September 20, 2024

Central Bank Center
Lexington, KY

26TH 2024 CARDIOVASCULAR RESEARCH DAY UNIVERSITY OF KENTUCKY

September 20, 2024
Central Bank Center Ballrooms

8:30 am Guest Check-In | Breakfast

9:00 am Morning Session

9:00 am
Welcome
Alan Daugherty, PhD, DSc
Director, Saha Cardiovascular Research Center

9:05 am
Rapid Fire Presentations
Moderators: ***Lindsay Czuba, PhD***
Assistant Professor, College of Pharmacy
Scott Gordon, PhD
Associate Professor, College of Medicine

Liz Driehaus
Graduate Student
Wally Whiteheart, Mentor
"VAMP8 Deletion Mitigates Atherosclerosis by Modulating Platelet Inflammatory Cargo Release"

Nikitha Dharanipragada
Undergraduate
Nate Helsley, Mentor
"Deletion of Carnitine Palmitoyltransferase 1a from Adipocytes Leads to Insulin Resistance in Female Mice"

Vivek Pandey, PhD
Postdoctoral Fellow
Sanda Despa, Mentor
"O-GlcNAcylation Underlies the Activation of Sodium-Glucose Cotransporter 1 in Diabetic Hearts"

Morning Session - continued

9:25 am

The Gill Heart and Vascular Institute Early Career Awards

Moderator: ***Cheavar Blair, PhD***
Assistant Professor, College of Medicine

Satoshi Koyama, MD, PhD
Postdoctoral Fellow
Broad Institute at MIT and Harvard
"Diagnosing Millions of Hearts: Harnessing Genetic Data for Cardiovascular Insights"

Jessica Caldwell, PhD
Postdoctoral Fellow
University of California, Davis
"Sex-dependent Cardiac Cyclic-AMP Signaling and Arrhythmias in the Failing Heart"

Joshua Travers, PhD
Postdoctoral Fellow
University of Colorado
"Cell Painting and Machine Learning Distinguish Healthy from Failing Human Cardiac Fibroblasts"

10:25 am
Break

Morning Session - continued

10:45 am
Rapid Fire Presentations
Moderators: ***Ila Mishra, PhD***
Assistant Professor, College of Medicine
Ryan Temel, PhD
Associate Professor, College of Medicine

Nicole Marker
Medical Student
Sibu Saha, Mentor
"Surgical Treatment of Cardiac Tumors: A Single Center Experience"

Abhilash Prabhat, PhD
Postdoctoral Scholar
Brian Delisle, Mentor
"Feeding Behavior Underlies the Circadian Rhythm in the Autonomic Input to the Heart and Heart Rate"

Maggie Murphy, PhD, RD, LD
Assistant Professor
Pediatrics
"Obesity Predicts Left Ventricular Mass in Youth Undergoing Ambulatory Blood Pressure Monitoring"

11:05
Poster Pitches

11:15 am
Poster Judging and Poster Session
Odd-numbered posters are judged during the first 10 minutes. Presenters remain at their poster throughout the session.

12:15 pm Lunch

12:30 pm
Nancy Brown
Chief Executive Officer
American Heart Association
"Advancing Health and Hope for Everyone, Everywhere"
Introduction: ***Alan Daugherty, PhD, DSc***
Director, Saha Cardiovascular Research Center

1:15 pm
Break

1:30 pm Afternoon Session

1:30 pm
Trainee Presentations
Moderator: ***Ming Gong, PhD***
Professor, College of Medicine

Gregory Milburn
MD, PhD Student
Ken Campbell, Mentor
"Mechanical Unloading Restores PKA-Mediated Phosphorylation of Sarcomeric Proteins in Patient with Heart Failure"

Garrett Elmore
Graduate Student
Jon Satin, Mentor
"Interventional Cardiomyocyte-Restricted RAD Knockout Improves Cardiac Dysfunction in a Murine Model of Dilated Cardiomyopathy"

Afternoon Session - continued

2:00 pm
Poster Pitches

2:10 pm
Poster Judging and Poster Session

Even-numbered posters are judged during the first 10 minutes. Presenters remain at their poster throughout the session.

3:05 pm
Alumni Presentation
Brandon Fornwalt, MD, PhD

Vice President of Cardiology
Tempus Labs
"What I Would Tell My Younger 25-Year-Old Self"
Introduction: ***Gregory Graf, PhD***
Associate Director, Saha Cardiovascular Research Center

3:30 pm
Gill Heart and Vascular Institute Award for Outstanding Contributions to Cardiovascular Research
Svati Shah, MD

Associate Dean for Translational Research
Director, Duke Center for Precision Health
Duke University
"Heart Failure Omics: Metabolism and Beyond"
Introduction: ***Hisashi Sawada, PhD***
Assistant Professor, College of Medicine

4:15 pm Awards Presentation

4:30 pm Networking Reception

CARDIOVASCULAR RESEARCH PRIORITY AREA
HealthCare GILL HEART & VASCULAR INSTITUTE
Saha Cardiovascular Research Center
Saha Aortic Center

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Research Day is provided by the following units:

Featured Speaker

Nancy Brown

CEO

American Heart Association

Nancy Brown is Chief Executive Officer of the American Heart Association (AHA), which is celebrating its 100th birthday in 2024 and launching its second century of advancing health and hope for everyone, everywhere. Its unwavering mission: To be a relentless force for a world of longer, healthier lives. Under Nancy's leadership as CEO since 2008, the AHA has become a global authority on cardiovascular and brain health as well as overall health and well-being, active in more than 100 countries. Forging powerful partnerships – together with more than 35 million volunteers, supporters, and staff – Nancy champions equitable health for all and is committed to innovation at the intersection of science, technology and public health on behalf of patients and their families.

Highlights include:

» Investing **more than $5.9 billion** to accelerate scientific discoveries in cardiovascular and cerebrovascular care and driving innovation using new technologies, including **artificial intelligence**.

» Expanding the Association's **global leadership** in resuscitation science and CPR/ AED education and training, including launching the **"Nation of Lifesavers"** movement to empower everyone to save a life.

» Empowering **women's health** through the Go Red for Women® movement, Research Goes Red registry and the Go Red For Women Venture Fund—the largest known dedicated women's health venture fund in the U.S.

» Funding innovative science in **brain health** and advancing solutions to build life -long cognitive skills and prevent brain disorders and diseases.

» Ensuring longer, healthier lives for all patients by elevating heart and stroke **quality improvement programs and registries** in more than 2,900 hospitals and nearly 2,000 clinics across the U.S. as well as 14 countries.

» Advocating for **policies and legislation that support healthy communities** and access to affordable, equitable care through the AHA's expansive You're The Cure grassroots network and Voices for Healthy Kids.

» Investing in and creating innovative commercial solutions through **AHA Ventures**, Cardeation Capital, and the Association's family of Social Impact Funds that support entrepreneurial businesses and community leaders to create equitable, sustainable health solutions.

» Building a healthy, high-performing and engaged workforce by partnering with Fortune 500 CEOs, through the **AHA CEO Roundtable**, to improve the health and well-being of employees, families and communities.

» Leading an integrated approach to **food science initiatives**, including a multisector Health Care by Food initiative, and developing go-to-market solutions that improve health, food system sustainability and nutrition security.

» Defining **cardio-kidney-metabolic syndrome** for the first time and launching an initiative to provide treatment guidelines for the roughly 1 in 3 people affected in the U.S.

» Launching a world-class **institute for clinical trials** to accelerate solutions using data science, registries, and real-life studies.

As CEO, Nancy serves on numerous global leadership councils, including the **CNBC CEO Council, The Wall Street Journal CEO Council**, the **Yale CEO Summit** and Women Leaders in Healthcare.

Gill Heart and Vascular Institute Outstanding Contributions to Cardiovascular Research Award

Svati Shah, MD, MHS

Associate Dean for Translational Research
Director of the Duke Precision Genomics Collaboratory
Duke University

Dr. Svati H. Shah is a physician scientist and Associate Dean of Genomics and Director of Precision Genomics Collaboratory in the Duke School of Medicine; Vice-Chief of Translational Research and Director of the Adult Cardiovascular Genetics Clinic in the Division of Cardiology, Department of Medicine; Co-Director of Translational Research in the Duke Molecular Physiology Institute (DMPI); and a faculty member in the Duke Clinical Research Institute (DCRI). Her research focus is on metabolic and genetic pathways of cardiometabolic diseases, integrating diverse genomic, metabolomic and proteomic techniques for identification of novel mechanisms of disease and biomarkers. Her multi-disciplinary molecular epidemiology lab within the DMPI has quantitative and molecular components and leverages large biorepositories on to perform discovery studies using omics technologies, with subsequent functional validation for mechanistic insight.

Gill Heart and Vascular Institute Early Contributions to Cardiovascular Research Award

Jessica Caldwell, PhD

Postdoctoral Fellow
University of California, Davis

Dr. Caldwell, Ph.D., is a Postdoctoral Scholar at UC Davis specializing in advanced imaging methodologies to investigate arrhythmias at the tissue level. Her research spans from sub-cellular to whole-heart investigations, focusing on autonomic signaling and arrhythmia development. She targets the structural and functional remodeling of β-adrenergic receptor signaling in heart failure, with an emphasis on sex- and region-specific variations to uncover pivotal insights into arrhythmia triggers.

Satoshi Koyama, MD, PhD

Postdoctoral Fellow
Broad Institute at MIT and Harvard

Dr. Koyama is a cardiologist and scientist specializing in the genetic epidemiology of cardiovascular diseases. He trained as both a cardiologist and a genetic epidemiologist in Japan and is now working as a Postdoctoral Fellow at the Broad Institute. His primary research interest lies in identifying disease-causing and modifying factors through the analysis of large-scale biomedical data.

Joshua Travers, PhD

Postdoctoral Fellow
University of Colorado

Dr. Travers is a postdoctoral research fellow at the University of Colorado Anschutz Medical Campus Division of Cardiology where his research focuses on understanding and combating cardiac fibrosis and diastolic heart failure. He obtained his PhD from the Cincinnati Children's Hospital where he conducted research on the therapeutic potential of GRK2 inhibition in ischemic cardiomyopathy. His postdoctoral work, under the mentorship of Timothy McKinsey, focuses on the molecular mechanisms and cellular specificity of histone deacetylase inhibitors in treating diastolic dysfunction and heart failure with preserved ejection fraction. Dr. Travers was the 2022 ISHR Young Investigator Competition Award winner as well as a finalist for the AHA Katz Basic Research Prize, and through his NIH Pathway to Independence Award hopes to soon start his own research program advancing cardiovascular science and developing novel therapies for the treatment of cardiac fibrosis and heart failure.

Distinguished Alumni Presentation

Brandon Fornwalt, MD, PhD

Senior Vice President of Cardiology
Tempus Labs, Inc

Dr. Fornwalt graduated from the University of South Carolina Honors College in 2002 with an undergraduate degree in mathematics and marine science. He then worked in a free medical clinic for a year before starting an MD/PhD program at Emory and Georgia Tech. After finishing his degrees in 2010, he completed an internship in pediatrics at Boston Children's Hospital before becoming an Assistant Professor at the University of Kentucky. After four years on faculty in Kentucky, Dr. Fornwalt moved with his research team to Geisinger where he completed his diagnostic radiology residency and founded Geisinger's Department of Translational Data Science and Informatics, which focused on data-driven approaches to improving patient outcomes. In 2021, Dr. Fornwalt took a position at Tempus as the Senior Vice President of Cardiology where he leads the scientific and clinical aspects of Tempus' data-driven cardiology efforts. Dr. Fornwalt is also a part-time radiologist at UPMC Williamsport.

Poster Pitch Participants

Rubab Akbar
Post Doc
University of Kentucky

Garrett Anspach
Graduate Student
University of Kentucky

Dominic Armagno
Medical Student
University of Kentucky

Kyle Barker
Staff
University of Kentucky

Evelyn Bates
Graduate Student
University of Kentucky

Meredith Campbell
Graduate Student
University of Kentucky

Garrett Elmore
Graduate Student
University of Kentucky

Olivia Hage
Graduate Student
University of Kentucky

Julia Krpan
Graduate Student
University of Kentucky

Hannah Laney
Undergraduate
University of Kentucky

Lin Li
Post Doc
University of Kentucky

Sarisha Lohano
Undergraduate
University of Kentucky

Abdullah Masud
Post Doc
University of Kentucky

Shayan Mohammadmoradi
Post Doc
University of Kentucky

Raj Neupane
Post Doc
University of Kentucky

Sally Pauss
Graduate Student
University of Kentucky

Alex Pettey
Graduate Student
University of Kentucky

Roxana Ponce
Medical Student
University of Kentucky

Ezekiel Rozmus
Graduate Student
University of Kentucky

Caterina Squarci
Post Doc
University of Kentucky

Clairity Voy
Graduate Student
University of Kentucky

Ellen Woodward
Undergraduate
University of Kentucky

Trainee Podium Presentations

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

Liz Driehaus
Graduate Student
Wally Whiteheart, Mentor
"VAMP8 Deletion Mitigates Atherosclerosis by Modulating Platelet Inflammatory Cargo Release"

Nikitha Dharanipragada
Undergraduate
Nate Helsley, Mentor
"Deletion of Carnitine Palmitoyltransferase 1a from Adipocytes Leads to Insulin Resistance in Female Mice"

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Postdoctoral Fellow
Sanda Despa, Mentor
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Garrett Elmore
Graduate Student
Jon Satin, Mentor
"Interventional Cardiomyocyte-Restricted RAD Knockout Improves Cardiac Dysfunction in a Murine Model of Dilated Cardiomyopathy"

Poster Presenters

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Layne Voisard*[1], Dr. Rubab Akbar[2], Dr. Ila Mishra[2]

Presenter*, University of Kentucky: Department of Biology[1], University of Kentucky: Department of Internal Medicine, Endocrinology[2]

Abstract

Diabetes poses a significant global health challenge, impacting 530 million adults worldwide. Type 2 diabetes (T2DM) constitutes approximately 98% of all diagnosed cases globally, influenced by a complex interplay of metabolic syndrome (MS) factors such as obesity, insulin resistance, hypertension, and elevated blood lipid levels. These factors collectively contribute to the rising incidence of T2DM.

Asprosin, a recently identified adipokine, is closely associated with various MS conditions including T2DM, obesity, fatty liver disease, polycystic ovary syndrome (PCOS), and hypertension. Its physiological effects include stimulating hepatic gluconeogenesis and promoting feeding behavior through AgRP neuron activation. Given the widespread distribution of its receptor, Ptprd (Protein Tyrosine Phosphatase type δ), asprosin likely exerts additional metabolic functions beyond conventional pathways.

Ptprd is prominently expressed in pancreatic β cells. Treatment with asprosin impairs insulin production, increases inflammation, and induces apoptosis in these cells. Conversely, treatment with 7-BIA, a Ptprd antagonist, enhances insulin production and improves glucose tolerance. This suggests that mitigating asprosin-Ptprd signaling could potentially slow the progression of T2DM aggravated by obesity.

Additionally, our research indicates that asprosin interacts with oxytocin neurons in the hypothalamus. Mice lacking asprosin or with genetic Ptprd deficiency in these neurons exhibit low blood pressure. This underscores the role of asprosin-Ptprd signaling in linking T2DM, obesity, and hypertension.

While previous studies have shown that 7-BIA reduces appetite in mice, its effects on glucose regulation and hypertension remain unexplored. We hypothesize that 7-BIA could offer a unified treatment strategy for obesity, T2DM, and hypertension—a promising "one remedy for three maladies."

Our research aims to investigate the relationship between asprosin and MS, particularly exploring whether chronic treatment with 7-BIA, a Ptprd antagonist, can effectively address glycemic control, insulin resistance, blood pressure, food intake, and body weight in mouse models of diabetes and obesity. 7-BIA may be able to provide a viable therapeutic approach to simultaneously manage these interconnected metabolic disorders.

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Effect of Doxapram (a K2p Channel Blocker), Bacterial Endotoxin and pH on Heart Rate: Larval *Drosophila* Model

Elliott, E.R., Taul, A.C., Abul-Khoudoud, M.O., Hensley, N, and Cooper, R.L.
University of Kentucky

Two-P-domain K^+ (K2p) channels are responsible for maintaining a cell's resting membrane potential. K2p channels have varied expression in healthy tissue, but this is also altered in cancerous or diseased states; however, the precise correlation vs. causation as regards the alteration of K2p channel expression is still being investigated. The compound doxapram seems to block K2p channels and depolarize cells. Using *Drosophila*, the increased expression of the ORK1 K2p channel in cardiac and skeletal muscle was investigated. The heart rate in larval *Drosophila* is very sensitive to pH, and, since doxapram blocks a subset of K2p channel known to be acid-sensitive, it was postulated that doxapram would affect heart rate. A pH change from 7.1 to 6.5 increased the rate, while that from 7.1 to 7.5 decreased the rate. An amount of 0.1 mM of doxapram had no effect, but 0.5 of mM depressed *Drosophila* heart rates within five minutes. Exposure to 5 mM of doxapram immediately decreased the rate. Lipopolysaccharides (LPSs) from Gram-negative bacteria acutely increased the rate. LPSs activate K2p channels in the skeletal muscle of larvae and are blocked by doxapram. LPSs slightly reduce depression in the rate induced by doxapram. The overexpression of K2p channels in the heart and skeletal muscle depressed the heart rate and heightened pH sensitivity. At larval neuromuscular junctions, the overexpression in skeletal muscle increases the frequency of spontaneous quantal events and produces a more negative resting membrane potential. Research was previously conducted with funding support from a CURE fellowship and is now being further developed in a series of projects supported by the Beckman Scholars Program (ERE).

Implementing a time-restricted eating intervention in postmenopausal women

J. Matthew Thomas[1,2], Philip A. Kern[3], Dorothy D. Sears[4], Dara L. James[5], Samuel E. Armstrong[6], Cody Bumgardner[6], Aaron Mullen[6], Jean L. Fry[7], Courtney Murray[1], Julie S. Pendergast[1]

Affiliations:

[1]Department of Biology, College of Arts and Sciences, University of Kentucky, Lexington, KY
[2]Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY
[3]Department of Internal Medicine, College of Medicine, University of Kentucky, Lexington, KY
[4]College of Health Solutions, Arizona State University, Phoenix, AZ
[5]Edson College of Nursing and Health Innovation, Arizona State University, Phoenix, AZ
[6]Institute for Biomedical Informatics, University of Kentucky, Lexington, KY
[7]Department of Athletic Training and Clinical Nutrition, College of Health Sciences, University of Kentucky, Lexington, KY

Time-restricted eating (TRE) is an emerging intervention to improve metabolic health. Prior studies investigating TRE involved logging methods that were labor intensive for participants and/or researchers. As timed eating interventions become increasingly popular, easy and effective methods to monitor and reinforce daily meal times are needed. Here, we implemented an automated state-based text messaging system to support a TRE efficacy trial among metabolically unhealthy postmenopausal woman. Our objective was to investigate implementation of this text messaging system for monitoring meal times and promoting compliance. First and last meal times were collected using the text messaging system for 2 weeks at baseline and for 16 weeks after randomization to the control (maintain usual meal timing) or TRE intervention (consume all calories during 10h window with last meal before 8pm). All 19 participants completed the 18-week study and texted their meal times on 123.6 ± 2.8 days. Participants in the TRE group reduced their average calorie window by 3.13 h, from 13.0 ± 0.9 h at baseline to 9.9 ± 0.2 h during TRE. Participants complied with the TRE intervention 92.2 ± 5.1% of days during the 16-week intervention. We investigated whether daily texting of first and last meal times affected the calorie window of control participants. The calorie window of control participants was virtually unchanged from baseline, increasing by only 0.2 ± 1.1 h. Findings show that the state-based text messaging system is effective for implementing a TRE intervention and measuring ad libitum first and last meal timing in postmenopausal women.

Support: Research reported in this abstract was supported by the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute on Aging, and the National Center for Advancing Translational Sciences, of the National Institutes of Health, under award number R01DK124774, T32 AG078110, and UL1TR001998. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Title: Astrocytic mitochondrial transfer to brain capillaries in vivo increases with aging

Authors: *Velmurugan Gopal Viswanathan, Hemendra Vekaria, Samir Patel, Patrick Sullivan and Brad Hubbard*

Abstract: Intercellular mitochondrial transfer (IMT) is an intriguing biological phenomenon where mitochondria are transferred between different cells and notably, cell types. IMT is physiological, occurring in normal conditions, but also is utilized to deliver healthy mitochondria to cells in distress. Transferred mitochondria can be integrated to improve cellular metabolism, and mitochondrial function. Research on the mitochondrial transfer axis between astrocytes and brain capillaries *in vivo* is limited by the cellular heterogeneity of the neurovascular unit. To this end, we developed an inducible mouse model that expresses mitochondrial Dendra2 only in astrocytes and then isolated brain capillaries to remove all intact astrocytes. This method allows the visualization of *in vivo* astrocyte- endothelial cell (EC) and astrocyte-pericyte IMT. We demonstrate evidence of astrocyte-EC and astrocyte-pericyte mitochondrial transfer within brain capillaries. We also show that the aging enhances mitochondrial transfer from astrocytes to brain capillaries, revealing a potential link between brain aging and cellular mitochondrial dynamics. Finally, we observe that astrocyte-derived extracellular vesicles transfer mitochondria to brain microvascular endothelial cells, showing the potential route of *in vivo* IMT. These results represent a breakthrough in our understanding of IMT in the brain and a new target in brain aging and neurovascular metabolism.

CRISPR-Mediated Disruption of *DENND5B* Attenuates Triglyceride-Rich Lipoprotein Secretion from Human Enterocytes and Hepatocytes

Olivia Hage[1], Khaga R. Neupane[1], Alexander Karakashian [1], and Scott M. Gordon [1,2,*]

[1] Saha Cardiovascular Research Center, University of Kentucky, Lexington, KY, USA
[2] Department of Physiology, University of Kentucky, Lexington, KY, USA

Background and Hypothesis: Elevated plasma triacylglycerides (TAGs) are an established risk factor for atherosclerotic cardiovascular disease, and are regulated by dietary absorption via the small intestine and endogenous production by the liver. Our laboratory previously reported that *Dennd5b*-deficient mice are protected from diet-induced hepatic steatosis, hyperlipidemia, and atherosclerosis. Electron microscopy imaging of the intestinal epithelium of whole-body *Dennd5b*$^{-/-}$ mice reveals significant lipid accumulation in intracellular chylomicron secretory vesicles, suggesting a post-Golgi chylomicron secretion defect. Given that *Dennd5b* is expressed by the two major triglyceride-rich lipoprotein (TRL)-secreting organs (liver and small intestine), we hypothesized that disruption of *Dennd5b* attenuates TRL secretion in both of these cell types in humans.

Methods and Results: To determine if *DENND5B* disruption impairs TRL secretion in human cells, we generated *DENND5B*$^{-/-}$ intestinal epithelial (Caco-2) and hepatocyte (HepG2) cell lines using CRISPR gene editing. Western blots verified DENND5B protein reduction in *DENND5B*$^{-/-}$ cells. The impact of *DENND5B* deficiency on TRL secretion was quantified by measuring the cellular and secreted TAG content of *DENND5B*$^{+/+}$ and *DENND5B*$^{-/-}$ cells under standard growth conditions and after oleic acid loading. Caco-2 cells were differentiated on membrane supports for 21 days prior to experiments. *DENND5B*$^{-/-}$ Caco-2 cells had significantly reduced TAG secretion compared to wild-type Caco-2 cells (-67.8%, $p<0.0001$). *DENND5B*$^{-/-}$ HepG2 cells also had significantly reduced TAG secretion (-91.0%, $p<0.0001$). Disruption of *DENND5B* in both cell lines reduced TAG secretion without affecting cellular TAG content. Immunofluorescence microscopy revealed intracellular DENND5B protein localization consistent with cytoskeletal involvement that persisted with oleic acid loading. This finding is bolstered by co-immunoprecipitation of Dystonin and Tubulin Beta Class I with DENND5B from wild-type HepG2 lysates grown under standard conditions. Tubulin is the most significant component of microtubules. Dystonin facilitates linking of membrane-bound cargo to the Dynein/Dynactin motor complex that shuttles targets along microtubules.

Conclusions: These data support the hypothesis that DENND5B plays a role in TRL secretion from human enterocytes and hepatocytes. Its mechanism may involve post-Golgi vesicular transport along the cytoskeleton. These studies provide new model systems for investigating the molecular function of DENND5B in TRL secretion from human cells.

This research was funded by the American Heart Association Pre-Doctoral Fellowship to Olivia Hage, and NIH grant R01DK133184.

Title: The Impact of Hepatic G5G8 on the Development of MASLD Phenotypes

Authors: Rachael A. Morgan, Erika L. Savage, Victoria P. Noffsinger, Robert N. Helsley, and Gregory A. Graf.

Background: Accumulation of free cholesterol in hepatocytes leads to an exacerbation of metabolic dysfunction-associated steatotic liver disease (MASLD) in mice and humans. The ATP-binding cassette subfamily G members 5 and 8 (ABCG5 and G8) are obligate heterodimers that coordinate the secretion of cholesterol into bile. The purpose of this work is to determine the contribution of biliary cholesterol secretion to the development of MASLD and associated metabolic disturbances in mice lacking hepatic G5/G8.

Methods: Eight-week-old male and female control (*G5G8*$^{F/F}$) and littermate ABCG5/G8 liver-specific knockout (LKO; *G5G8*$^{AlbCre+}$) mice were fed the Gubra Amylin Nash (GAN) diet containing 2.0% cholesterol (w/w) for 16 weeks. Body weights were measured weekly, and body composition was measured by MRI at the beginning and end of the study. Mice were necropsied after a 4 hour fast, and tissues, bile, and serum were collected for lipid analysis, histology, bulk RNA-sequencing, and protein expression by immunoblotting.

Results: No differences were observed in terminal body weight, yet liver weight was ~20% greater in LKO mice as compared to controls. Fasting glucose did not differ, insulin levels and HOMA-IR values were elevated in LKO mice by 30-50%. Total and esterified cholesterol levels increased by >100% in both male and female LKO, with no changes in triglycerides. Only female LKO mice accumulated significantly greater free cholesterol than both male and female control mice (0.69±0.65 vs. 4.91±2.52 μg/mg tissue). Hepatic cholesterol accumulation coincided with elevations in serum ALT levels and inclusion of inflammatory loci in histology sections from LKO mice. RNA sequencing of hepatic tissue confirmed substantial enrichment of transcripts in multiple inflammatory pathways.

Conclusion: These results show that ABCG5/G8 are important for protecting against cholesterol-induced liver damage. Ongoing studies are examining the lipid composition in bile and serum and quantifying the degree of inflammation and fibrosis in the liver.

Thermoneutrality Does Not Affect Aortic Aneurysms But Reduces Atherosclerosis in Hypercholesterolemic Mice

Liyuan Zhu,[1] Bowen Li,[1] Deborah A. Howatt,[1] Alan Daugherty,[1,2,3] Hong S. Lu[1,2,3]

[1] Saha Cardiovascular Research Center, [2] Saha Aortic Center, [3] Department of Physiology, University of Kentucky

Background: Thermoneutrality (TN) is the ambient temperature range for which energy is expended primarily to maintain basal metabolic rate. Although mouse studies are predominantly performed at room temperature (RT, 20-22°C), thermoneutrality is achieved at ~30°C in mice. It has not been defined whether thermoneutral conditions affect development of aortic aneurysms in mice. Therefore, this study compared the effects of RT and TN on development of angiotensin II (AngII)-induced aortic aneurysms in mice.

Methods: Male C57BL/6J mice were injected with an adeno-associated viral vector (AAV) expressing a gain-of-function mutant of mouse PCSK9 ($2x10^{11}$ GC/mouse) and GalNAc LDL receptor antisense oligonucleotides (4 mg/kg/week) to deplete LDL receptors and fed a Western diet. After confirmation of their hypercholesterolemic condition, mice (RT, N=9; TN, N=10) were infused with AngII (1,000 ng/kg/min) via subcutaneously implanted mini osmotic pumps. The mice were housed in cages that were either RT (20 °C) or TN (30 °C) during 4 weeks of AngII infusion.

Results: Body weights were not different between the two groups. All mice, irrespective of the housing temperature, were hypercholesterolemic; however, plasma total cholesterol concentrations did not differ between the two groups (1208 ± 148 mg/dl *vs* 1192 ± 120 mg/dl, P=0.9). One mouse in the RT group succumbed to abdominal aortic rupture (11%, 1/9 mice), while two mice in the TN group died due to either ascending or abdominal aortic rupture (20%, 2/10 mice). The maximum diameters of ascending aortas were not different between the TN and RT groups (2.0 ± 0.4 mm *vs* 2.4 ± 0.5 mm, P = 0.11). Maximal diameters of abdominal aortas were also not different between the two groups (2.8 ± 0.3 mm *vs* 2.1 ± 0.4 mm, P=0.15). Despite the lack of changes in AngII-induced aortic aneurysms, atherosclerotic lesion sizes were different in the thoracic aortic region (19.4 ± 3.0% *vs* 6.3 ± 1.4%, P = 0.001).

Conclusion: Thermoneutral temperature had no significant effect on aortic aneurysm development. In contrast, atherosclerosis was attenuated at a thermoneutral temperature.

Key Words: Temperature; thermal neutral; Atherosclerosis; Aortic aneurysm; hypercholesterolemia

Direct and indirect effects of epinephrine in inducing arrhythmogenesis under low-glucose conditions

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Severe hypoglycemia accounts for up to 10% of deaths in young people with insulin treated type 1 diabetes. Previous work in Dr. Fisher's lab has demonstrated that severe hypoglycemia-induced sudden death is mediated by cardiac arrhythmias. The mechanism by which severe hypoglycemia causes arrhythmias is incompletely characterized. It is postulated that in response to hypoglycemia, an increase in the counterregulatory hormone epinephrine may underly hypoglycemia-induced fatal arrhythmia. β-adrenergic agonists have been shown to increase spontaneous diastolic Ca^{2+} release from sarcoplasmic reticulum via ryanodine receptors (RyR2) in cardiomyocytes (via β-AR-cAMP-Epac-PI3K-Akt-NOS1-CaMKII-RyR pathway), triggering arrhythmogenesis. However, it is not known if diastolic Ca^{2+} leak mechanism contributes to arrhythmogenesis during hypoglycemia when the substrate availability is low. As another mechanism of action, epinephrine also induces the release of endothelin-1 (ET-1), a potent vasoconstrictor that increases intracellular Ca^{2+} by activating L-type Ca^{2+} channels and Ca^{2+}-induced Ca^{2+} release by reducing SR Ca^{2+} uptake in cardiomyocytes. The role of ET-1 in arrhythmogenesis during hypoglycemia is also not known. Hence, we hypothesized that, under low glucose conditions, (1) epinephrine increases diastolic Ca^{2+} leak in isolated rat cardiomyocytes, and (2) ryanodine receptor (RyR2) inhibition using dantrolene, or ET-1 receptor inhibition using bosentan hydrate reduces epinephrine-induced arrhythmia in isolated rat hearts. Isolated cardiomyocytes were loaded with Fluo-4 and incubated in 0 Ca^{2+} Normal Tyrode's (NT) buffer containing either 5 mM (control) or 0.6 mM glucose (low-glucose). For spontaneous Ca^{2+} sparks and leak measurements in confocal microscope, the myocytes were perfused with either 5 mM glucose, or 0.6 mM glucose +/- epinephrine (20 nM). Combined low-glucose + epinephrine treatment had the highest Ca^{2+} leak and rate of occurrence of arrhythmogenic Ca^{2+} waves, while treatment with low-glucose alone increased the frequency of occurrence of spontaneous Ca^{2+} sparks. In isolated rat hearts, epinephrine (1 μM) significantly increased the duration of arrhythmia under low-glucose (0.1 mM) conditions compared to control conditions (5 mM glucose). RyR2 receptor inhibition with dantrolene (40 μM) increased, and ET-1 receptor inhibition with bosentan hydrate (10 μM) significantly reduced the duration of Epinephrine-induced arrhythmia in isolated hearts under low-glucose conditions. In conclusion, this study shows that under low-glucose conditions, epinephrine induces non-spark mediated 'silent' diastolic Ca^{2+} leak that may underlie arrhythmogenesis during hypoglycemia. Our study also shows that, in addition to direct effects, epinephrine-induced arrhythmogenesis may indirectly be mediated by ET-1.

Plasminogen Activator Inhibitor-1 Deficiency Augments Angiotensin II Induced Cardiac Fibrosis in Mice

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Background

Cardiac fibrosis, characterized by the increased deposition of collagen fibers in the heart, is a key driver of heart failure. As reported by many, Angiotensin II (AngII) and Plasminogen activator inhibitor-1 (PAI-1) play vital roles in cardiac fibrosis formation in humans as well as mice. Whole body deficiency of PAI-1 (PAI-1 -/-) in conjunction with AngII infusion for 28 days results in large fibrotic remodeling of the myocardium in mice. This study aims at identifying the histopathological processes contributing to this overt fibrotic pathology.

Methods and Results

PAI-1 -/- mice and their wild-type littermates (PAI-1 +/+) were infused with AngII for 28 days. In situ imaging identified large white webbing on the epicardium of PAI-1 -/- hearts. Histologically, Masson's trichrome labeled collagen deposition in remodeled areas. Collagen deposition in PAI-1 -/- mice, compared to PAI-1 +/+ mice, was increased 6-fold with a focus in the epicardium (17.5 ± 1.3% vs 2.8 ± 0.8% of total heart area). Immunohistochemistry for cardiac troponin-I revealed that loss of cardiomyocyte co-localized with collagen deposition. Importantly, Prussian blue staining revealed the fibrotic areas were colocalized with ferric iron accumulation, indicating previous bleeding. To determine the initial time of injury, mice were infused with AngII for 24 hours. Hematoxylin and Eosin staining confirmed large red blood cell accumulation in the myocardium after this short period of AngII infusion.

Conclusion

These data indicate that as soon as AngII infusion occurs, PAI-1 protects against cardiac fibrosis by maintaining hemostasis. Deficiency in PAI-1 leads to cardiac hemorrhage as early as 24 hours after AngII infusion, which may be associated with the large fibrotic deposition in the chronic phase.

Acknowledgment

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Stroke after CABG: A Single Center Experience

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Sibu Saha

Objective

Coronary artery bypass graft surgery is the tenth most common surgery in USA. Despite the improvements in medical management stroke remains a devastating complication after coronary artery bypass graft surgery and is associated with significant mortality and morbidities.

Methods

Study was approved by the IRB. This is a retrospective chart review with 3,126 cases treated at the UK Healthcare from 2016 to 2023 who underwent a CABG procedure. Patients for this study were identified by searching the UKHC electronic medical records.

Results

Out of 3,126 patients who underwent a CABG procedure, stroke was diagnosed in 41(1.3%) patients. Age ≥61 years, male sex, and white were characteristics that were strong predictors of stroke after CABG. In addition, preoperative characteristics such as diabetes mellitus, hyperlipidemia, history of atrial fibrillation and especially, hypertension, were commonly observed in every patient with stroke after CABG, having incidence of 75.6%, 87.8%, 26.7% and 95% respectively. Mortality for stroke patients was 10 (24.4%) at 30 days, and 2 (4.9%) at 5 years, having a total of 12 (29.3%) deaths. Furthermore, postoperative characteristics such as respiratory failure were observed in our study as a common factor in patients who died after stroke after CABG with incidences 92.7%.

Conclusions

With this retrospective study, we are able to observe that UK Healthcare has a stroke incidence after CABG that is very similar to other institutions. Careful assessment and management of the different preoperative, intraoperative, and postoperative factors specific to this institution and its population should be implemented to reduce complications after CABG.

From intact cell to myofibrils: a muscle mechanics techniques comparison

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Cardiac muscle performs rhythmic contraction and relaxation to pump blood. This muscle function is made possible by the fundamental unit of the muscle, the sarcomere. Contraction begins when an action potential triggers L-type calcium channels and calcium enters a cardiomyocyte. The principal protagonists of muscle contraction are myosin motors on the thick filament that cyclically interact with actin filaments, allowing the muscle to shorten. After contraction, the muscle can relax when the calcium levels in the cell decrease. Perturbation of this cycle leads to a large number of diseases. We are interested in Heart Failure with preserved Ejection Fraction (HFpEF) where heart does not fill properly between beats due to impaired relaxation. Our hypothesis is that impaired relaxation in HFpEF begins at the sarcomere level. Sarcomeric relaxation has been studied using different scales of tissue preparations ranging from intact whole muscle fibers to single cardiomyocytes, and to single myofibrils composed of 10 to 20 sarcomeres. In this work, different tissue preparation techniques are systematically compared to determine which one is more suitable for studying relaxation in HFpEF samples. The use of single myofibrils with fast switching activation and deactivations solutions is found to be the perfect candidate for future studies.

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Title: Role of Social Determinants of Health and the Renin-Angiotensin-Aldosterone System in Risk for Hypertensive Disorders of Pregnancy and Related Adverse Outcomes.

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Background: Hypertensive Disorders of Pregnancy (HDP) pose significant risks to both maternal and fetal health. Conditions such as gestational hypertension, pre-eclampsia, and eclampsia are influenced by social determinants of health, including socioeconomic status, access to prenatal care, and racial disparities. Additionally, the renin-angiotensin-aldosterone system (RAAS), which regulates blood pressure and fluid balance, plays a role in the pathophysiology of HDP.

Methods: This study examined the interplay between social determinants, particularly socioeconomic factors and access to care, and RAAS in HDP. Pregnant individuals enrolled at the University of Kentucky, especially those at high risk, provided data. Clinical information, socioeconomic status, and RAAS biomarkers were analyzed to assess their impact on HDP development and progression. Data were collected at two gestation points, and pre-eclampsia outcomes were recorded at delivery. Analyses were conducted by insurance status and race.

Results: Women with Medicaid had 2.1x higher risk of developing preeclampsia compared to women with private insurance, with identical rates of gestational hypertension. Lower renin and higher aldosterone levels were found in the private insurance cohort. The Medicaid cohort had increased incidence of previous preeclampsia, T2DM, Renal disease, tobacco and illicit drug use. Preeclampsia incidence was 11.2%, with 14.7% in the Medicaid cohort and 6.8% in the private insurance cohort. Medicaid cohort had elevated BMI (34.74, $p \leq 0.0074$), with private insurance cohort 1- and 5-Minute Apgar scores greater than Medicaid and trending towards significance ($p = 0.0544$). Tested values such as SBP, DBP, hospital LoS, etc. were not significant.

Conclusions: Social determinants, especially socioeconomic factors, can significantly impact HDP risk and outcomes. This is despite both groups being given prophylactic treatment for preeclampsia due to risk. This could potentially skew data towards similar outcomes and results. The RAAS's role in HDP pathophysiology offers potential intervention avenues in the future.

How changes in expression of genes forming K+ and Na+ channels influence physiological behaviors within Drosophila melanogaster models

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Two-pore-domain K+ channels (K2P) are responsible for maintaining the resting membrane potential of cells. Prior to further identification, these were referred to as leak channels. There appears to be 15 known types of K2P channels in humans and 11 known types in *Drosophila melanogaster*, as well as six subfamilies, although little is known about the expression of these subtypes in various animal tissues or the impact of altered expression on cellular physiology. The *Drosophila melanogaster* model allows for selective misexpression of certain neuron subsets, providing insight into individual cell types and the animal's physiology as a whole. It is established that glial cells within the nervous system play an important role in the development and function of the nervous system, as they release gliotransmitters and cytokines. Prior research on overexpressed glial K2P channels and their impacts on behavior and neuronal function was limited, but yielded results that were uncharacteristic of the model. This project expands upon prior research conducted of *Drosophila* pan-glia cells and motor neurons to examine the effects of K2P overexpression on behavior and physiology. After conducting various assays, it was concluded that overexpression in motor neurons had the most prominent effects on *Drosophila* functioning; with glial, sensory, cardiac and chordotonal neurons also generating statistically significant differences in *Drosophila* activity. Due to these findings, it seems that overexpression of K2P channels has an ability to influence *Drosophila* behaviors.

The regulatory role of asprosin in hypertension

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Asprosin is a novel adipokine, identified through the study of genetic disease called neonatal progeroid syndrome (NPS). So far, two spatio-temporally distinct functions of asprosin have been discovered. Asprosin cell-autonomously induces hepatic glucose release and stimulates appetite via the activation of agouti- related protein (AgRP) neurons of the hypothalamus. Asprosin performs these two spatio-temporally distinct functions via two different receptors. Ptprd (Protein Tyrosine Phosphatase type δ), a membrane bound phosphatase receptor mediates asprosin's orexigenic function, while a G-protein coupled receptor, Olfr734 (mouse ortholog of OR4M1), acts as the hepatic receptor for its glucogenic function. In this ongoing study we are assessing the role of asprosin in regulation of blood pressure (BP). Our preliminary results show that asprosin deficient female mice (NPS) present with significantly lower BP, which can be completely rescued with intra-nasal treatment of recombinant asprosin. Further, at the mechanism level, our preliminary data shows that asprosin's hypertensive effects are mediated by Ptprd signaling in the oxytocin neurons. Both male and female mice, with genetic loss of *Ptprd* from oxytocin^{+} neurons (*Oxy-cre*$^{+}$*;Ptprd*$^{flox/flox}$) had significantly lower MAP (mean arterial pressure) when compared to wild type littermate controls (*Oxy-cre*$^{+}$*;Ptprd*$^{+/+}$). This study identifies a novel function of asprosin and represents a new avenue for therapeutic development for treatment of hypertension.

Cardiac Hemorrhage Precedes Angiotensin II-induced Cardiac Fibrosis in Plasminogen Activator Inhibitor-1 Deficient Mice

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Background

Plasminogen activator inhibitor-1 (PAI-1) regulates hemostasis and induces tissue fibrosis. We detected visually discernable fibrosis in the myocardium of whole-body PAI-1 deficient (PAI-1 -/-) mice infused with angiotensin II (AngII). This was confirmed in tissue sections of myocardium that revealed perivascular and interstitial fibrosis. Therefore, despite PAI-1 being an inhibitor of extracellular matrix-degrading enzymes, its absence profoundly promoted myocardial fibrosis. In agreement with these observations in mice, cardiac fibrosis has been detected in humans with complete PAI-1 deficiency. However, the underlying mechanisms by which PAI-1 deficiency leads to cardiac fibrosis remain unclear.

Methods and Results

To investigate the basis for PAI-1 deficiency leading to cardiac fibrosis, PAI-1 -/- and their wildtype (PAI-1 +/+) littermates were infused with either saline or AngII (1,000 ng/kg/min) for 7 or 28 days. Gross inspection within 7 days of AngII infusion revealed readily discernable hemorrhage in the hearts of PAI-1 -/- mice, but not in their wild type littermates. Tissue sections from these mice showed extravasated erythrocytes in a distinctive distribution within the myocardium. The red blood cell accumulation within the myocardium was not associated with an obvious loss of integrity of coronary arteries. Instead, erythrocytes were diffusely present throughout the myocardium with the largest accumulations in the epicardium. Immunostaining for CD68 and troponin I revealed that cardiac hemorrhage was associated with macrophage accumulation and loss of cardiomyocytes. Additionally, perivascular and interstitial fibrosis, detected by Masson's trichrome staining, was augmented in AngII-infused PAI-1 -/- mice as early as 7 days of infusion, and progressively increased by 28 days of infusion. The distribution of erythrocytes at 7 days corresponded with the pattern of interstitial fibrosis detected by 28 days of infusion in PAI-1 -/- mice. This relationship was verified in mice infused with AngII for 28 days by the coincident staining of fibrosis and ferric iron, which indicates erythrocyte degradation.

Conclusions

PAI-1 deficiency-induced cardiac hemorrhage and fibrosis have a spatial and temporal mode of association, implicating the potentially mechanistic importance of PAI-1 deficiency-induced cardiac hemorrhage in cardiac fibrosis.

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Activation of estrogen receptor alpha increases the amplitude of the eating behavior rhythm in female mice

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The loss of circulating estrogens after menopause increases the risk of cardiovascular disease in women. Estrogens are cardioprotective because of their direct effects on vasculature, including vasodilation and their indirect effects on lipids and insulin sensitivity. We previously found that estradiol treatment of ovariectomized female mice improved cardiometabolic risk in mice by regulating the daily rhythm of eating behavior, but the mechanism is not known. Estrogens signal via estrogen receptors to regulate gene expression and membrane properties in the nervous system to regulate behaviors. The objective of this study was to investigate the role of estrogen receptor alpha (ERα) in the regulation of eating behavior rhythms and diet-induced obesity in mice. To investigate the effects of ERα activation on daily rhythms underlying diet-induced obesity, we ovariectomized female mice and implanted them with pellets containing the selective ERα agonist, propyl pyrazole triol (PPT), or vehicle as a control. Ovariectomized females implanted with control pellets rapidly gained body weight and fat mass when fed high-fat diet, whereas PPT-treated females were entirely resistant to diet-induced obesity. PPT-treated females ate slightly fewer calories than controls, but both groups had similar levels of activity. The feeding efficiency ratio of PPT-treated mice was markedly lower than that of control mice, suggesting that activation of ERα reduces the conversion of calories to body mass during high-fat diet feeding compared to control-treated ovariectomized females. Consolidating food intake to the active phase may be a mechanism that reduces feeding efficiency and thus inhibits diet-induced obesity in females. Consistent with this hypothesis, we found that PPT-treated females fed low- and high-fat diet had extraordinarily high amplitude eating rhythms that peaked at night. For example, 90% of eating events in PPT-treated females occurred during only 7 hours in the active phase. Together these findings demonstrate that ERα is a potent regulator of the eating rhythm. Thus, studying ERα signaling in the brain could reveal the neural circuitry underlying the daily eating rhythm.

Gene Expression and Fatty Acid Profiling of Metabolically-driven Human Hepatocellular Carcinoma

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Background: Hepatocellular carcinoma (HCC) is the most common form of liver cancer and the third leading cause of cancer death worldwide. As hepatitis B remains the foremost risk factor for HCC, metabolic dysfunction-associated steatotic liver disease (MASLD) is the fastest-growing etiology of HCC. The focus of this work is to identify novel gene and fatty acid associations in human MASLD-driven HCC that may be exploited for therapeutic benefit.

Methods: Human HCC tumor (n=8) and adjacent non-tumor samples (n=8) were obtained from the Biospecimen Procurement and Translational Pathology Shared Resource Facility at the University of Kentucky Markey Cancer Center. All patients met cardiometabolic MASLD criteria and were negative for viral hepatitis. Hematoxylin and eosin (H&E) staining was used for pathological determination of tumor and adjacent nontumor tissue. Lipids were extracted using a methyl-tert-butyl ether extraction method and subjected to lipidomics by the West Coast Metabolomics Center. RNA was isolated, purified, and used for bulk sequencing. Data were analyzed using paired nonparametric analyses via a Wilcoxon or Mann-Whitney test, where appropriate.

Results: Histological analysis by H&E showed significant lipid vacuole accumulation in HCC tumors relative to nontumor tissue. Lipidomics analyses revealed significant increases in long-chain nonesterified monounsaturated fatty acids (MUFAs; C16:1, C18:1, C20:1) and MUFA-enriched phospholipids (PC30:1, PC32:1, PE32:1, and PC36:1) in tumors relative to nontumor tissue. No significant differences were observed in nonesterified polyunsaturated fatty acids (PUFAs; C18:2, C20:4, and C22:6), PUFA-enriched phospholipids (C36:4, C38:4, C38:6, C40:6), or in fatty acid esters of hydroxy fatty acids (FAHFAs; C38:2, C38:4, C38:6). However, both MUFA- (C14:1, C18:1) and PUFA-enriched acylcarnitines (C18:2, C18:3) were collectively reduced in human tumors. Differential analysis of RNA sequencing revealed 854 genes down regulated and 850 genes upregulated in tumors versus nontumor tissue. Consistently, fatty acid oxidation genes (*CPT1A*, *CPT2*, *ACADL*, *ACADM*, *ACADS, HADHA*) were significantly lower in tumor versus nontumor tissue. Genes involved in *de novo* lipogenesis were largely dysregulated (e.g. no differences in *SREBF1* or *FASN*; increases in *ACLY, ACACA,* and *SCD1*; decreases in *ACSL1*) in tumor versus nontumor tissue.

Conclusions: These results suggest HCC tumors exhibit a reduced capacity to undergo mitochondrial β-oxidation resulting in accumulation of free- and esterified-MUFAs with a concomitant reduction in MUFA-carnitines. Current studies are underway to determine mechanisms by which MUFAs and the impairment of hepatic MUFA catabolism through FAO promotes the development of HCC and tumor growth in mice.

Intermediate Signalling in Restoration of Dilated Cardiomyopathy and Improvement of Cardiac Dysfunction

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Background and Significance:

Heart disease is the leading cause of death within the Commonwealth of Kentucky and in the United States. Dilated cardiomyopathy (DCM) is a prevalent form of heart disease characterized by ventricular chamber enlargement and impaired systolic function, leading to heart failure. RRAD protein contributes to myocardial function; deletion of cardiomyocyte RRAD (cRadKO) results in a stable positive inotropic state of the heart that is well-tolerated in health. The present study tests cRadKO alterations to acute function, and to signalling intermediaries in the setting of DCM.

Methods:

A DCM murine model of heart failure known as *muscle lim protein knockout* (MLPKO) was used. Cardiomyocyte RADko was induced at 10 weeks of age in MLPKO mice to create double cRadKO+MLPko mice (abbreviated dKO). Male and female mice were used. Western blot analysis was performed to investigate the regulation of calcium cycling within cardiomyocytes by probing for SERCA, pTHR17/PLN, and RYR2. To probe intermediaries of heart remodelling and pro-hypertrophic signalling we assessed Phospho-P38, Phospho-Akt, Phospho-GSK3β, Phospho-PKCα, and Phospho-SAPK/JNK.

Results:

Calcium cycling proteins (SERCA, pThr17/PLN, and RYR2) exhibited relative increases in signal in the dKO mice compared to the MLPKO mice. These increases were observed in both sexes, consistent with enhancement of calcium handling and regulatory mechanics in the dKO mice relative to the MLPKO mice. In addition, the dKO mice showed relative increases in phosphorylated signaling proteins- Phospho-P38, Phospho-Akt, Phospho-GSK3β, Phospho-PKCα, and Phospho-SAPK/JNK- when compared to MLPKO mice across both sexes.

Conclusions:

Induction of RAD deletion after the onset of DCM improved cardiac dysfunction and attenuated pathological remodeling, as evidenced by enhanced signaling of key proteins involved in hypertrophic signaling and Ca^{2+} handling. The increased phosphorylation of the pro-hypertrophic proteins in the dKO mice suggests a complex interplay of signaling pathways that contribute to the observed amelioration of cardiac dysfunction. Changes in phosphorylation-status of signalling intermediaries might contribute to cellular adaptation and pro-adaptive mechanisms driven by cRadKO.

Critical Role of Testosterone in the Circadian Rhythm of Mouse Blood Pressure

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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. Previous studies have found if change food intake pattern can alter BP rhythm in several animal species. However, it is mostly unknown whether, and if so, via what mechanism, male sex steroids regulate the circadian rhythm of BP. Additionally, whether the BP rhythm regulation by male sex steroids is associated with food intake rhythm still need to be elucidate. The current study investigates the role of male steroids on BP/food intake circadian rhythm and clock gene per2 oscillations.

Approach and Results - We monitored 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 and clock genes mRNA expression by real-time qPCR (RTqPCR) in various tissues. The preliminary results of the ex vivo monitoring found that the peripheral oscillators in aorta, thymus and white adipose tissue were phase advanced to various extents by orchiectomy. Our preliminary analysis of RTqPCR showed orchiectomy altered Bmal1 and Per2 mRNA levels in aorta, WAT and thymus compared to sham surgery group.

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 and clock genes mRNA expression in various peripheral tissues to different extents.

Lipid Droplet Accumulation in the Aging and Alzheimer's Diseased Brain

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Background: Apolipoprotein E4 (*APOE4*), the strongest genetic risk factor for late-onset Alzheimer's Disease (AD), is associated with neuroinflammation, lipid droplet (LD) accumulation, and reduced clearance of amyloid beta. In the AD brain, glial cells such as microglia and astrocytes become reactive and can become lipid laden. Previously, *APOE4* has been associated with increased accumulation of LDs in these cells. However, LD accumulation in humanized ApoE targeted replacement mice, a commonly used model of late-onset AD risk, has not been investigated. We hypothesize that E4 expressing glial cells will have increased accumulation of LDs, as compared to E3 and E2.

Methods: Humanized ApoE mice were crossed with 5xFAD mice (EFAD mice) from which brains were harvested from both young (~4 months) and aged (~15 months) mice. In addition to an age comparison, a western diet was given to older mice for 6 months before harvesting at 15 months. Immunohistochemistry was performed using markers for LDs (Plin2), microglia (IBA1), and astrocytes (GFAP) on whole brain sections. Using the Indica HALO platform, we correlated LD load with cell-type colocalization.

Results: Staining age-matched controls revealed E4 microglia trended towards higher levels of LDs compared to their E2 and E3 counterparts. The E4 mice also had more amyloid-beta plaques, specifically within the hippocampus and isocortex, which were surrounded by LD burdened astrocytes and microglia. Finally, we found LDs were not only found in these glial cells but also in neuron-dense regions of the brain, a surprising finding given that the literature primarily discusses glial LD accumulation.

Conclusion: Preliminary data based on IHC and image quantification of cell colocalization suggests a trend toward higher LD content in E4 brains compared to their E3 and E2 counterparts. Additionally, E4 brains contain more amyloid plaques than E3 and E2 brains. Additional staining using neuronal-specific makers (ex. MAP2) will need to be performed to understand neuronal LDs.

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Spatially-Explicit Simulations Predict How Different Modes of MyBP-C Function Modulate Isometric Twitches

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Multiple groups are trying to develop sarcomere-based therapies for Heart Failure with reduced Ejection Fraction. Trials that attempted to activate myosin with omecamtiv mecarbil showed minimal benefit, in part because the increased contraction compromised diastole. Cardiac Myosin Binding Protein-C (cMyBP-C) regulates both contraction and relaxation under physiological conditions and could be a more effective therapeutic target. However, cMyBP-C's complex function makes it difficult to study in biological experiments.

Here we used FiberSim, a spatially-explicit model of half-sarcomeres (https://campbell-muscle-lab.github.io/FiberSim/) to investigate how different potential modes of cMyBP-C function modulate contractile properties. In the model, cMyBP-C molecules are restricted to 9 stripes in the C-zone of the half-sarcomere where they have the appropriate stoichiometry (3 cMyBP-C molecules to 18 myosin molecular per 43 nm thick filament repeat). The cMyBP-C molecules can transition between a null state (no effect) and two states that respectively stabilize myosin in its suppressed super-relaxed / interacting heads motif / OFF configuration or bind to available sites on the thin filament. cMyBP-C molecules that are bound to actin increase thin filament activation via cooperative effects.

As shown in Fig 1, the time-course of isometric twitch contractions is prolonged when cMyBP-C molecules bind to the thin filament. Peak force is reduced when cMyBP-C stabilizes the SRX state. Simulations in which some cMyBP-C molecules bind actin and some stabilize myosin in the suppressed state have smaller slower twitches.

Ongoing work is testing how cMyBP-C modulates afterloaded twitches that may be more representative of myocardial function in vivo.

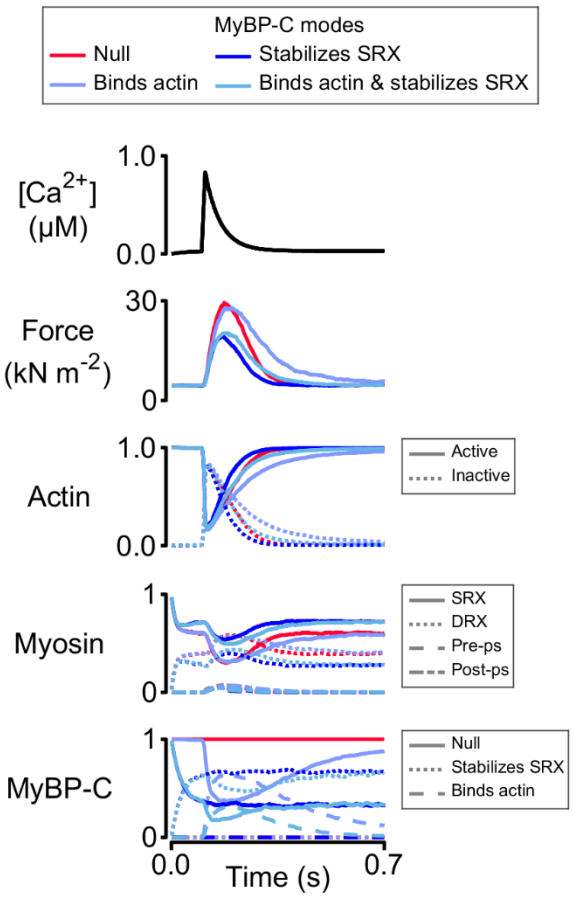

Figure 1: FiberSim-based simualtions of isometric twitches.

Lipids and Lesions: The Impact of Proteolytic Modifications on Lipoproteins and Atherosclerosis Risk

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Background: Atherosclerosis, a leading cause of CVD, is tightly associated with high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol levels. While these lipoproteins are known for their roles in cholesterol transport, emerging evidence suggests that they have functional properties beyond their cholesterol carrying capacity that affect their atherogenicity. For example, HDL associates with Alpha-1-antitrypsin (AAT), a protease inhibitor that targets elastase, a protease that is believed to modify lipoproteins in plaque and accelerate plaque progression. AAT-deficient (AATD) individuals lack this protease inhibitor, leaving elastase activity unchecked, which may affect their risk for developing atherosclerosis. Our lab recently published that an AAT-mimetic peptide that targets to HDL can stabilize lesion progression, suggesting an atheroprotective role for HDL-associated-AAT. However, clinical studies conflict over the relationship between AATD and CVD, highlighting the need for further investigation into the mechanistic relationship between elastase and circulating lipids.

Purpose: Here, we propose to characterize the effects of elastase-mediated damage to lipoproteins in circulation with the goal of pinpointing the role of AAT in protection against atherosclerotic cardiovascular disease. We hypothesize that elastase will degrade HDL and LDL *in vitro* and *in vivo,* and AAT-deficiency will result in altered susceptibility to atherogenesis due to proteolytic modification of circulating lipoproteins.

Methods and Results: Size-exclusion chromatography and lipid assays demonstrate that *Serpina1*$^{-/-}$ (AAT deficient) mice display an altered lipid profile characterized by reduced plasma triglyceride and increased HDL-cholesterol. Administration of an HDL-targeting AAT-mimetic peptide partially corrects this dyslipidemia, suggesting that alterations in circulating lipoproteins are caused by increased elastase activity in this strain. To examine the impact of elastase on lipoproteins *in vitro*, *Serpina1*$^{-/-}$ mouse plasma was treated with a dose range of human neutrophil elastase for 1 hour at 37ºC. SDS PAGE and Western blotting demonstrate dose-dependent degradation of ApoA1 and ApoB in *Serpina1*$^{-/-}$ mouse plasma. Analysis of protein susceptibility to elastase cleavage indicates a possible preferential degradation of lipoprotein-associated proteins compared to non-lipoprotein associated proteins. In the future, we will compare lesion size and morphology in *Serpina1*$^{-/-}$ mice and *Serpina1*$^{+/+}$ mice to assess the effect of AATD on atherosclerosis.

Conclusions: *Serpina1*$^{-/-}$ mice display an altered lipid profile which may be driven by increased circulating elastase activity in this strain. These proteolytic modifications may influence lipoprotein metabolism and impact atherosclerosis risk. Discovering the role of AAT and elastase in lipoprotein modification and lesion progression will lead to insight on novel functions of HDL and LDL and inform the development of more efficient therapeutics for CVD.

Rad Regulation of L-type Calcium Channels

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Background: Rad is key to beta-adrenergic regulation of the L-type channel. Deletion or phosphorylation of Rad disinhibits the channel, resulting modulation of the channel, where conductance is elevated, V1/2 is negatively shifted, and decay kinetics are faster. L-type channels possess the capacity to facilitate in response to repeated depolarizations, resulting in increased macroscopic conductance and slower decay kinetics. Elevated CaMKII activity downstream of increased cytosolic calcium is the putative mechanism underlying calcium dependent facilitation.

Aims: To determine the mechanism through which Rad governs facilitation of the L-type channel.

Methods: Using the whole-cell configuration of the patch clamp technique, we paced isolated myocytes hearts with and without cardiac restricted deletion of Rad (cRadKO). Cells were paced with step-clamp (0mV, 200ms) and ramp (-80-+60mV, 200ms) protocols at a frequency of 1/2 Hz and 1/3 Hz respectively.

Results: cRadKO resulted in elevated facilitation compared to wildtype controls. Use of barium as charge carrier or the equimolar replacement of EGTA with BAPTA eliminated calcium dependent facilitation (CDF) in cRadKO. 10uM KN-93 in the intracellular solutions eliminated CDF in cRadKO. As flux increases in the absence of Rad, the degree of facilitation decreases.

Conclusions: cRadKO results in elevated facilitation which is calcium dependent and attenuated by CaMKII inhibitor KN-93. In the absence of Rad, as flux increases, the degree of facilitation decreases, possibly representative of heterogeneity in channel clustering in the absence of Rad.

This work was supported by NIH HL072936 (DAA & JS), DoD W81XWH-20-1-04 (DAA & JS), amd WU-13-238 (JS).

Role of the X chromosome gene, *Kdm5c*, in sexual dimorphism of AngII-induced aortopathies

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Background: Aortopathies, including thoracic and abdominal aortic aneurysms, are sexually dimorphic diseases occurring at a greater prevalence and severity in males than females. Due to the limited treatment options, aortopathies are life threatening conditions resulting in high morbidity and mortality from aortic rupture. Recently, we demonstrated that an XX sex chromosome complement protected female mice from AngII-induced aortopathies. In addition, we showed that a gene on the X chromosome known to escape X-inactivation in mice and humans, namely *Kdm5c*, was more highly expressed in aortas of XX than XO female mice. In this study, we hypothesized that gene dosage effects of *Kdm5c*, an X chromosome gene that escapes X inactivation, protects females against aortopathies.

Methods: We bred *Kdm5c* floxed heterozygous females (*Kdm5c*$^{fl/+}$) to germ-line Cre expressing transgenic male mice (B6. Ctg(CMV-cre)1Cgn/J) to obtain female mice that are either wild-type (*Kdm5c*$^{+/+}$) or globally hemizygous for this gene (*Kdm5c*$^{+/-}$). In terms of gene dosage, the *Kdm5c*$^{+/-}$ female mouse is analogous to an XY male mouse that has only one copy of this gene. At two months of age, female mice of each genotype (on a C57BL/6 genetic background) were injected intraperitoneally with an adeno-associated virus carrying a gain of function mutant of proprotein convertase subtilisin/kexin type 9 (AAV-PCSK9) to induce hypercholesterolemia (which augments susceptibility to aortopathies). Mice were fed Western diet through study endpoint. One week after AAV injection, mice were infused with AngII (1,000 ng/kg/min) by osmotic minipump for 28 days and we monitored the disease development via ultrasound.

Results: Surprisingly, at baseline prior to AngII infusion, *Kdm5c*$^{+/-}$ females exhibited significantly dilated ascending and abdominal aortic lumen diameters compared to *Kdm5c*$^{+/+}$ females. Following AngII infusion, internal diameters of the ascending and abdominal regions of aortas from *Kdm5c*$^{+/-}$ females were increased, but this effect was not observed in *Kdm5c*$^{+/+}$ females. Furthermore, at study endpoint, maximal external diameters of ascending and abdominal aortas were significantly greater in AngII-infused *Kdm5c*$^{+/-}$ females compared to *Kdm5c*+/+ females. As an additional measure for aortopathy, we quantified aortic weights and aortic arch area; both of these parameters were significantly greater in hemizygous *Kdm5c*$^{+/-}$ than *Kdm5c*$^{+/+}$ females.

Conclusion: These results suggest that gene dosage effects of the X chromosome gene, *Kdm5c*, protect females from the development of aortopathy. Future studies should identify downstream targets regulated by the X chromosome gene, *Kdm5c*.

De Novo Elastic Fiber Synthesis by Smooth Muscle Cells Following Aortic Dissection in Mice

Sohei Ito, Hong S. Lu, Alan Daugherty, Hisashi Sawada

Introduction

Aortic dissection (AD) is a life-threatening disease characterized by blood accumulation in the aortic wall with subsequent development of a false lumen and enhanced susceptibility to rupture. Despite considerable attention to the initiation of AD onset in previous studies, substantial knowledge gaps exist regarding the pathophysiology following AD. Elastic fibers are a crucial component of the extracellular matrix in maintaining the structural integrity of the aortic wall. However, it remains unknown whether and how the synthesis of elastic fibers is reactivated after the onset of AD.

Methods and Results

β-aminopropionitrile (BAPN) was administered to 4-week-old male C57BL/6J mice to induced AD. Four weeks of BAPN administration generated acute AD with mild dilatation of a false lumen filled with fresh thrombus, whereas 12 weeks of BAPN administration led to chronic AD exhibiting pronounced false lumen aneurysm formation with substantial thickening of the vascular wall. qPCR revealed that, despite a slight decrease at the acute phase of AD, elastin mRNA abundance increased significantly in the chronic phase of AD compared to vehicle controls. I*n situ* hybridization demonstrated that elastin mRNA was distributed mainly in the vascular wall circumscribing the false lumen. In Verhoeff's iron hematoxylin staining, numerous elastic fibers were observed in the vascular wall of the false lumen. Compared to native elastic fibers around the true lumen, elastic fibers in the false lumen wall had more layers, but were more densely packed, thinner, and less organized. These results suggest that, while structurally different, elastic fibers are newly synthesized in the false lumen wall following AD. Of note, de novo elastic fiber formation was not observed in mice that died of false lumen rupture in the chronic phase. To assess biomechanical properties of de novo elastic fibers, vascular wall elasticity was measured by ultrasonography in mice without AD and mice with AD at acute or chronic phase. Compared to control aortas, the elasticity was elevated significantly at the chronic phase of AD, suggesting improved stabilization of the vascular wall. Next, immunostaining for aortic cell markers was performed to determine the responsible cell type generating de novo elastic fibers. The vascular wall of the false lumen was comprised predominantly of cells expressing smooth muscle cell (SMC) markers; αSMA and MYH11, and these cells were colocalized with elastin mRNA. These data suggest that SMCs are the major cell type of the false lumen wall and the source of de novo elastic fibers.

Conclusion

In response to AD progression, elastic fibers are newly synthesized by SMCs, contributing to the stabilization of the false lumen vascular wall.

Title: **ECMO as a Rescue Measure for Post Cardiotomy Circulatory Collapse: A Single Center Experience**

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Introduction:
Nearly 500,000 open heart operations are performed in the United States each year. These are high risk procedures with reported complications including post-cardiotomy circulatory collapse. It is reported in the literature that 2-3% of patients who undergo open heart surgery fail to wean from cardiopulmonary bypass (CPB). Extracorporeal Membrane Oxygenation (ECMO) is one of the measures used to salvage these patients. Outcomes following initiation of this therapy, though, are often poor. The aim of the study was to review our institution's experience with ECMO in the management of post cardiotomy circulatory collapse and analyze the outcomes of patients who were treated with ECMO therapy.

Methods: This study was conducted following IRB approval. We performed a retrospective descriptive study of all patients ages 18-90 who underwent an open-heart procedure requiring ECMO support from April 1, 2014, to December 31, 2022. There were no specific exclusion criteria. Subjects for this study were identified by searching the electronic medical records database on the CPT codes for open heart procedure requiring ECMO. We then performed an in-depth chart review for each patient and analyzed the data.

Results: We identified 45 patients who fit our inclusion criteria out of a total of 6,346 open heart procedures. The study population consisted of 33 males and 12 females. Most patients were Caucasian (40) followed by Black or African American (4) and Asian American (1). The average patient age was 59.9 years with the youngest being 32 years and the oldest 84 years. The operations performed included Heart Transplant (17), CABG (12), AVR (6), MVR (6), Aortic Root Replacement (5), Tricuspid Ring Annuloplasty (4), and HVAD placement (1). Some patients underwent multiple procedures during the same operation. Patients spent an average of 6.8 days on ECMO therapy. 23 patients died during their stay in the hospital, while 22 patients survived and were discharged to Home (7), Rehabilitation Facility (12) , Long Term Acute Care Hospital (2), or Detention Center (1). There were no complications related to ECMO device therapy.

Conclusions: Without intervention, outcomes following post cardiotomy circulatory collapse are fatal. A variety of techniques exist to salvage these critically ill patients. ECMO therapy is a valuable option for these patients. Our study shows a 48.8% survival rate following initiation of ECMO therapy. This along with the remainder of our data provides valuable insight into the use of ECMO as a salvage therapy for patients suffering from post-cardiotomy circulatory collapse at our institution.

Acknowledgements:

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Distinct Aortic Pathological Features in Mice with Smooth Muscle Cell-specific Deletion of Fibrillin-1

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Background: Fibrillin-1 (FBN1) is a crucial structural protein of elastic fibers; global mutations of this protein lead to aortic root and proximal ascending aortic aneurysms, exhibiting significant sexual dimorphism. FBN1 is abundant in smooth muscle cells (SMCs), the predominant cell type in the aortic wall. This study determined the effect of SMC-specific deletion of FBN1 on aortic pathologies in mice.

Methods: SMC-specific FBN1 deleted (SMC-FBN1-/-) mice were generated, and their aortic phenotypes were compared to their wild-type littermates (SMC-FBN1+/+) using ultrasonography, in situ, and ex vivo imaging, micro-CT, proteomics, and histological analysis.

Results: Quantitative PCR and immunostaining confirmed the absence of *Fbn1* mRNA and FBN1 protein in the aortic media, while it remained abundant in the endothelia and adventitia of SMC-FBN1-/- mice. Ultrasound monitoring observed luminal dilations in the ascending aortic region at 4 weeks of age, which progressively expanded with age in SMC-FBN1-/- mice compared to their SMC-FBN1+/+ littermates. micro-CT scanning confirmed expansions from the distal ascending region to the proximal descending thoracic aortic region at 12 weeks of age, while no apparent dilations were observed in the aortic root, proximal ascending region, and abdominal aortic region of SMC-FBN1-/- mice. The aortic phenotype was consistent in both male and female mice. Proteomics analysis in mice at 4 weeks of age revealed a reduced abundance of extracellular matrix organization-related proteins in SMC-FBN1-/- mice, compared to their SMC-FBN1+/+ littermates. Histologically, elastic fibers with comparable laminar layers were formed in SMC-FBN1+/+ and -/- mice; however, SMC-FBN1 deletion led to thinner and fragmented elastic fibers.

Conclusions: SMC-specific deletion of FBN1 induced distinct aortic pathologies in the distal ascending aorta through the descending thoracic aortic region, irrespective of sex. Although SMC-specific FBN1 deletion did not prevent elastic fiber formation, it impaired aortic wall integrity.

Key Words: smooth muscle cells; fibrillin-1; aorta; extracellular matrix; mouse

The PPARγ Coregulator Landscape Across Five Different Fat Pads Based on Dietary Fat Intake: Negative Indications of Lipids that Change the Transcriptional Targets

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Rosiglitazone (Rosi) treats type 2 diabetes by improving insulin resistance. Its use has been halted due to a subset of patients developing congestive heart failure (CHF). The mechanisms by which this occurs are unknown. Rosi targets PPARγ, a nuclear receptor that is highly expressed in adipose tissue and, to a lesser extent, in cardiac tissue. An important mechanism of PPARγ regulation is through coregulators that can alter transcriptional activity through protein-protein interaction to drive PPARγ toward specific pathways. We hypothesized coregulator interaction with PPARγ may be regulating its activity differently in obese conditions, inducing CHF through adipose-derived peripheral signaling. To address this, we fed C57BL/6 mice a normal chow (NCD) or high-fat (HFD) diet for 16 weeks. Following this, we injected mice with Rosi every 48 hours for 4 weeks. Interestingly, in both NCD and HFD Rosi mice, we found significantly increased heart weights (p-value<0.05), and various changes in weights of five adipose depots. To understand the roles of the PPARγ coregulator interactome in obesity, we used our state-of-the-art PamGene PamStation Nuclear Hormone Receptor chip to measure the interaction of PPARγ with 155 coregulators in five adipose depots and the liver. In adipose depots increasing in weight due to Rosi treatment, such as retroperitoneal white adipose tissue (WAT) and mesenteric WAT, more coregulators are associated with the PPARγ complex. Interestingly, depots that did not change in weight with Rosi treatment, such as epididymal WAT and inguinal WAT, showed a reduction of coregulators leaving the PPARγ complex, indicating differential PPARγ regulation. Our novel data suggests that the coregulator interactome of adipose depots represent potential therapeutic targets to ascertain the mechanism by which Rosi treatment and adipose signaling may cause CHF in a subset of patients, and how treatment responses are influenced by dietary patterns.

Impact of Cardiomyocyte-Restricted Deletion of RAD on Voluntary Exercise and Cardiac Remodeling

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Background: RAD regulates calcium current through the $Ca_v1.2$ channel. Cardiomyocyte-restricted deletion of RAD (cRADKO) improves calcium channel activity and systolic function in healthy mice, but the impact of cRADKO on voluntary exercise and its impact on cardiac communication with extracardiac tissue is unexplored. An athlete's heart has overlapping features of dilated cardiomyopathy including LV chamber dilation with a decreased ejection fraction.

Hypothesis: cRAD deletion will pre-adapt the heart to exercise.

Methods: cRADKO mice were injected with tamoxifen at 6 weeks and placed on running wheels at 9 weeks. To measure exercise, we used the Progressive Weighted-wheel-Running (PoWeR) model of mouse exercise training; it provides voluntary exercise without stressing the mice. We collected echo data at the start and end of the 8-week training time. We are measuring cardiomyocyte cell size, cardiac fibrosis, and capillary density. To evaluate extra-cardiac effects we harvested soleus, gastrocnemius, and plantaris muscles to measure muscle fiber type, fiber size, and myonuclear density.

Results

We found no significant difference in voluntary exercise activity between the wildtype and the cRADKO mice. Exercise training effects were sexually dimorphic. Only male WT mice showed a significant dilation and reduced ejection fraction (EF). Male cRADKO mice showed elevated EF at baseline and cRADKO prevented changes in EF or chamber dimension. cRADKO LV thickness increased compared to WT. Female mice showed no changes in heart structure or in vivo function with exercise.

Future Directions and Expected Results

WT and cRadKO indistinguishable total exercise eliminate differential exercise as a variable, thus permitting evaluation of gene knockout effect. cRadKO in sedentary healthy background does not alter any known signaling pathways. To assess cRadKO effect on physiological hypertrophic signaling I will be measuring candidate signaling intermediaries. Athletes heart masquerades as DCM and is mainly seen in males. Our WT mice on PoWeR recapitulated human athletes heart, and cRadKO pre-adapted the heart to exercise.

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Title: Identification of the Molecular Determinants for the Circadian Regulation of the Human *KCNH2* promoter

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Background: *KCNH2* encodes a voltage-gated potassium channel (Kv11.1), which conducts the rapid delayed rectifier potassium current (I_{Kr}) critical for ventricular repolarization in the heart. Circadian clock factors regulate the expression of the *KCNH2* mRNA transcript in the heart. However, the molecular mechanisms for the circadian expression of *KCNH2* transcripts have yet to be defined.
Hypothesis: A conserved cis-regulatory element in the core *KCNH2* promoter drives circadian regulation of human *KCNH2* (h*KCNH2*) promoter activity.
Objective: Identify the molecular determinants of the circadian regulation of h*KCNH2* promoter activity and determine the impact of single nucleotide polymorphisms (SNPs) in this region.
Methods: Real-time bioluminescence (LumiCycle, Actimetrics) and Dual Glo luciferase assays were employed to identify critical cis elements that regulate the circadian expression of the human *KCNH2* promoter using promoter-luciferase reporter constructs. We studied several deletion and mutant h*KCNH2* promoter reporter vectors transiently transfected in a myogenic cell line (C2C12 cells). Bioluminescence was measured at 10-minute intervals for 7-10 days. Data were analyzed for circadian characteristics (period, phase and amplitude). Mutant constructs included both engineered and constructs containing naturally occurring rare and common SNPs.
Results: Lumicycle data demonstrated that the h*KCNH2* promoter reporter showed robust circadian and overall activity in C2C12 cells. Deletion and mutational analysis of the h*KCNH2* promoter-reporter construct identified a highly conserved tandem E-box element within 1 Kb of the exon 1 start site that was critical for circadian and overall regulation of h*KCNH2* promoter activity. Cells expressing h*KCNH2* promoter-reporter constructs with different SNPs in this tandem E-box element showed SNP-specific effects. Surprisingly, a relatively common SNP decreased h*KCNH2* circadian promoter activity by 66% ($n = 6$, $p=0.0049$).
Conclusion: We identified a conserved tandem E-box in the proximal promoter of h*KCNH2* Exon1 necessary for circadian and overall h*KCNH2* promoter activity. Several SNPs in this region suggested a functional impact that might lead to decreased levels of I_{Kr}.

Thrombotic Mechanisms in People Living with HIV at Initial Diagnosis

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Background: One of the leading causes of death in people living with a human immunodeficiency virus (HIV) infection (PLWH) is thrombotic events. Multiple thrombotic risk factors are prevalent in PLWH, including decreased Protein S (PS) (~60% of PLWH) and elevated von Willebrand factor (VWF). VWF is a procoagulant protein that we recently showed binds and inhibits PS when it unfolds. PS is a critical cofactor for the anticoagulant activated Protein C (APC). We hypothesize VWF-mediated PS inhibition contributes to thrombotic risk in PLWH. We compared PS activity to thrombin generation and microclot formation in PLWH.
Methods: Citrated plasma was collected from PLWH at initial diagnosis (n=5) and healthy controls (n=8). Thrombin generation, PS activity, and microclot formation were measured.
Results: We began by optimizing an assay to measure PS activity in plasma. Tissue factor (TF)-initiated thrombin generation was measured in the presence or absence of exogenous APC. Since APC activity is PS dependent, the effect of APC indicates plasma PS cofactor activity. Multiple concentrations of APC, TF, and phospholipid (PL) were tested. APC was either preincubated in plasma or added with a PL-TF activation mixture. Results showed that 30μM PL, 6.8pM TF, and 5nM APC were ideal, and that APC had a larger effect when added with the PL-TF mixture. 5nM APC was ideal because higher concentrations showed PS independent activity.

We used this assay to compare thrombin generation in the presence and absence of APC and vortexing, a technique which allows us to unfold plasma VWF. In the absence of APC, plasma from healthy controls showed elevated thrombin generation compared to PLWH, marked by significantly increased endogenous thrombin potential (ETP) (HC=1473$\pm$253, PLWH=1230$\pm$189, p=0.0451). Trends towards elevated peak thrombin and maximal velocity in healthy controls were also observed. In the presence of APC, these differences disappeared, though the lagtime was slightly prolonged in PLWH. These results indicate that APC had less activity in PLWH than healthy controls, supporting the hypothesis that reduced PS activity contributes to increased thrombin generation in PLWH. Ratios comparing the presence and absence of vortexing showed significantly less change in ETP (HC=0.86$\pm$0.11, PLWH=1.04$\pm$0.03, p=0.0242), peak thrombin (HC=0.85$\pm$0.11 HIV=1.06$\pm$0.09 p=0.0424), and maximal velocity (HC=0.70$\pm$0.14 HIV=1.08$\pm$0.09 p=0.0121) in the absence of APC. In the presence of APC, these differences again disappeared. Microclots, measured using the amyloid protein-binding fluorescent dye Thioflavin T, were not different between PLWH and controls, suggesting that the fibrinolytic system is functioning properly in PLWH.
Conclusions: These data support the hypothesis that decreased PS activity contributes to increased risk of thrombotic events in PLWH. Future directions include running D-dimer assays on these samples and collecting samples from PLWH after they have been started on antiretroviral therapy (ART) to compare the levels of thrombin generation, microclots, and D-dimer pre- and post- ART. These results suggest that monitoring and correcting VWF and PS levels in PLWH in clinical settings could decrease their risk of a thrombotic event.
Funding: This work was supported by NHLBI grant R35HL150818

Increased Microclots Are Associated with Long Covid

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Background: Some individuals are unable to recover from acute conditions of COVID-19 and develop long-term complications, including tissue damage, auto-immunity, and persistent inflammation for 12 months or more following infection, collectively known as “long Covid”. Thrombotic markers, including elevated von Willebrand factor (VWF) and platelet-neutrophil aggregates, are associated with long Covid, though large thrombotic events are uncommon. We hypothesize that small thrombi, “microclots,” are prevalent in long Covid, and tested this hypothesis, utilizing plasma samples from a cohort of long Covid patients and healthy controls.

Methods: A total of 310 plasma samples were used, including 140 long Covid patients (each at baseline and Week 10) and 30 healthy controls. Microclots were measured using Thioflavin T, a fluorescent dye that binds to amyloid fibrils.

Results: We have previously used this assay to show that microclots are significantly increased in acute Covid patients, compared to uninfected controls. This study compared microclots in long Covid patients and healthy controls. At baseline, microclots were elevated ~20% in patients compared to controls (1.22±0.78 vs. 1.00±0.25, respectively; $p<0.0001$). At week 10, this difference had reduced, but was still significant (Long Covid: 1.11±0.40; Controls: 1.01±0.25; $p=0.0046$), suggesting that microthrombosis decreases over time, though not in every patient.

We next assessed demographic effects on microclot measurements. First, there was no difference between males (n=62) and females (n=90) at baseline (Males=0.396±2.5; Females = 0.967±8.68; $p=0.46068$) and Week 10 (Males=0.295±1.4; Females = 0.459±2.69; $p=0.38846$). Similarly, we found no statistical difference ($p=0.09313$) between Hispanic (n=18) and non-Hispanic (n=134) individuals. Lastly, there were no significant racial differences when analyzing White (n=112), Asian (n=20) ($p=0.43489$), and Black/African American populations (n=3).

Conclusions: The results support our hypothesis that microclots are associated with long Covid. Future studies will identify mechanism(s) of clot formation in these patients. This work was supported by Pfizer, Inc.

Exenatide, a GLP-1 receptor agonist, and its influence on the Diabetic Heart: Does Timing Alter Clock Gene Expression?

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Abstract

Type 2 diabetes mellitus (T2DM) affects approximately 383 million people globally, with estimates reaching nearly 600 million by 2035. T2DM is linked to autonomic dysfunction, which increases the risk of cardiovascular complications. Circadian rhythms, governed by clock genes, are crucial for regulating blood pressure, heart rate, and vascular function, and their disruption heightens cardiovascular risk. While glucagon-like peptide-1 receptor agonists (GLP-1RAs) are effective in lowering hemoglobin A1c and body weight in T2DM, their impact on circadian gene regulation remains underexplored.

This study investigates whether exenatide, a short-acting GLP-1RA, can restore normal circadian gene oscillations in diabetic mice, focusing on the heart and liver. Previous research shows that db/db mice exhibit circadian rhythm disruptions, with exenatide administered at ZT0 restoring normal rhythms and reducing hypertension, whereas ZT12 administration worsened these parameters. In this study, key clock genes (bmal1, clock, cry1, cry2, per1, per2, rev-erbα) were examined. This project aims to highlight the potential importance of timing in GLP-1RA treatment in a clinical setting and its ability to modulate circadian gene expression.

Cardiac Sarcoidosis and Amyloidosis Display Site Specific Decreased Phosphorylation of Myosin Binding Protein C

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Infiltrative cardiomyopathies are a subset of restrictive cardiomyopathies characterized by the aggregation of abnormal proteins, cells, or materials within the extracellular matrix of the heart. The two most common types of infiltrative cardiomyopathy are cardiac sarcoidosis and amyloidosis. Cardiac sarcoidosis and amyloidosis are the result of accumulation of immune cells or insoluble protein fibrils, respectively, within the myocardium. This results in increased fibrosis, thickening of the heart walls, and heart failure. These infiltrative cardiomyopathies primarily impact the extracellular matrix, but it is unclear whether cardiomyocyte contractile function is also impaired. Within the cardiomyocyte, contractility can be regulated via phosphorylation of sarcomere proteins. Specifically, dephosphorylation of myosin binding protein C (MyBPC) can impair contractility. MyBPC has been shown to be hypophosphorylated in dilated cardiomyopathy (DCM). MyBPC phosphorylation status was evaluated to investigate if infiltrative cardiomyopathies display similar hypophosphorylation as observed in prior DCM studies. Left ventricle samples from patients with cardiac sarcoidosis and amyloidosis were analyzed by western blot. Phospho-specific antibodies were used to assess phosphorylation of MyBPC at Ser273, Ser282, and Ser302. Compared to non-failing donors, amyloidosis and sarcoidosis myocardium had decreased phosphorylation of MyBPC at Ser273 and Ser282. However, only sarcoidosis myocardium had decreased phosphorylation at Ser302 compared to non-failing and amyloidosis myocardium. The decreased phosphorylation at Ser273 and Ser282 may reflect altered protein kinase A (PKA) activity in amyloidosis and sarcoidosis while the decreased phosphorylation at Ser302 in sarcoidosis may be due to altered protein kinase D (PKD) activity. Additional kinase assays as well as myofibril ATPase measurements will provide a deeper understanding on how these differences in phosphorylation impact sarcomere function. These results are similar to findings in DCM myocardium which suggests that many of the sarcomeric alterations seen in DCM are conserved in infiltrative cardiomyopathies.

Spatially-Explicit Contraction Model Predicts That Filament Compliance Affects Time Course of Relaxation

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Relaxation is an integral part of the beat-to-beat function of the heart. Impaired relaxation leads to diastolic dysfunction and eventually a form of heart failure. The relaxation time course is often studied using single myofibrillar preparations with 10 to 30 sarcomeres. It has been shown that the relaxation time course has two phases, with force dropping linearly and then exponentially. Previous work by our group modeled this behavior using a distribution-type Huxley-based contraction model of a single half-sarcomere with a series element. The half-sarcomere was allowed to shorten against the series element, and it was shown that the relative motion of the thick and thin filaments contributed to the biphasic relaxation. In the current work, FiberSim was used to model the effects of filament compliance on relaxation without a series elastic element. FiberSim is a spatially explicit model of a half-sarcomere that tracks the location and status of each contractile protein in a myofilament lattice. Initial simulations showed that the relaxation profile became biphasic as the thick and thin filament stiffnesses decreased at full activation. After performing similar simulations at a range of calcium concentrations, it was determined the linear slope and exponential rate were greater at lower thick and thin filament stiffnesses. Although the biphasic relaxation was observed in the simulations, the resulting filament extensions were approximately 5%, which is about 10 times greater than observed in experiments. The future work is focused on investigating biphasic relaxation time course with comparable filament extensions measured in experiments. This will help determine the extent to which filament compliance plays a role in muscle relaxation, providing greater insight into diastolic performance.

Funding: Commonwealth Undergraduate Research Experience (CURE) Fellowship

Modulators of myocardial passive tension are not changed in right ventricle and atria from patients with heart failure but sarcomere phosphorylation is decreased

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Heart failure with reduced ejection fraction is primarily the result of impaired left ventricular contraction. However, some patients may develop right ventricle failure concurrently or secondarily to left ventricle failure. While the left ventricle is the focus of attention of most pre-clinical studies, understanding the molecular impacts of heart failure on the right atrium and ventricle may provide insights on the pathogenesis of patients with biventricular heart failure. Paired right atrium and ventricle samples were collected from nine patients with heart failure and seven non-failing organ donors. Molecular modulators of passive tension and active contraction were examined using histological and western blotting techniques to investigate how heart failure impacts the right side of the heart.

The right atrium of both failing and non-failing hearts had increased fibrosis and tubulin compared to their respective right ventricles. There was no difference in right ventricle fibrosis between failing and non-failing myocardium. Fibrosis and tubulin modulate the stiffness of the myocardium, suggesting the right atria may be stiffer than the right ventricle, but stiffness of these chambers does not change with heart failure. Phosphorylation of myosin binding protein C (MyBPC) has been shown to increase contractile function of sarcomeres and to be depressed in the left ventricles of patients with heart failure. Similarly, both the right atria and ventricles of patients with heart failure had hypophosphorylation of MyBPC. While the atria have been shown to have less MyBPC than ventricles, both failing and non-failing myocardium had increased relative MyBPC phosphorylation compared to their ventricles.

These data suggest that while the right atria is likely stiffer than the right ventricle, there is not a large shift with heart failure. Additionally, depressed contractile function of the right ventricle in heart failure may reflect dephosphorylation of MyBPC and decreased force generation within the sarcomere. Restoration of MyBPC phosphorylation or small molecule myosin activators may be potential routes of improving right heart function in patients with heart failure.

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Proteomics and Lipidomics Analysis of Mesenteric Lymph from Mice with Impaired Chylomicron Secretion

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Background: Postprandial triglyceride (TAG) levels are an independent predictor of cardiovascular risk in humans. Chylomicrons (CMs) are the primary carrier of dietary TAG and they travel to circulation via intestinal lymph. The protein component of lipoproteins, including chylomicrons, can impact their metabolism in the plasma. While previous findings have discovered roles of several proteins associated with lipoproteins, the protein composition of newly secreted CM's, prior to entry in the circulation where they are rapidly metabolized, has not been thoroughly studied using modern mass spectrometry methods. Furthermore, the lipid composition of newly released chylomicrons has not been extensively reported.

Objectives: 1) Determine the protein and lipid composition of mesenteric lymph during fasting and after a lipid bolus, 2) identify proteins in lymph that may impact the metabolism of newly secreted chylomicrons prior to entry into the circulation, and 3) determine the impact of disruption of intestinal CM secretion in the composition of mesenteric lymph.

Methods and Results: Mouse mesenteric lymph was collected under fasting conditions and after infusion of a mixed lipid bolus into the duodenum of wild type and *Dennd5b*$^{-/-}$ mice. This study revealed that, compared to wild type mice, *Dennd5b*$^{-/-}$ mice exhibit significantly reduced TAG content in the lymph (5 h, -94%, $p<0.001$). Electron microscopy imaging and analysis on the lymph samples revealed that the lymph of wild type mice had both greater number and larger sized lipid particles in comparison to the lymph of *Dennd5b*$^{-/-}$ mice. In addition, lipidomics analysis of fasting and postprandial lymph showed significant increases in triglycerides and free fatty acids in wildtype, but not *Dennd5b*$^{-/-}$ mice. Shotgun proteomics revealed significant changes in the protein content of lymph that was collected at fasting and 4 hours after lipid bolus administration. Gene ontology analysis for differentially expressed proteins revealed that intralipid infusion affects the expression of proteins involved in several lipoprotein metabolism pathways, only in wild type mice. 14 proteins were observed to be significantly increased in response to lipid including: Saa1 (+200%, $p<0.01$), Mttp (+1236%; $p<0.01$), and Pla2g12b (+7346%; $p<0.01$). These protein changes were confirmed by western blotting analysis of lymph. The Saa1 effect was of particular interest. Because Saa1 is known to bind to lipoproteins, we used size-exclusion chromatography to analyze the distribution of Saa1 protein across lipoprotein fractions in lymph. This demonstrated that Saa1 in lymph is associated primarily with very large size fractions that typically contain chylomicrons and to a lesser extent with HDL-containing fractions. In oil gavage studies in mice, we found that plasma Saa1 is elevated at 4 hours post-gavage and is primarily associated with HDL. To determine the source of post-gavage Saa1, mouse intestinal tissue was analyzed by western blot and immunofluorescence for Saa1. Western blotting and immunofluorescence demonstrate that intestinal Saa1 is increased in response to high fat diet.

Conclusions: This study suggests that wildtype mice have lipid-poor ApoB-containing lipoproteins in lymph during fasting conditions. We identified several proteins that respond to dietary lipid ingestion and may contribute to postprandial lipoprotein metabolism in the lymph and periphery. In wildtype mice, a lipid bolus increases lymphatic Saa1-3 concentrations and Pla2g12b that may remodel CM's prior to reaching the circulation. These effects were not observed in *Dennd5b*$^{-/-}$ mice, suggesting that these responses to dietary lipid are dependent on successful CM secretion from intestinal epithelium.

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Right Ventricular Dysfunction: An Early Sign of Anthracycline Induced Cardiotoxicity - Case Series

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Abstract

Background: Anthracycline-induced cardiotoxicity is clinically distinguished by a reduction in left ventricular ejection fraction (LVEF) exceeding 10% and an LVEF below 50%. Due to these guidelines, alterations in right ventricular (RV) structure and function are often neglected as indicators of cardiotoxicity. In this report, we present two cases of anthracycline-induced cardiotoxicity that initially manifested as RV dilation and dysfunction.

Case presentations: Patient One, A 41-year-old woman with a history of sub-massive pulmonary embolism and obesity, was diagnosed with right pulmonary artery sarcoma and treated with surgical resection, radiation therapy, and adjuvant doxorubicin. Months after starting chemotherapy, she experienced progressive dyspnea on exertion and lower extremity swelling; a follow-up TTE demonstrated normal LV size and function, RV dilation, and RV pressure and volume overload. Since LVEF did not fall under the definition of cardiotoxicity, she continued doxorubicin treatment. Her clinical condition worsened, leading to severe RV dilation, reduced function, and new ECG abnormalities. Eventually, a cardiac MRI revealed reduced biventricular function and RV volume overload, with a final diagnosis of biventricular failure due to chemotherapy.
Patient Two, a 21-year-old male diagnosed with osteosarcoma at age 12, underwent surgical resection, endoprosthesis reconstruction, and adjuvant chemotherapy with cisplatin and doxorubicin. A 7-year post-chemotherapy follow-up echocardiogram noted increased RV pressure suggestive of pulmonary hypertension. Follow-up TTE showed normal LVEF with borderline normal GLS, visually normal RV size and systolic function, and borderline elevated RVSP of 35 mmHg. Subsequent cardiac MRI revealed reduced right and left ventricular function and non-ischemic cardiomyopathy.

Conclusions: These cases illuminate a critical gap in the current diagnostic criteria and definition of cardiotoxicity, emphasizing the necessity for more comprehensive echocardiographic approaches to assess cardiotoxicity. Specifically, they underscore the importance of including changes in right ventricular (RV) structure and function, which are often overlooked but can serve as early indicators of cardiotoxicity.

Infiltrative Cardiomyopathies Display Decreased Phosphorylation of Thick and Thin Filament Regulatory Proteins

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Infiltrative cardiomyopathies are a subset of restrictive cardiomyopathies characterized by the aggregation of abnormal proteins, cells, or materials within the extracellular matrix of the heart. This results in increased fibrosis, thickening of the heart walls, and heart failure. The two most common types of infiltrative cardiomyopathy are cardiac sarcoidosis and amyloidosis. These infiltrative cardiomyopathies primarily impact the extracellular matrix, but it is unclear whether cardiomyocyte contractile function is also impaired. Within the sarcomere, the thick filament can be regulated by phosphorylation of myosin binding protein C (MyBPC) while phosphorylation of troponin I (TnI) can regulate the thin filament. MyBPC and TnI have been shown to be hypophosphorylated in dilated cardiomyopathy (DCM) but have not been investigated in infiltrative cardiomyopathies.

Left ventricle samples from patients with cardiac sarcoidosis and amyloidosis were analyzed by western blot. Phospho-specific antibodies were used to assess phosphorylation of MyBPC at Ser273, Ser282, and Ser302. TnI phosphorylation was measured using Phos-tag gel electrophoresis. Compared to non-failing donors, amyloidosis and sarcoidosis myocardium had decreased phosphorylation of MyBPC at Ser273 and Ser282. However, only sarcoidosis myocardium had decreased phosphorylation at Ser302 compared to non-failing and amyloidosis myocardium. Additionally, both amyloid and sarcoid myocardium had decreased TnI phosphorylation compared to non-failing donors. The decreased phosphorylation of MyBPC at Ser273 and Ser282 and TnI may reflect altered protein kinase A (PKA) activity in amyloidosis and sarcoidosis. Decreased phosphorylation at Ser302 was only observed in sarcoidosis and may be due to altered protein kinase D (PKD) activity in this disease. Additional kinase assays as well as myofibril ATPase measurements will provide a deeper understanding of how these differences in phosphorylation impact sarcomere function. These results are similar to findings in DCM myocardium which suggests that many of the sarcomeric alterations seen in DCM are conserved in infiltrative cardiomyopathies.

An estrogen-olfactomedin negative feedback loop may drive nicotine consumption in a sex-specific manner

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Estrogen has been shown to be implicated in nicotine use disorder such that increased levels of estrogen are associated with a higher likelihood to develop nicotine use disorders and increased nicotine consumption. Estradiol (E2) treatments in ovariectomized (OVX) female rats resulted in the increase of only the beta isoform of the estrogen receptor (ER) in brain reward circuitry area, the nucleus accumbens core (NAcore). From existing datasets, we identified the olfactomedin (OLFM) genes as responsive to estrogen, expressed in the brain, and having a hormone function. Estrogen treated uterine cells showed an increase in expression of OLFM1 and OLFM2. Interestingly, nicotine suppressed the estrogen-induced increase in OLFM1. We confirmed that this mechanism is ERβ and OLFM1 specific by performing a chromatin immunoprecipitation assay. We found that OLFM1 promoter was enriched by ERβ and not ERα. We treated OVX female rats with E2, nicotine, and E2 and nicotine combined and examined gene expression in the brain reward areas. We found that Olfm1 and Olfm2 were significantly increased in the NAcore and the ventral tegmental area. Again, nicotine suppressed the E2-induced OLFM expression in these areas. Finally, we performed extensive PamGene analysis with neuroblastoma cell line treated with the same treatments. This showed us that the combination of estrogen and nicotine resulted in kinase activity distinct from that seen with estrogen or nicotine alone. Furthermore, we found that the ERβ interactome was majorly altered by estrogen and nicotine combination, whereas the ERα interactome didn't have as much change. These findings lead us to suggest that the ERβ activation of olfactomedins might provide a feedback loop for nicotine consumption and potentially open up a new pathway for therapeutic targeting.

Title: Elucidating the "Gap Junction" of *GJA1* circadian regulation

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Background: Gja1 encodes connexin 43 (Cxn43), a key component of cardiac gap junctions that facilitate synchronized cardiac contraction by enabling efficient depolarization. The expression of Gja1 mRNA in the heart follows a circadian rhythm, cycling approximately every 24 hours. However, the underlying mechanism driving this circadian expression remains unclear.
Hypothesis: A conserved cis-regulatory element within the Gja1 promoter is hypothesized to control the circadian regulation of Gja1 promoter activity in both mouse and human (hGJA1) genes.
Objective: To identify the conserved cis-regulatory elements responsible for the circadian regulation of Gja1 promoter activity.
Methods: We employed real-time bioluminescence (LumiCycle, Actimetrics) to investigate the circadian expression of cloned mouse (mGja1) and human (hGJA1) promoter-luciferase reporter constructs in transiently transfected C2C12 myogenic cells. Bioluminescence was recorded at 10-minute intervals over 7-10 days, and the data were analyzed for circadian parameters, including period, phase, and amplitude.
Results: The LumiCycle data demonstrated that both mGja1 and hGJA1 promoter constructs exhibited circadian bioluminescence in C2C12 cells. We then generated a deletion mutant of the hGJA1 promoter-reporter construct, removing base pairs 1777 to 2155 upstream of exon 1 (ΔhGJA1). Notably, the ΔhGJA1 construct showed a ~16-fold increase in circadian bioluminescence amplitude compared to the full-length hGJA1 construct (3.6±0.40 counts/second, Control; 59.17±4.1 counts/second, ΔhGJA1, $p<0.05$). Additionally, the acrophase of the ΔhGJA1 construct occurred approximately 3.5 hours earlier than that of the full-length hGJA1 construct (n=10-15 dishes per condition).
Conclusion: We have identified a conserved region within the hGJA1 promoter that appears to repress circadian promoter activity. Ongoing studies are being done to pinpoint the minimal cis-regulatory element responsible for this repression.

Does time of administration matter? Investigating the effects of exenatide, a GLP-1 receptor agonist, on clock gene expression in the diabetic brain.

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Clock genes create and regulate circadian rhythms through a series of interlinked transcription-translation feedback loops. These loops generate oscillations in gene expression and protein levels that repeat approximately every 24 hours. These genes play a crucial role in controlling various physiological and behavioral patterns such as sleep-wake cycles, hormone release, body temperature, and metabolism. The clock genes also play a crucial role in regulating the cardiovascular system through the body's circadian rhythm. Clock genes control the 24-hour circadian rhythm in cardiovascular tissues, including the heart and blood vessels. They also influence daily fluctuations in blood pressure, with typically higher levels during the day and lower at night. Clock genes also affect heart rate and vascular tone changes throughout the day, coordinating with activity levels and metabolic needs. Type 2 diabetes has been shown to disrupt these oscillations and, by extension, contribute to worsened outcomes most of the time involving the cardiovascular system.

We have previously demonstrated that *db/db* mice exhibit circadian rhythm disruption, manifesting as loss of food intake rhythm, nondipping or reverse dipping blood pressure, and blunted 24-h oscillation of sympathetic nervous system activity biomarkers. Moreover, we demonstrated that ZT0 administration of exenatide can effectively restore food intake rhythm, reestablish normal blood pressure circadian rhythm and reduce hypertension, and lower sympathetic overactivity. In contrast, ZT12 administration of exenatide worsens food intake pattern, blunts blood pressure circadian rhythm or causes a reverse dipping pattern, and exacerbates irregular sympathetic activity in *db/db* mice.

The current study measures changes in clock mRNA expression in the brain and muscle after treatment with exenatide at ZT0 or ZT12 in *db/db* mice. Both of these tissues heavily depend on the cardiovascular system. Real time PCR was utilized to determine changes in clock gene mRNA expression (Bmal1, Clock, Per1, Per2, Cry1, Cry2, Rev-erbα Rorc) at ZT5 or ZT17 without or with treatment with exenatide.

Preliminary results have demonstrated a sleep-enhancing effect with ZT0 administration of exenatide. Surprisingly, the cortex of *db/db* mice showed the most significant restoration, in part or full, of day-to-night gene expression. After ZT0 injection of exenatide, there was a day to night difference in BMAL1, PER1, REV, RORC. While these findings are promising, the implications by which ZT0 exenatide-induced changes to clock gene activity have on sleep quantity and quality, requires additional experiments. I would like to thank University of Kentucky and the Commonwealth Undergraduate Research Experience (CURE) program for giving me the amazing opportunity pursue my research endeavors.

Title: The Impact of Highly Effective Modulator Therapies on the ABCG5 and ABCG8 Sterol Transporters

Authors: Meredith Campbell, Isha Chauhan, Vicky Noffsinger, and Gregory A. Graf

Background: Sitosterolemia is characterized by excess sterol accumulation, thereby contributing to facets of cardiometabolic disease. Sitosterolemia arises from mutations in the ATP-binding cassette (ABC) transporters, ABCG5 and ABCG8, which form a heterodimer crucial for sterol secretion from the liver and small intestine. Highly Effective Modulator Therapies (HEMTs) are used for the treatment of Cystic Fibrosis by rescuing the function of the ABC transporter, CFTR, and may interact with additional ABC transporters including ABCG5 and ABCG8. The overarching goal of this work is to determine if HEMTs can also rescue the function of known sitosterolemia-causing mutations in ABCG5 and ABCG8.

Methods: Lentiviral Transduction of Human HepG2 hepatocytes created cells expressing ABCG5 (G5) only, ABCG8 (G8) only, or both ABCG5 and ABCG8 (G5/G8). These cells were then treated with three HEMTs (Ivacaftor, Tezacaftor, Elexacaftor), independently or in combination doses of 1µM to 20 µM. Immunoblotting was performed to measure differences in ABCG5 and ABCG8 protein levels following treatment with HEMTs.

Results: Treatment with HEMTs alone, or in combination, had no significant effect on total ABCG8 protein levels. However, individual treatment with Ivacaftor or Elexacaftor but not Tezacaftor increased immature ABCG5 protein levels and induced expression of a high molecular weight (~150 kDa) band which is currently unknown. Intriguingly, combination treatment with all three HEMTs caused a much greater increase in the immature, mature, and high molecular weight bands of ABCG5.

Conclusion: Highly Effective Modulator Therapies impact the abundance of the mature and immature forms of ABCG5. HEMTs also revealed an unidentified high molecular weight form of ABCG5, possibly indicating post-translational modification(s), the formation of an ABCG5 homodimer, or heterodimer of unknown composition. Further identification of these high molecular weight bands by proteomics may lead to a better understanding of the impact as well as the binding patterns of the proteins.

Atrial Fibrillation After CABG, a Single Center Study

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Objective:
Post Operative Atrial fibrillation (POAF) is a common complication following coronary artery bypass grafting (CABG). This retrospective, observational study aims to examine the incidence and outcomes of POAF after CABG in our institution.

Methods and Materials:
Institutional Review Board approval was obtained for the retrospective review of patients who underwent CABG and developed POAF as a complication between 2006 and 2022. The study population includes patients who underwent first time CABG only, also excluding previous AF.
2671 patients (58.72 y/o, 29.97 BMI, m/f: 74.8% male, 93.1% white, 3.9% black, 2.9% other race/non-specified) were identified for this study. 238 patients (60.31 y/o, 29.45 BMI, m/f: 73.9% male, 92.9% white, 6.3% black, .8% other race/non-specified) developed POAF, while 2433 patients (58.56 y/o, 29.98 BMI, m/f: 74.9% male, 93.2% white, 3.7% black, 3.1% other race/non-specified) did not develop POAF.
The following outcomes were analyzed for this study: 30-day, 2-year, and 5-year mortality rates, hospital length of stay (LOS), intensive care unit (ICU) LOS, diagnosis of infection, post-operative bleeding, shock, embolism, renal failure, respiratory failure, sepsis, and stroke.

Results:
At our institution, POAF occurred in 8.9% of patients. Complications were also more prevalent in patients with POAF compared to those without. These included higher rates of infection (7.56% vs. 5.75%), post-operative bleeding (5.04% vs. 2.84%), renal failure (14.71% vs. 9.37%), and stroke (5.11% vs. 2.04%). Patients with POAF experienced a longer average LOS in both the ICU (5.26 days) and the hospital overall (10.94 days) compared to those without AF (ICU LOS: 5.18 days, hospital LOS: 10.25 days). The 30-day, 2-year, and 5-year mortality rates for patients who underwent CABG and developed AF were 1.3%, 7.8%, and 24%, respectively.

Conclusion:
Our study analyzed the impact of POAF on patient outcomes following CABG, including increased rates of complications and prolonged hospitalization/ICU stay. These findings emphasize the importance of monitoring and targeted intervention for AF after CABG.

Insulin Receptor Knockout Potentially Affects Liver Fibrosis through Regulating the Transcriptional Response to TGFβ in Human Hepatic Stellate Cells

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The hepatic stellate cell is the major cell type promoting liver fibrosis development. In addition, insulin stimulation is essential for regulating hepatic stellate cell (HSC) fibrogenesis activity[1]. However, the role of insulin receptor (INSR) in regulating HSC responsiveness to fibrotic environment is not entirely understood. This motivates us to explore HSC cellular pathways and functions that are regulated by insulin signaling. Thus, we removed functional INSR in an HSC cell line, LX2, with CRISPR Cas9 technology. We validated the INSR knockout with Western Blotting. To measure the functional changes, we performed a cell growth assay and a cell migration assay on scramble control LX2 and INSR KO LX2 with TGFβ treatment. We found that the loss of INSR leads to increased cell growth and migration in LX2 cells. To explore the pathways that are affected by losing INSR, we treated the scramble control LX2 and INSR KO LX2 with TGFβ for 24 hours and extracted the RNA for RNA sequencing. The RNA sequencing data showed that the gene expression profile changed significantly. The Gene Ontology pathway analysis uncovered the affected cell functions and pathways, including fibrosis-associated pathways like collagen-activated signaling and smooth muscle cell differentiation pathways. Other affected pathways include insulin receptor signaling, cellular response to insulin stimulation, SMAD protein signaling, collagen metabolic process, integrin-mediated cell adhesion, and Notch signaling. To explore the pathway changes at a kinase activity level, we performed a kinome analysis by using our PamGene Pam Station technology. We identified that serine/threonine kinases like ERKs, MTOR, and P38 are hypo-activated, whereas the protein/tyrosine kinases, such as Fes, FLT4, and EphA1, are hyperactivated with the loss of functional INSR. These findings show the kinase activity changes in INSR regulation of HSC responsiveness to TGFβ.

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Phosphodiesterase Effect on RAD-Modified Contractile Function

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Background:

Ras associated with diabetes (RAD) is a protein that regulates L-type calcium channels (LTCC) in the t-tubules of cardiomyocytes (CMs). In the absence of RAD, trigger calcium release through the LTCC is amplified, elevating calcium dynamics and allowing CMs to contract faster and stronger. Because RAD ablation is broadly cardioprotective, RAD is an intriguing target for therapeutic heart failure (HF) drugs. Phosphodiesterases (PDEs) comprise a class of proteins that hydrolyze cyclic adenosine monophosphate (cAMP), blunting cAMP activation of protein kinase A (PKA). In cardiomyocytes, PKA phosphorylates calcium-handling proteins including RAD to elevate calcium dynamics, promoting contractile function. The PDE isoform PDE1 is upregulated in HF, and it shares LTCC localization with RAD. ITI-214, a novel PDE1 inhibitor, is presently in phase II clinical trials to treat HF.

Hypothesis:

CMs will not respond to ITI-214 treatment in the absence of RAD.

Goals:

To determine whether PDE inhibition operates synergistically with RAD deletion.

Methods:

We analyzed calcium transient (CaT) and sarcomere length (SL) traces in CMs harvested from RAD-knockout ($RAD^{-/-}$) and wild-type (WT) mice. CMs were primed with isoproterenol to increase intracellular cAMP and treated with ITI-214 to inhibit PDE1 or with IBMX to inhibit PDE3, PDE4, and PDE5.

Results:

PDE inhibition increases total calcium release, the rate of calcium release, and the rate of calcium reuptake in WT mice, but not in $RAD^{-/-}$ mice. PDE1 inhibition increased total sarcomere contraction in WT and not $RAD^{-/-}$, but PDE3,4,5 inhibition only increased total sarcomere contraction in $RAD^{-/-}$. PDE1 inhibition also increased sarcomere contraction velocity in WT but not $RAD^{-/-}$, but PDE3,4,5 inhibition did not affect sarcomere contraction velocity in the WT or $RAD^{-/-}$ CMs. PDE1 inhibition increased sarcomere relaxation velocity in both WT and $RAD^{-/-}$ CMs, but PDE3,4,5 inhibition had no effect on sarcomere relaxation velocity in either WT or $RAD^{-/-}$ CMs.

Conclusions:

Our data support the hypothesis that $RAD^{-/-}$ CMs will not respond to PDE1i by ITI-214. However, the effects of PDE1 inhibition and PDE3,4,5 inhibition are remarkably similar, raising concerns that these effects are being masked by a time-dependent response to a high isoproterenol dose. Further experiments will be conducted with a lower isoproterenol dose.

Contribution Of Platelet Granule Cargo Uptake And Secretion To The Initiation And Progression Of Aortic Aneurysms

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Aortic aneurysms present as aortic widening and often catastrophic rupture. While precipitated by a thrombo-inflammatory environment, underlying pathological mechanisms are unclear and adequate treatment is lacking. Antithrombotics have been implied as therapy, but few studies have defined how platelets contribute to aortic aneurysms. We studied the role of platelet granule cargo uptake and secretion in aortic aneurysm formation.
Aneurysms were induced in mice defective in platelet α-granule biogenesis (Nbeal2$^{-/-}$, Serglycin$^{-/-}$), endocytosis (Arf6$^{-/-}$, VAMP2/3$^{\Delta}$), and exocytosis (Munc13-4^{Jinx}) via: *i.p.* injection of an AAV vector with a mouse PCSK9 gain-of-function mutation; western diet; and continuous *s.c.* infusion of angiotensin II. Thoracic and abdominal aneurysm formation was assessed with ultrasound. At 4 and 12 weeks, or after rupture, aortas were harvested, analyzed, and prepared for histology.
During the normocholesterolemic study, 30% (3/10) of Nbeal2$^{-/-}$ and 21.4% (3/18) of Munc13-4^{Jinx} mice suffered aortic rupture, compared to 0% (0/26) of wildtype mice. Hypercholesterolemia exacerbated this in the Nbeal2$^{-/-}$ mice, where 67% (6/9) of the mice died, of which all (n=5) males. In the survivors, the inner diameter of neither the thoracic nor abdominal aorta of Nbeal2$^{-/-}$ mice changed over time, in both normo- as well as hypercholesterolemic conditions. *Ex vivo*, Serglycin$^{-/-}$ mice showed a clear, more distributed, widening of the thoracic and descending aorta instead of local aneurysm formation with an increased maximum outer diameter of the ascending region. Munc13-4^{Jinx} mice had an all-or-nothing phenotype; either early-stage massive aneurysm formation or rupture, or a more diffused phenotype. Mice with platelet endocytosis defects survived and under normocholesterolemia, the differential increase in the inner diameter of both the thoracic and abdominal aorta was higher in female Arf6$^{-/-}$ mice at early stages. Similarly, VAMP2/3$^{\Delta}$ mice had the largest inner thoracic diameter increase after 28 days. Arf6$^{-/-}$ and VAMP2/3$^{\Delta}$ mice showed a smaller maximum outer diameter of the descending aorta as wildtype. In a hypercholesterolemic environment, *in vivo* aneurysm formation was attenuated in the Arf6$^{-/-}$ and VAMP2/3$^{\Delta}$ mice. Preliminary histology shows reduced elastin breaks in all genetic strains, especially in Nbeal2$^{-/-}$ and VAMP2/3$^{\Delta}$ mice.
Our data show that platelet granule cargo exerts significant but perhaps contrasting effects on aortic aneurysm formation. By comparing phenotypes, more detailed mechanistic insights into the role of platelets in aortic aneurysms are possible.

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Title: Altering ApoE in mice expressing human amylin in the pancreas exacerbates brain microvascular and parenchymal amyloid pathology

Background:
The *APOE* ε4 allele is the most prominent genetic predisposition for sporadic Alzheimer's disease (AD). Amylin, a neuroendocrine hormone co-secreted with insulin from the pancreas, is increased in blood in AD and readily forms neurotoxic homo- and hetero-oligomers with β-amyloid in AD. Here, we investigated whether mice humanized for amylin and ApoE demonstrate ApoE isoform-specific alterations in cerebrovascular amylin deposition and β-amyloid homeostasis.

Methods:
Mice humanized for ApoE3 or ApoE4 and amylin (ApoE3HIP and ApoE4HIP) and amylin without ApoE expression (ApoE-KO-HIP) were tested for behavior deficits before brain microvessel isolation and amylin/β-amyloid quantification. GFAP-amylin colocalization in the brain was quantified using immunohistochemistry (IHC), double-immunofluorescence was used for amylin-ApoE colocalization, and immunoprecipitation experiments were conducted to confirm brain amylin-ApoE binding interactions.

Results:
ApoE4HIP mice demonstrated worsened behavioral deficits vs. E3HIP mice. IHC of ApoE4HIP and ApoE3HIP brains revealed increased deposits of GFAP and amylin in ApoE4HIP mice. Amylin in brain parenchyma was higher in ApoE4HIP vs. ApoE3HIP mice while ApoE-KO-HIP mice demonstrate reduced amylin in microvessels and parenchyma. β-amyloid 40 levels were elevated in ApoE4HIP brain and microvessels.

Conclusions:
Our data suggest ApoE may function as a transporter of amyloid-forming amylin in the brain with amylin binding ApoE4 stronger than ApoE3. The increased affinity of amylin for ApoE4 coincides with worsened brain amyloid pathology (in parenchyma and microvasculature), disrupted β-amyloid homeostasis, increased astrogliosis suggesting neurodegenerative insult, and functional impairments due to elevated amylin amyloid burden. These data may implicate the amylin-ApoE interaction as a mechanism underlying ApoE4-specific neuropathology.

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Role of Glucose-6-phosphate dehydrogenase (G6PD) in platelet function

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Glucose-6-phosphate dehydrogenase (G6PD), the key enzyme in the pentose phosphate pathway (PPP), is essential for producing NADPH and pentoses. G6PD deficiency is the most common human enzymopathy and affects about 400 million people worldwide. Patients with G6PD deficiency have an increased cardiovascular disease risk after age 60. An early study (Hofmann *et al*, 1981) reported that platelets from G6PD deficient patients showed increased aggregation with hyper-responsiveness to ADP. Despite these findings, a comprehensive understanding of the role of G6PD in platelet function is still lacking. In this study, we aim to elucidate the impact of G6PD deficiency on platelet function using a humanized G6PD Mediterranean (Med) mutation conditional knock-in mouse model, which closely mimics the human condition. G6PD Med-mutant mice have normal mean platelet volumes. Tail bleeding assay demonstrated a significant reduction in bleeding time in both male and female G6PD Med-mutant mice compared to wild-type (WT) controls, indicating enhanced platelet activity. *Ex vivo* thrombin-induced clot contraction assay revealed faster clot contraction for G6PD Med-mutant platelets than for the WT counterpart, corroborating the hyperactive platelet phenotype. More studies are underway to gain deeper insights into the effects of G6PD deficiency on platelet function. A comprehensive understanding of the molecular mechanisms underlying the platelet hyperactivity in G6PD deficiency could inform therapeutic strategies and improve clinical outcomes for affected individuals.

Title: The Impact of Intestinal Carnitine Palmitoyltransferase 1a on the Development of Obesity Phenotypes in Response to High Fat Diet

Authors: Victoria Noffsinger, Isha Chauhan**,** Erika Savage, Meredith Campbell, Rachael A. Morgan, Beau Forester, Robert N. Helsley, and Gregory A. Graf.

Background: Carnitine palmitoyltransferase 1a (Cpt1a) esterifies long chain fatty acids to carnitine allowing their entry into mitochondria for subsequent β-oxidation and the production of cellular energy. Cpt1a is commonly called the liver isoform, but the abundance of transcripts encoding the enzyme is greater throughout the intestinal tract in both mice and humans. Lipid absorption is known to be an active metabolic process, but the role of macronutrient metabolism in nutrient absorption and utilization is poorly understood. Therefore, we examined the impact of intestinal Cpt1a deficiency in the development of metabolic phenotypes in response to a high fat diet.

Methods: Eight-week-old male and female control ($Cpt1a^{fl/fl}$) and intestinal-specific knockout ($Cpt1a^{IKO}$; $Cpt1a^{Vil\text{-}Cre+}$) mice were fed high diet (60% kCal) for 16 weeks. Body weights were measured weekly, body composition was measured by MRI, and glycemic control assessed by glucose tolerance test and HOMA-IR. Mice were necropsied after a 12 hour fast, and tissues and plasma were collected for lipid analysis, histology, bulk RNA-sequencing, and protein expression by immunoblotting.

Results: No differences were observed in terminal body weight in male mice, but females were modestly protected from diet-induced obesity. HOMA-IR values suggested improved insulin sensitivity irrespective of sex, but glucose tolerance did not differ between genotypes in either males or females. The absence of Cpt1a resulted in increased fasting plasma triglycerides and increased circulating triglycerides 1 hour following an oral fat gavage. Differences in plasma cholesterol were not observed. Histological analysis of the proximal small intestine did not reveal morphological differences in villus length, width or distance to crypts. RNA sequencing analysis indicates alterations in a host of lipid metabolizing enzymes and the increase in genes that mediate β-oxidation.

Conclusion: These results indicate that intestinal Cpt1a influences the development of obesity in female, but not male mice. Improvements in insulin sensitivity and the increased plasma triglycerides following ingestion of fat suggest that the loss of CPT1a diverts dietary fat from oxidative metabolism in the gut and promotes lipid absorption.

Title: Uncovering the Role of Cysteine String Protein- α in Mouse Platelets

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Background: Platelets use SNARE-mediated exocytosis to modulate the vascular microenvironment via the release of cargo molecules from three types of granules: dense, α, and lysosomal. This process is complex and is regulated by several protein-protein interactions that are not completely characterized. To understand how the process of exocytosis is regulated, we have examined Cysteine String Protein-α (CSPα), a potential SNARE regulator and assessed how it affects thrombosis and hemostasis. This abundant protein is the only detectible isoform from its respective family in platelets.

Objective: To address the role of CSPα in platelet exocytosis.

Methods: To address CSPα 's role, we examined the platelet and hemostatic phenotype of CSPα$^{-/-}$ mice using several *in vitro* and *in vivo/ex vivo* models. An IDEXX hematology analyzer was used to measure platelet counts and sizes. Platelet activation and morphology were examined using cytometry. ATP secretion was analyzed using a chemiluminescent assay. Hemostasis was assessed using tail bleeding and a BioFlux microfluidics system. The levels of brain and platelet SNARE machinery were quantified by western blotting. Protein localization was examined by 3-Dimensional Structured Illumination Microscopy (3D-SIM) and subcellular fractionation. Biochemical studies were done to examine CSPα's association with lipid rafts in platelets. Cyrosectioning and immunostaining were done of the femurs and spleens of CSPα$^{-/-}$ mice to image and quantify megakaryocyte numbers and collagen I deposition.

Results: CSPα$^{-/-}$ platelets have defective α and dense granule release with minimum effects on lysosomal granule secretion. Consistent with the secretion defects, CSPα$^{-/-}$ mice had a significant tail bleeding defect and attenuated thrombus under flow at low-shear rates. Microscopy experiments show that CSPα co-stains with markers for both α and lysosomal granules. CSPα is not present in lipid rafts and is found mostly in triton-soluble membrane fractions. Deletion of CSPα only effects proteins the brain SNARE machinery there was no reduction in platelet SNARE machinery proteins. Hematology data indicates that CSPα$^{-/-}$ mice have normal platelet size and counts, but leukocytes counts were significantly lower in CSPα$^{-/-}$ mice. Immunostaining of the femurs and spleens in CSPα$^{-/-}$ mice showed a significant reduction in megakaryocyte numbers and increased collagen I deposition.

Conclusions: These experiments demonstrate that CSPα has a role in thrombosis and hemostasis and also hematopoiesis as well. These data fill gaps in our knowledge of CSPα's physiological role and our understanding of how platelet exocytosis is controlled.

Platelet function and gene expression after autologous or allogeneic stem cell transplantation

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Background: Hematopoietic stem cell transplant (HSCT) is an advisable option for leukemia and lymphoma patients. But whether autologous or allogeneic stem cell transplant is more beneficial is still controversial. Platelets play a vital role in blood clotting and wound healing, relying on molecular transport. Development of RNA sequencing technologies makes it possible to seek individual strategies for patients with HSCT. The aim of this study is to understand the transcriptome profiles in platelets of leukemia and lymphoma patients.

Objective: Genes from platelets are altered after autologous or allogeneic stem cell transplantation and may correlate with disease response.

Methods: Platelet RNA sequencing was conducted in leukemia and lymphoma patients (n=8) before and after autologous or allogeneic stem cell transplant using Illumina NovaSeq 6000 sequencing. Statistical analyses, including GO, KEGG, and GSEA enrichment analyses, were applied to the sequencing data.

Results: After allogeneic stem cell transplant, 6 genes were downregulated, and 58 genes were upregulated. Autologous stem cell transplant resulted in 7 downregulated genes and 8 upregulated genes in leukemia and lymphoma patients. Notably, the IL6_JAK_STAT3_signaling pathway genes were significantly upregulated in both autologous and allogeneic stem cell transplant treatment.

Conclusions: In summary, platelet gene expression profiles differ before and after autologous or allogeneic stem cell transplant in leukemia and lymphoma patients. The upregulation of IL6_JAK_STAT3_signaling suggests potential implications for diverse chemotherapy strategies.

Keywords: Autologous stem cell transplantation, Allogeneic stem cell transplantation, platelets, pathway, genes.

Circulating Lipids may Provide a Survival Benefit in the Obese with Sepsis

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Objectives: Clinical data show that overweight or moderately obese patients have increased survival rates compared to those in a normal weight range for many acute disease states including sepsis. Among the theories proposed to explain obesity-mediated protection in sepsis is the availability of higher energy reserves from excess adipose tissue in the obese. The objective of this study was to explore whether higher levels of circulating lipids in the obese provide a survival benefit in sepsis.
Approach: *Preclinical Arm*: A preclinical model of sepsis induced by cecal slurry (CS) injection was utilized to test the concept that inhibiting adipose tissue lipolysis (atglistatin 0.2 mmol/kg, 3x daily) in diet-induced obese (DIO, 20 weeks high-fat diet) mice would dampen the survival benefit in the obese. CS was injected intraperitoneally to mice followed by antibiotic and fluid resuscitation, beginning at 12h and continuing twice daily for 5 days. Triglyceride (TG) and free fatty acid (FFA) levels were measured in the plasma, and survival was monitored for 10 days. *Clinical Arm*: Clinical data from the Acute Respiratory Distress Network Studies 10 and 12 Statins for Acutely Injured Lungs from Sepsis (ARDSNet-SAILS) trial was used to conduct a secondary analysis regarding the effect of obesity and lipid-lowering statins on sepsis survival.
Results: *Preclinical Arm*: DIO mice had improved survival compared to lean mice ($p=0.0043$) and were better able to maintain circulating TG and FFA levels at homeostasis. In lean mice, TG levels acutely decreased while FFA levels acutely increased. Atglistatin treatment in DIO mice significantly decreased plasma lipid levels and reduced survival time compared to vehicle-treatment, although the survival difference did not achieve significance due to high mortality in both groups ($p=0.0807$). Atglistatin treatment in normal chow-fed mice also reduced plasma TG and FFA levels, and survival compared to vehicle-treatment (20% vs. 100% survival, $p=0.0262$). *Clinical Arm*: Obese sepsis patients showed the expected positive survival benefit compared to normal weight sepsis patients; however, this did not achieve significance in this secondary-use study which was not powered for this analysis (OR = 0.81, 95% CI 0.53-1.24).
Conclusions: This study indicates that maintaining higher plasma lipid levels may be advantageous in the setting of sepsis and may account for the survival benefit observed in obese patients.
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Loss of the v-SNARE VAMP8 Protects Against Aortic Aneurysm: Implications of Impaired Platelet Cargo Release

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Introduction and Objective: As vascular sentries, platelets, and their ability to release a host of bioactive molecules, are critical for vascular homeostasis as well as hemostasis. Despite data linking platelet activation to abdominal aortic aneurysms (AAA) and rupture, the underlying mechanisms remain poorly understood. This study addresses the hypothesis that VAMP8, the primary v-SNARE controlling platelet exocytosis, significantly contributes to AAA formation.

Approach and Results: A retrospective, single-center, multiple comparisons study of adult aneurysmal patients admitted to UKHealthCare between 2004 and 2023 revealed a significantly lower platelet count in AAA patients compared to healthy individuals. These results were also observed in experimental aneurysm models using an AngII-infused hypercholesterolemic mouse model, suggesting increased platelet consumption. Our results indicated a noticeable accumulation of platelets at elastin break sites and false lumen formation in the abdominal aortas of aneurysmal mice. The platelet accumulation showed significant colocalization with VAMP8, alongside a marked upregulation of VAMP8 in the thrombus segments of the aneurysmal tissue compared to control**.** WT and VAMP8$^{-/-}$ mice were infused with AngII (1,000 ng/kg/min) or saline two weeks post PCSK9 AAV infection for 28 days using subcutaneously implanted Alzet mini-osmotic pumps. Our results revealed that VAMP8$^{-/-}$ mice are protected against AngII-driven aortic rupture. Both *in vivo* and *ex vivo* aortic diameter analysis indicated that VAMP8 deficiency profoundly attenuated AngII-induced AAA compared to controls. Bulk RNA-seq analysis on washed platelets isolated from VAMP8$^{-/-}$ compared to WT mice revealed distinct transcriptomic profiles with significant enrichment in pathways related to aortic remodeling and aneurysm pathogenesis. Further, RNA-seq analysis of suprarenal aortas and platelets (5-day, AngII infusion) showed that VAMP8 deficiency significantly reducing extracellular matrix degradation and increasing aortic wall stability, as observed by the downregulation of matrix metalloproteinases (MMPs) and the upregulation of collagen synthesis genes.

Conclusion: VAMP8 deficiency results in the profound attenuation of aortic aneurysms, potentially via enhanced ECM stability, introducing a novel paradigm for understanding the impact of platelet cargo secretion in aortopathies.

EFFECTS OF NEONATAL ESTRADIOL TREATMENT ON REGULATING SEXUAL DIFFERENTIATION OF DAILY RHYTHMS UNDERLYING DIET-INDUCED OBESITY IN FEMALE MICE

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Sex differences of the brain and behavior often require the combination of organizational and activational effects of sex hormones. The activational effects of estrogens in adult female mice protect daily eating and activity rhythms from disruption by high-fat diet feeding and inhibit diet-induced obesity. However, male mice have disrupted eating rhythms when fed high-fat diet and this causes obesity. Treatment of adult male mice with estradiol does not protect rhythms from disruption by high-fat diet, as it does in females. In this study, we sought to determine whether the organizational effects of estradiol masculinize the daily eating and activity rhythms underlying diet-induced obesity in female mice. We injected female mice with estradiol daily for the first five days after birth. As adults, these female mice were ovariectomized and implanted with tubing containing either estradiol or oil. We found that adult treatment of female mice with estradiol inhibited diet-induced obesity. Adult, but not neonatal, estradiol treatment increased activity but had no effect on food consumption. Interestingly, adult and not neonatal estradiol treatment restored high-amplitude activity rhythms in ovariectomized female mice fed high-fat diet. Together, these results demonstrate that neonatal estrogen treatment does not masculinize daily rhythms that regulate diet-induced obesity.

Glucocorticoid Receptor β and Human Left Ventricular Cardiomyocytes: Glucocorticoid Resistance in the Heart

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Cardiovascular disease is a chronic disease, and it is the leading cause of death worldwide. Recently published work has suggested that glucocorticoid resistance may negatively impact cardiac cellular physiology. It is warranted that the mechanisms underlying glucocorticoid resistance in the heart be discovered. Glucocorticoids are steroid hormones produced in the zona fasciculata of the adrenal cortex. They have two known receptors: the dominant isoform, GRα, and the dominant negative isoform, GRβ. Current literature suggests that GRβ does not bind to glucocorticoids. Its mechanism of action is to antagonize the activity of GRα at its' target genes. Increased GRβ activity is also associated with glucocorticoid resistance, leading to several chronic, stress-related, inflammatory, immune, and metabolic diseases. We hypothesized that increasing GRβ plays a role in the negative effects of the nuclear receptor PPARγ on fat accumulation within the heart, leading to adverse cardiac outcomes such as myocardial infarctions and congestive heart failure. Human left ventricular cardiomyocytes (AC16 cells) with CRISPR KO or overexpression of GRβ were treated with PPARγ agonist rosiglitazone to determine. The findings indicate that GRβ is a positive regulator of PPARγ transcriptional signaling, and their co-signaling may be a factor in developing congestive heart failure. Our study indicates that inhibiting GRβ may prevent glucocorticoid resistance in cardiac tissue to reduce fat accumulation. A deeper understanding of GRβ's responsiveness to cardiovascular and metabolic treatments and whether it might serve as a therapeutic target for cardiovascular diseases is needed.

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Hypertriglyceridemia promotes aortic aneurysm formation and rupture in angiotensin II infused mice

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Plasma total cholesterol (TC) and triglyceride (TG) are reported to be positively associated with the risk of abdominal aortic aneurysm (AAA). Our group discovered that hypercholesterolemia augments angiotensin II-induced AAA in mice. We also found that remnants of triglyceride-rich lipoproteins (TRLs) may be crucial for AngII-induced AAA. Recently, it was reported that hypertriglyceridemia (HTG) caused by inducible lipoprotein lipase deficiency (iLpl-/-) results in uptake and accumulation of TRL lipid in aortic endothelial cells in vivo. Based upon these findings, we hypothesized that HTG stimulates AAA formation.

Methods: Adult male and female Lplf/f.beta-actin-Mer/Cre/Mer 1/0 and Lplf/f mice were administered 75 mg/kg/day tamoxifen for 5 consecutive days. Mice were fed either a standard diet (SD) throughout the study or a Western-type diet (WD) starting 1 wk after the completion of tamoxifen administration and continuing for a total of 5 wks. Mini osmotic pumps were implanted in mice 2 wks after completion of tamoxifen administration and delivered saline or AngII at 1,000 ng/kg/min for 4 wks.

Results: AngII infused and SD fed iLpl-/- versus Lplf/f mice had elevated plasma TG and TC levels but similar abdominal aortic external diameter and AAA incidence. Since the SD was low in fat, a high fat WD was then fed to AngII-infused mice. Plasma TG and TC were increased in female iLpl-/- versus Lplf/f mice. However, plasma lipid concentrations could not be measured in male iLpl-/- because 10 of 11 animals had died from aortic rupture. In contrast, none of the male Lplf/f mice died and only 4 of 11 had abdominal aortic dilation. Female compared to male iLpl-/- mice were protected from AngII-induced aortic rupture (1/11 died) and only 1 of 9 females had abdominal aortic dilation. To eliminate the possibility that HTG alone caused aortic rupture in male mice, the study was repeated with the addition of saline infusion. In agreement with the first study, AngII plus WD lead to death by aortic rupture in all male iLpl-/- mice (9/9). In contrast, iLpl-/- male mice infused with saline had markedly greater survival (1/5 died of unknown cause).

Conclusions: HTG causes aortic aneurysm development in AngII-infused male iLpl-/- mice. Thus, treating HTG could reduce AAA risk.

Revolutionizing our understanding of specific platelet-pathogen interactions

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Aside from their classical role in maintaining hemostasis, platelets are considered first-line vascular guardians that influence both the innate and adaptive immune systems. This is mediated by direct interactions with immune cells or by releasing pre-stored cytokines and chemokines upon interacting with pathogens like viruses. While this establishes platelets as potential players in the response to pathogens, it remains unclear what platelets can do with pathogens and how important that is to immune responses, especially against viruses. Platelets express several surface receptors that could interact with different viruses. To understand the mechanisms of HIV-1's interaction with platelets, we chose the EcoHIV model. While EcoHIV is an established model for neuroAIDS, its effects on platelets are ill-defined. Our results indicate that EcoHIV behaves differently from HIV-1 and is cleared from circulation after 48 h post-infection. The EcoHIV course of infection resembles an HIV-1 infection under the effects of combined antiretroviral therapy (cART) since infected mice stayed immunocompetent and the virus was readily detected in the spleen. EcoHIV-infected mice failed to become thrombocytopenic and showed no signs of platelet activation. One explanation is that mouse platelets lack the EcoHIV receptor, murine Cationic Amino acid Transporter-1 (mCAT-1). No mCAT-1 was detected on their surface, nor was any mCAT-1 mRNA detected. Thus, mouse platelets would not bind or become activated by EcoHIV. However, impure virus preparations, generated by Polyethylene Glycol (PEG) precipitation, do activate platelets, suggesting that nonspecific PEG-precipitates may contain other platelet activators (e.g., histones and cell debris). Our data do not support the concept that platelets, through general surface proteins such as DC-SIGN or CLEC-2, have a wide recognition for different viruses and suggest that direct platelet/pathogen interactions are receptor/ligand specific.

Small Molecule Compounds for Heparin Reversal and Their Use in Heparin Quantitation

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Background: Heparin is a class of naturally occurring anticoagulants frequently used to prevent blood clots. Heparins are rapidly reversed by protamine, but this approach has limitations, including protamine's short half-life and hemorrhagic risk if used in excess, emphasizing the need for alternative reversal agents. Here, we screened a library of 262 glycan-interacting small molecules of diverse chemical structures, to identify those capable of binding heparin and blocking its anticoagulant activity.

Methods: 262 compounds were screened for their ability to inhibit heparin anticoagulant activity in plasma-based assays. Two of the compounds possessed fluorescent properties and were further tested for their ability to measure heparin concentration in plasma.

Results: Initial screens were performed using a thrombin-initiated fibrin formation assay, which identified 7 compounds capable of reversing the anticoagulant activity of unfractionated heparin. Subsequent studies, utilizing fibrin formation and tissue factor- or kaolin-initiated thrombin generation, sought to assess their efficacy as protamine alternatives. Compounds GTC-3155 and GTC-3302 were most potent, completely reversing unfractionated heparin in all systems, but exhibited some heparin-independent anticoagulant activity at high concentrations (100 μM). GTC-3062 and GTC-3308 restored fibrin formation, partially restored thrombin generation, and did not have any apparent heparin-independent activity. These compounds were next evaluated against low molecular weight heparin. While all four compounds substantially reversed unfractionated heparin, only GTC-3155 and GTC-3062 were effective with low molecular weight heparin. Based on these results, GTC-3062, a quinazoline derivative shows the most promise for therapeutic use. GTC-3062 has acceptable metabolic stability in vitro, a plasma half-life of ~35min in C57BL/6N mice, and no apparent acute toxicity when injected up to 300 mg/kg. Direct binding of GTC-3062 to heparin was revealed as a hypochromic effect in UV absorbance and CD studies.

GTC-3155, a carbazole derivative, also possesses fluorescent properties, exciting at 350nm and emitting at 450nm, suggesting potential use for heparin detection and quantification. GTC-3155 binds directly to heparin, as revealed in fluorescence studies. Heparin binding, either in buffer or human plasma, reduced the emission in a dose-dependent manner (0.2-2U/mL), allowing for quantitation within this range. Thus, GTC-3155 may be useful for monitoring heparin dose, and for measurement of non-anticoagulant heparins, which cannot be monitored by anti-Xa activity.

Conclusions: Our data support the hypothesis that compounds which target the glycan moieties in heparin may be safer than protamine for reversing heparin anticoagulant activity. Notably, GTC-3062 partially reversed anticoagulation, restored fibrin formation, was effective against both unfractionated and low molecular weight heparins, and did not exhibit heparin-independent activity. The therapeutic potential of these compounds will next be evaluated using in vivo hemostatic and thrombotic mouse models.

Age-dependent Adrenal Stress Response in Sepsis

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Abstract

Sepsis is a life-threatening dysregulated host response to infection. The 2024 guidelines on corticosteroid use in critical illness recommend administering corticosteroids to adult patients with septic shock (conditional recommendation; low certainty). However, no recommendation is made for pediatric patients with sepsis due to weak evidence. Therefore, investigating the differences in adrenal function and the benefits of corticosteroid therapy between adults and younger populations is crucial. We hypothesize that developmental stages influence adrenal function, thereby affecting the efficacy of corticosteroid therapy in sepsis.

In this study, we evaluated adrenal function in 7-day-old, 3-week-old mice, and adult mice by examining scavenger receptor class B type I (SR-BI, a key receptor responsible for the uptake of cholesterol ester used in corticosterone synthesis) expression and performing adrenocorticotropic hormone (ACTH) stimulation test. We found that 7-day-old and 3-week-old mice exhibited lower adrenal SR-BI expression compared to adults, with 7-day-old mice having the lowest expression levels. After ACTH stimulation, both 3-week-old and adult mice exhibited increased corticosterone production, with adult mice showing a two-fold higher delta corticosterone than 3-week-old mice. However, 7-day-old mice showed no response to ACTH, with no increase in corticosterone production.

To investigate the efficacy of corticosteroid therapy in adult and young sepsis models, we induced sepsis using cecal ligation and puncture (CLP) in 7-day-old, 3-week-old and adult mice, followed by hydrocortisone injection. Our results demonstrated that corticosteroid therapy increased mortality in adult mice but had no significant effect on survival in 7-day-old and 3-week-old mice.

To better understand the molecular mechanisms underlying the differences in sepsis between adult and young mice, we performed RNA sequencing (RNA-seq) and identified differentially expressed genes (DEGs). In 3-week-old mice, upregulated DEGs enriched in ECM-receptor interaction, focal adhesion, Rap1 and PI3K-Akt signaling pathway, and platelet activity. And downregulated DEGs were predominantly enriched in pathways related to steroid hormone biosynthesis and metabolism.

In conclusion, 7-day-old and 3-week-old mice function less corticosterone than adult mice in response to ACTH stimulation or CLP-induced sepsis, suggesting that differences in adrenal responses may contribute to the varied effects of corticosteroid therapy on sepsis outcomes in different age groups. This highlights the potential need for age-specific corticosteroid therapy in the treatment of sepsis.

Exenatide administration time determines the effects on blood pressure dipping in *db/db* mice through modulation of food intake and sympathetic activity

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Abstract

Objective

Type 2 diabetics have an increased prevalence of hypertension and nondipping blood pressure (BP), which worsens cardiovascular outcomes. Glucagon-like peptide-1 (GLP-1) receptor agonists (RAs), a type 2 diabetes medication, also reduce BP. However, the mechanisms underlying the BP reduction and the influence of administration timing of GLP-1RAs on BP dipping are yet to be fully elucidated. The current study aims to address these knowledge gaps.

Methods

Exenatide, a short-acting GLP-1 RA, was intraperitoneally injected at the onset of the light (ZT0) or dark (ZT12) phase in male diabetic *db/db* mice and nondiabetic controls. BP and food intake were simultaneously monitored using radio-telemetry and BioDAQ cages, respectively. Food accessibility was controlled with programmable gates to limit access to the dark phase only (active time-restricted feeding, ATRF). Norepinephrine content was determined by HPLC in 6-hour urine samples collected over 24 hours under basal conditions, after ZT0 and ZT12 exenatide administration. Under ad libitum feeding and after ATRF, during the fasting phase, mecamylamine, a nicotinic acetylcholine receptor antagonist, was intraperitoneally injected at ZT0 alone, and in combination with exenatide; prazosin, an alpha-1 antagonist, was intraperitoneally injected at ZT0 alone, and in combination with exenatide.

Results

db/db mice exhibited nondipping BP and were hyperphagic compared to nondiabetic controls. ZT0 exenatide administration decreased light phase BP, restoring dipping BP. In contrast, ZT12 exenatide administration decreased dark phase BP, worsening BP circadian rhythm to reversed dipping. Exenatide-induced changes in BP rhythm corresponded with changes in food intake. After ATRF-induced rescue of BP rhythm, ZT0 exenatide administration's BP-lowering effects were abolished, whereas ZT12

exenatide administration's BP-lowering effects were still observed. Under ad libitum feeding, urinary norepinephrine content, a marker for sympathetic activity, was significantly reduced 6 hours following exenatide administration at both ZT0 and ZT12. The combination of exenatide and mecamylamine, administered at ZT0, did not result in further BP decrease than mecamylamine alone, with or without food accessibility. Furthermore, the BP-lowering efficacy from combined exenatide and prazosin ZT0 administration was not significantly different from prazosin alone, with or without food accessibility.

Conclusion

ZT0 exenatide administration restores BP dipping in *db/db* mice by inhibiting food intake and suppressing sympathetic activity during the light phase.

Plasmin generation in COVID-19 patients shows disruption in fibrinolytic system.

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Background:

Thrombosis can be diagnostically marked by D-dimer as a product of fibrinolysis breaking down clots, or by fibrin amyloid microclots detected by Thioflavin T (THT) fluorescent staining. Our laboratory previously measured both markers in plasma from COVID-19 patients and controls and noted that those with very high microclots always had very low D-dimer and vice versa. We hypothesized that this disparity reflects a deficiency in the fibrinolytic system. We tested this hypothesis by measuring the ex vivo activation of plasmin, the primary fibrinolytic enzyme.

Methods:

Plasma samples from two cohorts were included in this study: (1) University of Kentucky (UK): 52 COVID-19 outpatients, 20 COVID-19 inpatients, and 58 controls without COVID-19;

(2) Medical College of Wisconsin (MCW): 13 COVID-19 inpatients with samples pre- and post-recovery, 6 deceased, and 5 inpatient controls without COVID-19. D-dimer and microclot measurements were available for both cohorts. Plasmin generation, initiated with tissue factor (TF) and tissue plasminogen activator (tPA), was measured using a fluorescent plasmin substrate.

Results:

First compared was plasmin generation without thrombomodulin (TM), using pooled normal plasma. TM inhibits plasmin, reducing the endogenous plasmin potential (EPP), a measure of total plasmin activity, by 62.75±26.30% and peak plasmin by 58.29±17.51%.We next compared plasmin generation between populations. COVID-19 patients had lower EPP in outpatients (88.07±56.75%) and inpatients (86.65±38.23%) compared to controls (110.36±36.85%). Peak plasmin was lower in outpatients (76.29±42.18%) and inpatients (78.29±29.59%) compared to controls (92.97±21.67%). With TM, EPP was reduced in outpatients (32.64±29.19%) and inpatients (37.22±23.12%) compared to controls (55.16±43.22%). Peak plasmin was reduced in outpatients (24.67±20.30%) compared to controls (39.62±16.67%); but not in inpatients (42.69±19.29%).

We next compared plasmin generation parameters to D-dimer and microclot measurements. D-dimer trended negatively in EPP ($p=0.1028$) and peak plasmin ($p=0.1571$). Microclots had no correlation with EPP ($p=0.4344$) and trended positively with peak plasmin activity ($p=0.2069$). With TM, D-dimer had no correlation with EPP ($p=0.9892$) and peak plasmin activity ($p=0.2424$). Microclots had significant ($p=0.0011$) positive correlation with EPP and no correlation with peak plasmin. Both D-dimer and microclot were compared to the inhibition of plasmin activity. D-dimer had no correlation with EPP and had a significant

($p=0.0162$) negative correlation with peak plasmin inhibition. Microclot compared to % inhibition, had a significant ($p=0.0003$) negative correlation with EPP and no correlation with peak plasmin.

Conclusion:

The data shows that the fibrinolytic system is disrupted within COVID-19 patients. COVID-19 patient data had less total plasmin activity and inhibition of EPP and peak plasmin activity than controls. EPP was also shown to have a positive correlation with microclots, also supporting there being dysfunction in the fibrinolytic system of COVID-19 patients.

Profiling of the Renin Angiotensin-Aldosterone System (RAAS) Reveals Distinct Aldosterone Phenotypes in Human Pre-eclampsia

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Background: Pre-eclampsia is a leading cause of maternal and fetal death, and a significant driver of adverse outcomes of pregnancy. Mechanisms underlying the development of pre-eclampsia in humans are not well-understood. Activation of the RAAS with markedly increased secretion of aldosterone is a key feature of normotensive pregnancy, and thought to be impaired in pre-eclampsia. The purpose of our study was to perform RAAS profiling in pre-eclampsia, where we hypothesized that concentrations of aldosterone in serum of patients with pre-eclampsia would be reduced compared to pregnant patients without pre-eclampsia.

Methods: This is an IRB-approved, case-control study of 50 patients admitted to the hospital with pre-eclampsia and 50 gestational age-matched controls. Clinical characteristics (blood pressure, ultrasound findings, biochemical laboratory values) and blood samples were collected upon enrollment, and maternal and fetal outcomes were collected at delivery. Blood samples were analyzed for angiotensin I, angiotensin II, and aldosterone by LC-MS/MS using RAS Fingerprint™, which were used to calculate equilibrium-based biomarkers of RAAS activity.

Results: The median concentration of aldosterone in Pregnant Control patients was 4.5 fold higher than that of Pre-eclampsia Cases: median (interquartile range) of 620 (290.3 – 1242) versus 136.5 (50.6 – 385) pmol/L. Cases and Controls were further stratified by aldosterone concentrations below (Low Aldo) or above (Normal Aldo) the 25th percentile of Pregnant Control group. In Controls, low aldosterone level was observed in 13 of the 50 patients, and was linked with lower concentrations of angiotensin I and lower AA2-R, but no difference in concentrations of angiotensin II compared to Normal Aldo. In Cases, low aldosterone was observed in 34 of 50 patients, and was linked with lower concentrations of angiotensin I and angiotensin II, and lower AA2-R. In both Cases and Controls with Low Aldo, angiotensin peptide levels were increased compared to non-pregnant reference values, indicating activation of the RAAS in response to pregnancy. However only Controls with Low Aldo had aldosterone concentrations reflecting pregnancy, where Cases with Low Aldo had aldosterone levels more characteristic of non-pregnancy. In Cases with Low Aldo,

there was a higher incidence of pulmonary edema, absent end-diastolic flow, IUGR, and NICU stay. Of the 13 total cases of IUGR, 9 had aldosterone concentrations less than 90 pmol/L.

Conclusions: Consistent with previous studies, approximately 75% of patients with pre-eclampsia exhibit markedly reduced secretion of aldosterone, where concentrations are comparable to non-pregnant individuals and associated with lower concentrations of angiotensin II. In contrast, about 25% of pre-eclamptic patients retain some pregnancy-induced aldosterone secretion, and exhibit angiotensin II concentrations similar to Controls. These data suggest multiple mechanisms for pre-eclampsia in humans defined by the presence or absence of elevated aldosterone secretion.

Title:

Blood-brain barrier leakage but not microgliosis after traumatic brain injury is amplified in Angiotensin-II treated mice

Authors:

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Abstract:

The extent of brain damage accompanying traumatic brain injury (TBI) depends on the type and severity of insult and is modulated by age, sex, and comorbidities. Nearly half of all US adults suffer from hypertension. Not surprisingly, hypertension is the most common premorbid condition in people aged 50 or above hospitalized with a TBI. Hypertension has been linked to cerebrovascular damage, neuroinflammation and cognitive decline. Neurogenic hypertension is elevated blood pressure initiated by the local renin angiotensin system within the brain; often times involved in resistant hypertension (high blood pressure poorly responsive to common antihypertensives). Despite an overlap in several aspects of brain pathology induced by hypertension and triggered by TBI, little is understood about the impact of premorbid hypertension on outcomes following TBI. We hypothesize that hypertension induces mild blood-brain barrier leakiness and neuroinflammation, priming the brain for greater cerebrovascular damage after TBI. To test this hypothesis, mice were rendered hypertensive via subcutaneous infusion of 1000 ng/kg/min angiotensin-II (Ang-II) two weeks prior to and one week following induction of a moderate severity controlled cortical impact or sham injury. Vascular damage and microglia activation were examined using histology and immunohistochemistry. Quantification was performed using HALO image analysis software. Compared to injured normotensive mice, injured hypertensive mice exhibited significantly more IgG extravasation in the cortex and hippocampus denoting a potentiation of blood-brain barrier damage. Despite robust injury-induced microgliosis, CD68 immunostaining was not further increased by hypertension. Microbleeds were observed more in the cortex but much less in the hippocampi after injury and were equivalent in hypertensive and normotensive injured mice. The data presented here are preliminary findings of an ongoing study that we hope will contribute to laying the groundwork of identifying and characterizing hypertension as a potential premorbid risk factor for poor outcome after TBI.

Feeding behavior underlies the circadian rhythm in the autonomic input to the heart and heart rate

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Objective: The circadian pacemaker in the suprachiasmatic nucleus (SCN) of the hypothalamus drives circadian rhythms in feeding and activity. Several studies suggest the SCN regulates autonomic input to the heart to generate day-night rhythms in heart rate.
Hypothesis: We hypothesized that the daily rhythms in feeding behavior modified autonomic input to the heart to generate day-night rhythms in heart rate.
Methodology: Mice (n=6/sex) were implanted with telemetry probes to continuously record heart rate, core body temperature, and activity in thermoneutral housing conditions. Mice were housed in 12 h light: 12 h dark cycle (LD) with ad libitum access to food (ALF). In these conditions, mice primarily eat during the dark cycle. Mice were subjected to light phase time-restricted feeding (TRF) for 5 days. TRF mice were switched from LD to constant darkness (DD) and then returned to ALF. We quantified changes in autonomic input to the heart by injecting mice with the autonomic receptor inhibitors propranolol and methylatropine.
Results: ALF mice housed in LD had 24-hour rhythms in autonomic input to the heart, heart rate, and activity that all peaked in alignment during the dark cycle. One day after starting TRF, the phase of the 24-hour rhythms in heart rate but not activity shifted by 8-10 hours to align with feeding during the light phase. Autonomic blockade removes the day-night variation in the heart rate in mice under ALF but not TRF. Returning the TRF mice in DD to ALF showed the autonomic input to the heart and heart rate re-aligned with the free running rhythm in activity.
Conclusions: Day-night rhythms in heart rate and autonomic input to the heart align with activity in LD and DD in ad libitum fed but not time-restricted fed conditions. These data demonstrate that feeding behavior, not SCN-signaling or activity rhythms, drives day-night rhythms in autonomic input to the heart and heart rate. We also identified a 24-hour rhythm in the heart rate that appears to be independent of daily rhythms in core body temperature and autonomic receptor signaling to the heart.
Funding: This study is supported by Grants NHLBI R01HL153042 and NHLBI R01HL141343 to Dr. Brian Delisle.

Interventional cardiomyocyte-restricted RAD knockout improves cardiac dysfunction in a murine model of dilated cardiomyopathy

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Background: Heart failure (HF) is a growing burden, with prevalence expected to increase 46% by 2030 from 2012. In dilated cardiomyopathy (DCM), systolic heart failure occurs with reduced ejection fraction (HFrEF) and dilatation of the ventricles. Current therapies for HFrEF do not directly target hypocontractility and dysfunctional calcium handling. RAD (Ras associated with diabetes) is a key regulator of the L-type calcium channel (LTCC). Cardiomyocyte-restricted deletion of RAD ($cRAD^{\Delta/\Delta}$) of healthy mice has been shown to function as a calcitrope, stably bolstering cardiac systolic function by augmenting LTCC activity.

Hypothesis: Interventional $cRAD^{\Delta/\Delta}$ rescues cardiac dysfunction in the setting of DCM. Mechanistically, LTCC modulation results in positive inotropy.

Methods and Results: The muscle lim protein knockout mouse (MLPKO) is a murine model of DCM and HFrEF. The experimental timeline was to induce $cRAD^{\Delta/\Delta}$ after onset of DCM (2.5 months of age) and follow subjects for up to 2 months. Longitudinal echocardiography showed that $cRAD^{\Delta/\Delta}$ intervention provided significant rescue of systolic function. Patch clamp recordings of isolated cardiomyocytes of MLPKO with $cRAD^{\Delta/\Delta}$ demonstrated augmented LTCC activity, along with rescue of dysfunctional Ca^{2+} handling and sarcomere function. Bulk RNAseq of hearts demonstrated downregulated pathological signaling cascades and pro-hypertrophic gene expression which comported with the reduction in eccentric hypertrophy observed with gravimetrics, cardiac magnetic resonance imaging, and echocardiography. *RRAD* knockdown effects translate from mouse to human heart. Ventricle strips from HFrEF patients were treated with lentiviral shRNA targeting *RRAD* and recapitulated the inotropic and lusitropic effects observed in the mouse model.

Conclusions: Induction of $cRAD^{\Delta/\Delta}$ in MLPKO mice after onset of DCM rescued cardiac dysfunction and attenuated pathological remodeling. $cRAD^{\Delta/\Delta}$ intervention provided positive inotropy and lusitropy and reverted transcriptional signatures towards healthy myocardium. This study introduces the approach of targeting RAD regulation of the cardiomyocyte LTCC as a therapeutic strategy for systolic heart failure.

Obesity predicts left ventricular mass in youth undergoing ambulatory blood pressure monitoring

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Background: Left ventricular hypertrophy (LVH) is a risk factor for cardiovascular disease in adults. In youth, elevated blood pressure (BP) is associated with LVH, but independent effects of adiposity and hypertension on LVH are not well defined. The purpose of our study was to investigate relationships among LVH, LV mass, BMI, and other clinical parameters in pediatric patients referred for elevated BP. We hypothesized both BP and BMI to be associated with LV mass.

Methods: We conducted an IRB-approved retrospective chart review of all patients ages 6-18 years who underwent 24-hour ambulatory BP monitoring via the pediatric nephrology clinic at Kentucky Children's Hospital from August 2012-December 2023 excluding those with secondary HTN. Obesity was defined by BMI and weight-for-length percentiles according to CDC guidelines. LV mass was assessed by standard echocardiography. We compared LVMI and BP measures among groups stratified by obesity stage. Associations among BP, obesity, and LVMI were determined using descriptive statistics, correlational analyses, and multivariable logistic modeling using a backwards elimination variables selection criteria with $\alpha=0.05$.

Results: Of the 520 patients, 66% (n=342) were male, 76% (n=395) were obese, and 46% (n=238) had LVH. LVMI was strongly correlated with 24-hr systolic BP, however there was no difference in mean LVMI across BP severity category. In contrast, there was a near stepwise increase in LVMI across obesity stage with the strongest effect in girls – Normal: 29.7 $\pm$ 5.3 (girls) v. 34.8 $\pm$ 11.4 (boys) $g/m^{2.7}$; OB-1: 36.5 $\pm$ 6.6 (girls) v. 43.2 $\pm$ 10.3 (boys) $g/m^{2.7}$; OB-2: 41 $\pm$ 11.6 (girls) v. 44.2 $\pm$ 9.2 (boys) $g/m^{2.7}$; OB 3: 48 $\pm$ 11.6 (girls) v. 43.5 $\pm$ 9.4 (boys) $g/m^{2.7}$. LVMI was positively correlated with TSH, creatinine, uric acid, cystatin C, and inversely correlated with HDL. Multivariable logistic modeling revealed obesity stage and HDL cholesterol, but not BP or laboratory variables, as significant predictors of LVH.

Conclusions: We report a high incidence of LVH in youth with obesity and demonstrate a strong association between obesity and LVMI. Our data, in agreement with previous studies, indicate that adiposity is a significant determinant of left ventricular mass, and suggest the need for cardiac screening of youth with obesity even in the absence of elevated blood pressure.

O-GlcNAcylation underlies the activation of sodium-glucose cotransporter 1 in diabetic hearts

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Rationale: Type-2 diabetes (T2D) greatly increases the risk of developing heart failure. Recent evidence indicates that the cardiac expression and function of the sodium-glucose cotransporter 1 (SGLT1) is elevated in disease states, including T2D, leading to structural and functional remodeling of the heart. The mechanisms underlying SGLT1 activation in the diabetic heart are currently unknown.

Objective: To investigate if SGLT1 undergoes the O-linked attachment of β-*N*-acetylglucosamine (O-GlcNAcylation), a post-translational modification that is exacerbated in T2D, and its effect on SGLT1 activity in diabetic hearts.

Methods/Results: SGLT1 co-immunoprecipitates with O-GlcNAc in heart homogenates and HL-1 cardiomyocyte lysates. Enhancing global protein O-GlcNAcylation in HL-1 cells via si-RNA-mediated silencing of O-GlcNAcase (OGA), the enzyme that removes O-GlcNAc from proteins, resulted in increased amounts of O-GlcNAc that precipitate with SGLT1. SGLT1 function, measured as the SGLT1-mediated Na^+ influx, was significantly increased in HL-1 cells with silenced OGA. These data suggest that SGLT1 undergoes O-GlcNAcylation and this modification results in SGLT1 activation. Using rats that express human amylin in the pancreatic β-cells (HIP rats) as a model of late-onset T2D and their wild-type littermates as controls, we found that a larger fraction of SGLT1 co-immunoprecipitates with O-GlcNAc in hearts from T2D rats compared to controls. The SGLT1-mediated Na^+ influx was larger in myocytes from T2D *vs*. control rats. Increasing total O-GlcNAcylation with Thiamet-G in control myocytes resulted in a larger Na^+ influx. In reverse experiments, inhibition of O-GlcNAcylation with 6-diazo-5-oxo-L-norleucine crystalline reduced SGLT1-mediated Na^+ uptake in myocytes from T2D rats. Inhibition of O-GlcNAcylation in T2D myocytes reduced Ca^{2+} spark frequency and this effect was significantly less pronounced with SGLT1 blocked. These results suggest that O-GlcNAcylation affects myocyte Na^+ regulation by activating SGLT1, contributing to myocyte Na^+ overload and its downstream effects on sarcoplasmic reticulum Ca^{2+} leak in T2D.

Conclusion: Exacerbated O-GlcNAcylation underlies SGLT1 activation in diabetic hearts. Preventing O-GlcNAcylation may limit myocyte Na^+ overload and the frequency of pro-arrhythmogenic Ca^{2+} sparks in myocytes from T2D rats.

VAMP8 Deletion Mitigates Atherosclerosis by Modulating Platelet Inflammatory Cargo Release

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Background: Beyond their established roles in hemostasis and thrombosis, platelets are increasingly recognized for their contribution to chronic thrombo-inflammatory conditions within the vascular system, including atherosclerosis. Platelets actively participate in systemic responses by modulating their microenvironment through the exocytosis of cytokines and signaling molecules. Overexpression of VAMP8, the key v-SNARE protein involved in platelet exocytosis, has been linked to platelet hyperactivity and an elevated risk of coronary events in humans. This study investigates the role of VAMP8 in the progression of atherosclerotic lesions.

Methods and Results: To confirm platelet involvement in atherosclerosis, washed platelets from healthy WT mice were treated with 0.05 U/mL thrombin, and the platelet releasate was analyzed using highly-multiplexed CodePlex chip secretome proteomics. Our findings confirmed that platelets secrete several cytokines and chemokines associated with atherosclerosis, notably IL-1β. To further investigate the impact of impaired platelet exocytosis on atherosclerosis, we crossed VAMP8-deficient (VAMP8-/-) mice with ApoE-deficient (ApoE-/-) mice to create a hypercholesterolemic double-knockout (ApoE-/-/VAMP8-/-) model. These mice were fed a Western diet for 12 weeks to induce atherosclerosis. Atherosclerotic lesions were manually traced on the intimal surface from the ascending aorta to the descending thoracic aorta, 1 mm distal to the left subclavian artery, using Nikon NIS-Elements software (*en face* method). Our data indicated that VAMP8 deficiency significantly suppressed atherosclerosis development in male ApoE-/-/VAMP8-/- mice. We further validated these results by inducing hypercholesterolemia in mice aged 8-12 weeks via an adeno-associated viral (AAV) vector expressing a gain-of-function mutation (D377Y) in mouse PCSK9, followed by a Western diet. Atherosclerotic lesion development was also markedly reduced in VAMP8-/- mice in this model. Plasma total cholesterol levels did not differ between WT and VAMP8-/- groups in either model. Additionally, bulk RNA-seq analysis of washed WT versus VAMP8-/- platelets revealed significant downregulation of atherosclerosis-associated and inflammatory pathways, suggesting a potential impact on cargo release and its role in atherosclerosis development.

Conclusion: VAMP8 deletion significantly reduces atherosclerosis and affects the release of inflammatory cargo from platelets.

Surgical Treatment of Cardiac Tumors: A Single Center Experience

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Introduction: Cardiac tumors affect fewer than 1 in 2,000 people. 75% of primary tumors are benign and the most common primary tumor in adults is myxoma. As a regional referral center, UK healthcare provides 'standard of care' treatment for these patients. This study is a retrospective review of diagnostic procedures, surgical management, and outcomes in patients treated for tumors of the heart in our institution. We compare our management approaches, clinical and surgical outcomes with those reported in the literature.

Methods: The study population includes patients 7-79 years old that presented to University of Kentucky Healthcare for tumors of the heart from July 2004 - January 2023. With IRB approval, subjects for this study were identified by searching the University of Kentucky database on the CPT codes for tumors of the heart (benign neoplasm of the heart or malignant tumor). CCTS Data Warehouse has provided data based on CPT codes. All data was stored on REDCap.

Results: Operative treatment was offered to 52 people; 47 had resection and 5 had biopsy. The patient population consisted of 29 females and 23 males. The average patient age was 54 years old with the oldest being 79 and the youngest being 7. Most patients were caucasian (49) followed by unknown (2) and black/african american (1). The most common presentation symptoms were shortness of breath, dyspnea, and fatigue. The most common diagnosis methods were transthoracic echocardiogram, transesophageal echocardiogram, cardiac MRI, and CT scan. Surgical treatments included 47 resections and 5 biopsies; a certain number of people required closure of septum with or without a patch. The most common postoperative complication was respiratory insufficiency (22), bleeding (11), and sepsis (2). 48 patients were diagnosed with a benign neoplasm of the heart and 4 diagnosed with a malignant tumor. There were 49 primary tumors and 3 secondary tumors. The most common location of the tumor was the left atrium. The largest size number was 337.5 cm^3 and the smallest was 0.042 cm^3. The most common diagnosed cell type for the cardiac tumors was myxoma (32), followed by papillary fibroelastoma (11), fibroma (2), hemangioma (2), fatty infiltration, fibrosis and myocyte hypertrophy (1), metastatic hepatocellular carcinoma (1), metastatic neuroendocrine tumor (1), metastatic squamous cell carcinoma (1), and primary intimal sarcoma (1). The average length of stay in the hospital was 12.5 days with the longest being 59 days and shortest being 0.75 days. 39 patients were discharged home in a stable condition. The study population had one operative mortality (death within 30 days after surgery). 43 of the 52 patients treated are alive after 2 years.

Conclusion: Clinical outcomes such as discharge status, post-operative condition, and length of survival after procedures are non-inferior to those from other referral centers for such conditions. 83% of patients in this study surgically treated for cardiac tumors are alive after 2 years. This study shows surgical removal offers the best chance of cure for cardiac tumors.

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Deletion of Carnitine Palmitoyltransferase 1a from Adipocytes Leads to Insulin Resistance in Female Mice

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Background: Carnitine palmitoyltransferase 1 (CPT1) is the rate-limiting enzyme in mitochondrial fatty acid oxidation (FAO). Our laboratory and others have shown that CPT1a is the most abundant CPT1 enzyme in white adipose tissue (WAT) in mice and humans, prompting an investigation into its role in adipocyte biology.

Methods: CRISPR-Cas9N was used to delete endogenous CPT1a in murine 3T3-L1 fibroblasts. WT and CPT1a KO cells were used to study adipocyte differentiation and insulin responses *in-vitro*. For *in-vivo* studies, eight-week old male and female AKO (*Cpt1a*$^{\Delta Adipo}$) and littermate controls (*Cpt1a*$^{F/F}$) were placed on a high-fat diet (HFD; 60% kcal fat) for 16 weeks. Glucose and insulin tolerance tests were completed after 11 and 13 weeks on diet. Mice were necropsied after a 16 hour fast, and tissues and serum were collected for insulin and C-peptide analysis, bulk RNA sequencing, and protein expression by immunoblotting.

Results: Murine 3T3L1 KO cells exhibited increased adipocyte differentiation, which was accompanied by a ~50% increase in triglycerides and a 4-5 fold increase in expression of known adipogenic markers (*Cd36*, *Cidec*). Despite comparable IRβ phosphorylation, fully differentiated KO adipocytes had reduced Akt and Erk phosphorylation in response to insulin treatment, as compared to controls. Deletion of CPT1a from adipose tissue of female mice resulted in increased body weight and subcutaneous adiposity in response to HFD, as compared to littermate controls. Further, female *Cpt1a*$^{\Delta Adipo}$ mice displayed a 2-fold increase in fasting insulin and insulin to C-peptide ratios, which coincided with glucose intolerance and insulin resistance in these mice. Notably, no changes were observed in male mice across all parameters tested.

Conclusions: Deletion of CPT1a in adipose tissue promotes sex-specific responses in adiposity and insulin resistance. Future research will determine mechanisms by which substrates (fatty acids) and products (acylcarnitines) of CPT1a impact insulin signaling in adipocytes.

Thank you for joining us at the 26th annual Cardiovascular Research Day.

Please follow the QR code below for a quick survey of today's event:

We encourage you to save the date for next year's event at the Gatton Student Center on September 26, 2025.