in two independent murine models. Thus, our work demonstrates that specific targeting of GPVI to blunt platelet activation in AAA could be a potential therapeutic for a pathology with no current pharmacological treatments.

Reverse cholesterol transport pathway is altered by tyrosine kinase inhibition

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Graduate Student

Cardiovascular disease (CVD) is the leading cause of death in the U.S., where coronary artery disease (CAD) accounts for 42.1% of all CVD deaths [1]. Although high-density lipoprotein-associated cholesterol (HDL-C) is associated with reduced risk of CVD events, targeted therapy to increase HDL-C levels have been unsuccessful in altering CVD outcomes of atherosclerotic disease [2]. Single nucleotide polymorphisms in *SCARB1*, the gene that encodes HDL receptor Scavenger Receptor B1 (SR-B1), are associated with dyslipidemia and atherosclerotic cardiovascular disease [3, 4]. We were the first to identify inherited mutations in *SCARB1* that segregate with disease in a family with severe coronary artery disease and dyslipidemia, including elevated HDL [5]. Our findings suggest that HDL function (vs. HDL-C concentration) may be a promising target for cholesterol-based therapy. Here, we performed an unbiased high throughput drug screen with 788 FDA-approved compounds, using HepG2 cells to measure endogenous HDL binding. We identified five compounds that significantly increased HDL binding, of which, imatinib was the only compound to increase SR-BI expression. Imatinib is a *bcr-abl* tyrosine kinase inhibitor (TKI) that is a chemotherapeutic agent for chronic myeloid leukemia. Limited clinical evidence suggests a reduction in total cholesterol with *bcr-abl* TKIs, including imatinib and dasatinib [6, 7]. Additionally, high dose TKI treatment in atherosclerotic mice reduced total cholesterol (imatinib) [8] and atherosclerotic lesions (dasatinib) [9]. Yet, no data is available on the effects of TKIs on HDL and RCT. We have found that *bcr-abl* TKIs promote HDL binding and imatinib increased RCT protein expression *in vitro*, including SR-BI, ABCA1, and ABCG1. Furthermore, in wildtype C57Bl/6 mice on a high fat, high cholesterol diet, imatinib treatment was sufficient to decrease plasma total cholesterol, HDL-C and triglyceride levels and elevated only hepatic SR-BI. In summary, our data supports the exploration of TKI-mediated SR-B1 regulation, HDL metabolism, and RCT mechanism to identify new therapeutic targets for dyslipidemia and atherosclerotic CVD.

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Potential experimental model of coronary blood flow to cardiac work mismatch: An in vivo human approach.

Graduate Student

Model development of disease processes is complex. Models can mimic characteristics and predict responses, but are prone to generalization issues and often fail in species translation. We propose an acutely manipulated non-clinical in vivo human experimental disease model of supply-demand mismatch in the heart based on four premises: 1) humans do not require comparative physiological adjustments, 2) in vivo systems experiments better account for the complex internal milieu and local cell-to-cell signaling, 3) acute experimental perturbations tend to avoid chronic adaptations, and 4) non-medicated healthy subjects with no underlying co-morbidities can be studied in place of complex patients for safety and clearer mechanism of action identification. Our proposed experimental model is the cold stressed healthy older (> 65-year-old) adult; in this population we have been able to identify increases in left-ventricular wall stress (echocardiography; PMID: 2200358) and rate pressure product
(PMID: 20375268) without a corresponding decrease in coronary vascular resistance of the left anterior descending artery (Doppler ultrasound; PMID: 2200358). This mismatch is acute and mild – it does not produce indicators of cardiac ischemia. This healthy older population also has greater cold-induced left-ventricular wall stress, greater coronary vascular resistance in both baseline and cold stress conditions, and lower positive systolic and negative diastolic velocities (tissue Doppler) than healthy younger controls (PMID: 2200358 and PMID: 20375268). The supply-demand mismatch is not observed in healthy young subjects, as cold-induced left-ventricular work is lower and coronary vascular resistance appropriately decreases during cold stress (PMID: 2200358). Skin-surface cooling is mediated via a water-perfused suit which perfuses 15°C water through tubes that are in direct contact with the skin excluding the head, hands, and feet. This results in a 6-7°C decrease in mean skin temperature, which engages cutaneous thermoreceptors via TRP channel activation. Importantly, skin-surface cooling neither alters internal temperature nor significant heat production, and can be comfortably maintained for approximately 20 minutes. These 20-minute bouts can be reliably repeated when alternated with 20-minute thermoneutral water perfusion recovery periods (PMID: 19679742). Once the coronary supply-demand mismatch occurs during skin-surface cooling, then subsequent interventional treatments and perturbations can be tested. Several limitations exist in this proposed experimental model related to subject population age, cold-induced increases in preload and afterload (PMID: 19679742-and PMID: 17901119), and the mild asymptomatic changes compared to the disease state, but the translational benefits could outweigh these issues depending on the nature of the research question.

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Ankyrin-R Isoforms are Differentially Expressed in Cardiac Tissues

Graduate Student

Heart disease remains the leading cause of death in the United States with heart failure specifically accounting for 13.4% of all heart disease related deaths (1). In 2019, Andersson et al., utilized a multi-omics approach to evaluate participant samples from the Framingham Heart Study and showed that ankyrin-R (AnkR; encoded by *ANK1*) is associated with diastolic function, left ventricular remodeling and heart failure with preserved ejection fraction (2). Ankyrins are a family of proteins that link integral membrane proteins with the actin -spectrin cytoskeleton (3). Ankyrins-B/G have been extensively studied and identified within the heart and their dysfunction is associated with cardiac structural and electrical phenotypes (4,5). Ankyrin-R was first identified in red blood cells and has yet to be studied in the context of cardiac function and heart failure or arrhythmia disease. To study AnkR in the context of the heart we isolated perfused tissues from adult wild-type mice and performed immunoblot and qPCR analysis on ankyrin-R protein and *Ank1* mRNA expression. The large AnkR isoform is expressed in the heart, along with the brain, intestine, and spleen. Interestingly, only the heart showed expression of a small AnkR isoform that has previously been shown to interact with the sarcoplasmic protein obscurin (6). Notably, isolated cardiomyocytes express only the small AnkR isoform while cardiac fibroblasts express the canonical large AnkR isoform at both the protein and mRNA level. Canonical AnkR is diffusely expressed in the fibroblast membrane, cytoplasm, cytoskeleton, and soluble nuclear fractions. These preliminary results are the first to show canonical AnkR expression in the mouse myocardium specifically within the cardiac fibroblasts. Future studies will seek to define molecular, cell, and organ phenotypes related to AnkR in the heart.

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Carnitine Palmitoyltransferase 1a Modulates Sexually Dimorphic NAFLD

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Graduate Student

Background: Nonalcoholic fatty liver disease (NAFLD) affects almost 1 billion people worldwide and is associated with cardiometabolic risk factors such as obesity and dyslipidemia. Genome- and epigenome-wide association studies have associated variants and methylation status of carnitine palmitoyltransferase 1a (CPT1a) to perturbations in very low-density lipoprotein (VLDL) cholesterol and triglyceride levels. Here, we demonstrate hepatocyte-specific deletion of CPT1a in mice lowers plasma cholesterol and triglycerides while exacerbating NAFLD and associated inflammation in a sex-dependent manner.

Methods: Eight-week old *Cpt1a* floxed mice with the human apoB100 transgene ($Cpt1a^{fl/fl}/B100^{Tg}$) were administered control adenoassociated virus (AAV) or AAV encoding Cre-recombinase under control of a liver specific promoter (TBG-Cre). Control and *Cpt1a* liver-specific knock out (LKO) mice were placed on low-fat control or western-type diet (WTD; 42% kcal fat, 0.2% cholesterol) for 16 weeks. Body weights were recorded weekly and body composition by MRI was performed at the study midpoint and end. VLDL-secretion assays were completed by quantifying serum triglycerides up to 6-hours following intraperitoneal injection of the lipoprotein lipase inhibitor, Poloxamer 407 at 1,000 mg/kg. Tissues and plasma were collected and analyzed for lipid composition, bile acid metabolism, and gene and protein expression by QPCR and immunoblotting, respectively.

Results: Liver-specific *Cpt1a* deletion was confirmed by QPCR and immunoblot analysis. Male and female *Cpt1a* LKO mice displayed lower plasma cholesterol and triglycerides irrespective of diet. The reduction in plasma cholesterol was limited to the LDL pool in FPLC-fractionated plasma. Despite a reduction in steady-state serum lipids, VLDL-triglyceride secretion was accelerated in LKO mice. Hepatic triglycerides were also elevated in mice fed WTD and exacerbated in LKO mice across sexes. Loss of hepatic *Cpt1a* had no effect on hepatic cholesterol in male mice, but increased total and unesterified cholesterol by 2- and 2.5-fold, respectively, in females. These free cholesterol levels in female *Cpt1a* LKO mice were associated with increased Kupffer cell (*Clec4f*) and collagen (*Col1a1*) gene expression. As compared to WT mice, male LKO mice exhibit increased rates of cholesterol secretion into bile, *Cyp7a1* gene expression and muricholic acid production, thereby protecting these mice from diet-induced cholesterol accumulation and subsequent inflammation.

Conclusions: Liver-specific deletion of CPT1a reduces plasma LDL-cholesterol and triglycerides in male and female mice, despite having accelerated VLDL-secretion. Increases in hepatic cholesterol levels and inflammatory gene expression are only observed in female LKO mice. Male LKO mice are largely protective against hepatic cholesterol accumulation, likely due to their ability to secrete cholesterol into bile and to upregulate muricholic acid production.

Health literacy moderates the impact of a cardiovascular disease risk reduction intervention on diet quality among informal rural caregivers of people with chronic illnesses

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Background/Introduction: Rural caregivers of those with chronic illnesses have higher cardiovascular disease (CVD) risk than urban caregivers. Diet is a major lifestyle factor that contributes to CVD risk. However, interventions are often not constructed with health literacy (HL) in mind. We conducted a randomized controlled trial of a CVD risk reduction intervention called Rural Intervention for Caregivers' Heart Health (RICHH). The RICHH intervention was designed to be equally effective in caregivers with limited or adequate HL.
Purpose: To compare the impact of RICHH intervention on diet quality over 12-month in rural caregivers with limited versus adequate HL.
Methods: A total of 311 rural caregivers (54.8 ± 13.7 years old, 76% female) of individuals with chronic illnesses participated. The newest vital sign (NVS) and Healthy Eating Index-2015 (HEI-2015) were used to determine HL and diet quality, respectively. The HEI-2015 was computed based on food frequency questionnaires at baseline, 4-month, and 12-month. The RICHH intervention was delivered using videoconference technology by nurse interventionists once a week for 12 weeks followed by bi-weekly and monthly booster sessions. A piecewise linear mixed-effect model, controlling for age, education, smoking status, and perceived stress, was used to evaluate the impact of the intervention on diet quality between the two HL groups.
Results: There was a significant difference in improvement in the HEI-2015 total scores at 4 months between control and intervention in the limited HL group (beta estimate = 2.26, SE = 0.66, $P < 0.001$), but not in the adequate HL group (beta estimate = 0.22, SE = 0.33, P = 0.519). The difference in improvement between limited and adequate HL group was significant (beta estimate = 2.05, SE = 0.74, P = 0.006). The improvement in the limited HL group was sustained at 12-months (beta estimate = 8.82, SE = 2.98, P = 0.003).
Conclusions: The results of our study indicate that an intervention designed to address HL is effective in producing a sustained improvement in diet quality to reduce CVD risk in rural caregivers with limited HL.

Title: LRP1 knockout cells are more resilient to oxidative stress induced mitochondrial dysfunction and cellular damage: implications for neurovascular dysfunction in traumatic brain injury

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Staff

Abstract: Endothelial cells produce vasoprotective and vasorelaxant factors to maintain vascular homeostasis and function. Following traumatic brain injury (TBI), oxidative stress damages brain vascular endothelial cells and plays a crucial role in the initiation of cerebrovascular dysfunction. Our preliminary data demonstrate that mitochondrial dysfunction, which can be propagated by oxidative damage, is observed in isolated brain capillaries after mild TBI (mTBI). Studies have shown that endothelial-specific low-density lipoprotein receptor-related protein 1 (LRP1) deletion improves glucose metabolism and oxygen consumption in mice. LRP1, a large endocytic molecule, is significantly increased during ischemic brain injury. However, how LRP1 regulates mitochondrial dysfunction and reactive oxygen species (ROS) production, the two hallmarks of TBI pathophysiology, following brain injury is unknown.
We mimicked the oxidative TBI environment *in vitro* by treating the mouse embryonic fibroblast (MEF) cells with the extensively-used free radical generator, 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH). Under normal conditions, LRP1 knockout (LKO) itself did not alter mitochondrial function in MEF cells as compared to WT cells. However, AAPH treatment significantly decreased mitochondrial bioenergetics, measured as oxygen consumption rate using the Seahorse XFe96 analyzer, in WT cells, though AAPH did not affect mitochondrial function in LKO cells. The degree of oxidative stress-mediated mitochondrial fragmentation, measured by Mitochondrial network analysis (MiNA) using MitoTracker Green, was lower in LKO cells compared to WT cells.
In addition, LKO cells were found to have higher ROS buffering capacity compared to WT cells when treated with AAPH. Consistently, whole cell and mitochondrial Ca^{2+} levels were increased in WT cells compared to LKO cells with AAPH treatment. Hydroxyl radicals are known to cause lipid peroxidation and the production of 4-Hydroxynonenal (4HNE). AAPH treatment significantly increased 4HNE conjugates in WT cells, but not in LKO cells. Overall, cells deficient of LRP1 are more resistant to ROS induced mitochondrial dysfunction and cellular damage. Our future studies will include brain endothelial-specific conditional LRP1 knockout mouse model as well as LRP1 inhibitors to explore LRP1 mechanism following capillary dysfunction in mTBI.

Platelet secretion in wound healing: the essence of appropriate endocytosis, biogenesis, and de a ed re ease of -granules

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PostDoc

Wound healing comprises multiple processes that involves different cell types. Though platelet-rich plasma or growth factors are used to accelerate wound repair, detailed functional knowledge is lacking, partially due to insufficient mechanistic insights about platelet activity in the various wound healing phases. We aim to investigate the role of the platelet's secretory machinery in wound healing.

Two dorsal 4-mm full-thickness circular excisions were made on mice defective in platelet -granule biogenesis (Nbeal2$^{-/-}$ and Serglycin$^{-/-}$ mice), platelet exocytosis (Munc13-4^{Jinx} and VAMP8$^{-/-}$ mice), platelet endocytosis (Arf6$^{-/-}$ and VAMP2/3 mice) and wildtype mice (C57BL/6J). Wounds were measured daily to determine percentage wound healing compared to baseline. On day 3 and day 7, (partially healed) wounds were harvested with a 6-mm biopsy punch. For each animal, one baseline and one endpoint wound were prepared for histology. A panel of bioactive molecules was analysed from soluble extracts prepared from the other baseline and endpoint wounds.

After three days, a pro-inflammatory/pro-angiogenic phenotype was seen, along with increased tissue remodelling, which (partially) restored 7 days after wounding. Similar patterns were seen comparing cytokine and growth factor levels relative to percentage wound resolution. Pro-inflammatory and pro-angiogenic cytokines and growth factors such as IL-1 and -1, were increased in only partially healed wounds, whereas these levels decreased and/or returned to baseline in almost completely healed wounds. In C57BL/6J and Munc13-4^{Jinx} mice wound le els correlated negatively with wound resolution. Nbeal2$^{-/-}$ mice showed severely impaired wound healing with distinctive wound morphology when compared to wildtype mice. The platelet-specific Arf6$^{-/-}$ and VAMP2/3 mice, which have defects in endocytic trafficking and fibrinogen uptake/storage, also show remarkably slower wound healing compared to C57BL/6J mice, but only deviate from the second day after wounding. Disturbed tissue remodelling in Nbeal2$^{-/-}$ mice was also seen via divergent MMP-3, MMP-9, and TIMP-1 profiles in the wound. In contrast, serglycin deficiency did not alter wound healing, despite both Nbeal2 and serglycin playing important roles in platelet granule biogenesis and cargo packaging. Keratin content was, although on distinct days, decreased in wounds of both Nbeal2$^{-/-}$ (day 3) and Serglycin$^{-/-}$ (day 7) mice, and correlated positively and negatively with wound resolution, respectively. Interestingly, Munc13-4^{Jinx} mice lac ing dense granule release and ha ing delayed -granule release, presented with substantially faster wound healing, specifically in the first days after wounding. Although VAMP8$^{-/-}$ mice have a severe defect in the release of all granule types, *i.e.* dense granules, -granules, and lysosomes, their wound healing rate was similar as seen in wildtype mice, with a small defect at later timepoints.

We are the first to specifically study platelet granule secretion and their cargo to ultimately provide a broader understanding of platelets in the complete wound healing process. The combination of wound morphology/histology and a broad, multi-analyte cytokine and growth factor panel enabled us to provide a more in-depth and detailed analysis of our wound healing model. We suggest appropriate platelet granule

biogenesis and endocytosis with sufficient granule cargo levels to be essential in skin wound healing, resulting in a differential profile regarding angiogenesis and tissue remodeling. Moreover, our data indicates that defective dense granule release and/or delayed instead of explosi e -granule cargo release might be beneficial.

This work is supported by NIH/NHLBI (HL150818) and the VA.

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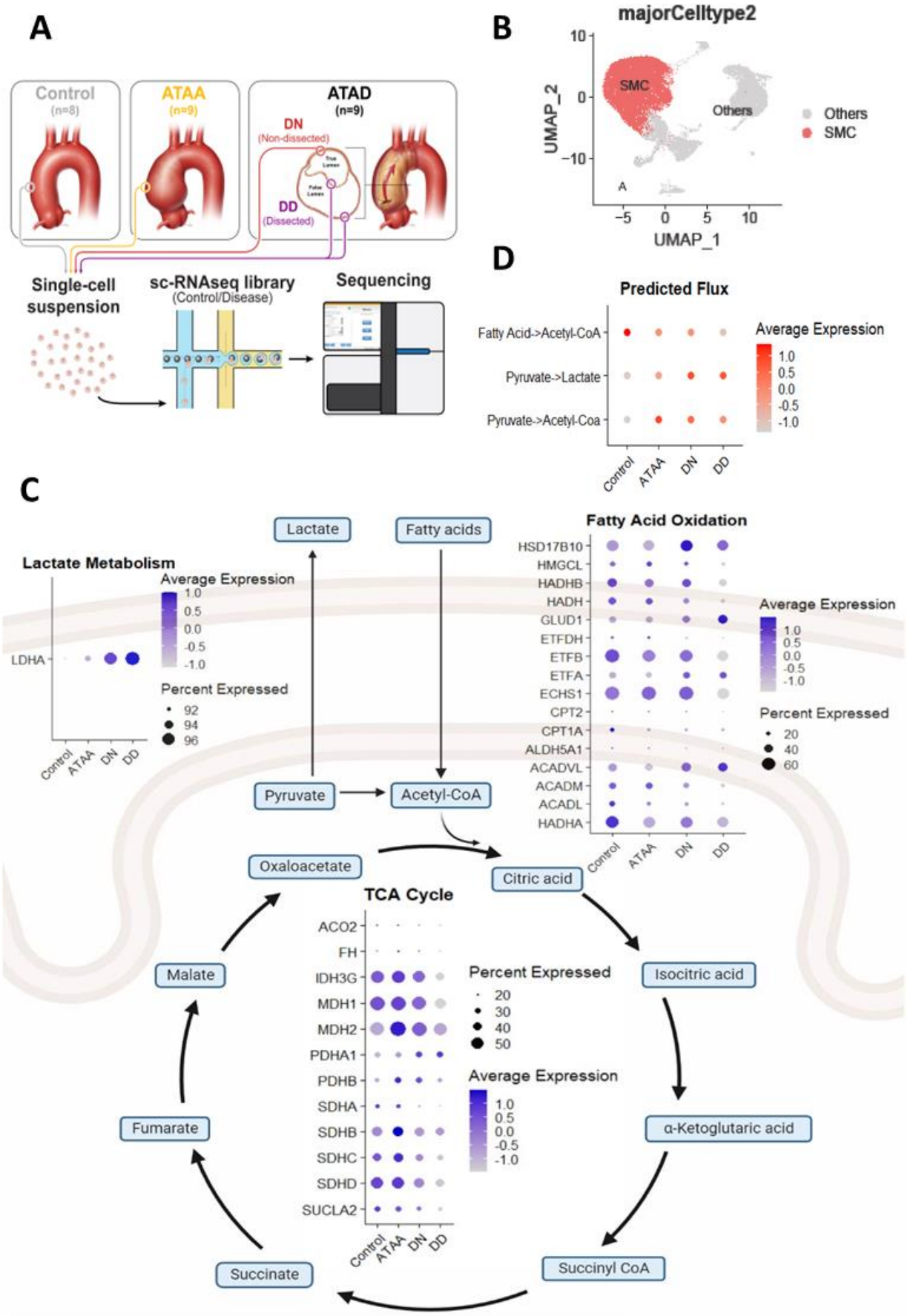
Mitochondrial Metabolism Dynamics in Sporadic Aortic Aneurysms and Acute Dissections

Graduate Student

ntroduction: Ascending thoracic aortic aneurysms (ATAA) and their progression to acute dissection (ATAD) are associated with high mortality. Our recent single-cell RNA sequencing (scRNA-seq) analysis revealed a shift from a compensatory to a decompensatory phenotype in smooth muscle cells (SMCs) in human aortic tissue as ATAA progresses to ATAD. Because the tricarboxylic acid (TCA) cycle plays a critical role in mitochondrial function and cellular metabolism, we hypothesized that TCA cycle activity is elevated in SMCs of human ATAA tissues and reduced in ATAD tissues compared to non-diseased controls.

Methods: We performed scRNA-seq analysis of ascending aortic tissue from 9 patients with ATAA without dissection, 9 patients with ATAD (dissected and non-dissected areas collected separately), and 8 organ donor control subjects (Fig 1A). Within the SMC clusters analyzed, we identified differentially expressed TCA-related genes between control, ATAA, and ATAD patients. Single-cell flux estimation analysis (scFEA) was performed to estimate metabolic flux variation in TCA cycle activity in SMCs.

Results: We observed an upregulation of TCA cycle enzyme coding genes (e.g., *SDHB, FH, MDH2*) in SMCs of ATAA compared to controls and downregulation of these genes in SMCs of ATAD compared to controls. Upon analyzing metabolic pathways (e.g., fatty acid β-oxidation and pyruvate metabolism) that contribute acetyl coenzyme A (acetyl-CoA) to the TCA cycle, we found similar mRNA abundance from β-oxidation-related genes (e.g., *ACADL, HADH, ECH1*) in ATAA and controls, but significantly downregulated expression in ATAD compared to controls (p<0.001). scFEA analysis confirmed significantly lower fatty acid oxidation activity in ATAD compared to controls (p<0.001). While expression of *PDHA1* (pyruvate dehydrogenase E1 subunit alpha 1, which catalyzes the conversion of pyruvate to acetyl-CoA) was similar across groups, the expression of lactate dehydrogenase coding gene *LDHA*, which converts pyruvate to lactate, was significantly increased in ATAD compared to ATAA (p<0.001) and controls (p<0.001). Through scFEA analysis, we observed significantly higher levels of pyruvate to lactate conversion in ATAD when compared to ATAA (p<0.001) and controls (p<0.001). **Conclusion:** Our data suggest TCA cycle activity increased in ATAA and decreased in ATAD. This shift may play a role in the transition from mitochondrial compensation to mitochondrial failure as it relates to the progression from normal aorta to ATAA to ATAD. Increased β-oxidation and lactate fermentation in ATAD may indicate disruption of acetyl-CoA supply from fatty acid oxidation and shift toward anaerobic glycolysis as a result of impaired TCA cycle in SMCs of ATAD.


A
Control
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DN
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sc-RNAseq library
(Control/Disease)
Sequencing
B
majorCelltype2
SMC
Others
UMAP_2
UMAP_1
D
Predicted Flux
Fatty Acid->Acetyl-CoA
Pyruvate->Lactate
Pyruvate->Acetyl-Coa
Average Expression
Control
ATAA
DN
DD
C
Lactate Metabolism
LDHA
Percent Expressed
Fatty Acid Oxidation
HSD17B10
HMGCL
HADHB
HADH
GLUD1
ETFDH
ETFB
ETFA
ECHS1
CPT2
CPT1A
ALDH5A1
ACADVL
ACADM
ACADL
HADHA
Lactate
Fatty acids
Pyruvate
Acetyl-CoA
Oxaloacetate
Citric acid
Isocitric acid
α-Ketoglutaric acid
Succinyl CoA
Succinate
Fumarate
Malate
TCA Cycle
ACO2
FH
IDH3G
MDH1
MDH2
PDHA1
PDHB
SDHA
SDHB
SDHC
SDHD
SUCLA2

Afferent signals contributing to sympathetic activation in a model of early life stress

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Early life stress (ELS) can have negative long-lasting impacts on mental and physical health. In this study we are exploring the connection between ELS and a high blood pressure, as related through the adipose afferent reflex (AAR). We model ELS through maternal separation and early weening (MSEW), and then the animals are put on either a high fat or low fat diet. The AAR connects white adipose tissue to the spinal cord, the brain, and back to the fat. Under normal conditions signals from the fat go to the brain and back triggering lipolysis. Under obesity induced hypertension, the signal is so overwhelmed that signals don't just go back to the fat, but they go to the heart and arteries, causing an increase in blood pressure. So, we study this increase in blood pressure as a sign that the AAR was activated. Many different substances can be used to trigger the AAR, previous studies focused on the use of capsaicin, an exogenous factor. In this study we explored how an endogenous factor, serotonin 5-HT, would trigger the AAR. We did this through in-vivo surgeries and in-vitro cell cultures. Future directions are to study different fat pads beyond the visceral and subcutaneous and see what responses they elicit.

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