28TH CARDIOVASCULAR RESEARCH DAY
UNIVERSITY OF KENTUCKY

9.18
2026

Abstract Book

University of Kentucky
Cardiovascular Research Day
September 18, 2026
Gatton Student Center

Table of Contents

Schedule	3
Sponsors	7
Speakers	8
Trainee Showcase	14
Lightening Talk	15
List of Attendees	16
Abstracts	20
Cardiovascular Research Day 2027	106
Event Survey	107

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Gatton Student Center
Grand Ballrooms

Check In @ 8:30 am

Opening Remarks @ 9:00 am

Alan Daugherty, PhD, DSc | Director, Saha Cardiovascular Research Center

Round 1 - Lightning Talks: Trainee Research Snapshots @ 9:05 am

Moderator: Gregory Graf, PhD

McKayla Dinkha | Undergraduate | Graf Lab
Sajeev Kaur | Postdoc | Gensel Lab
Raj Neupane | Postdoc | Gordon Lab
Garrett Anspach | Graduate Student | Helsley Lab
Abhilash Prabhat | Postdoc | Delisle Lab

Assistant Professor Showcase @ 9:15 am

Moderator: Hong Lu, PhD

Diego Lucero, PhD | Assistant Professor, Saha Cardiovascular Research Center
Decoding LDL Metabolism: From Gene Discovery to Molecular Mechanisms
Xu Xiao, PhD | Assistant Professor, Saha Cardiovascular Research Center
Intracellular Cholesterol Transport in Metabolic Disease
Brooks Lane, PhD | Assistant Professor, Biomedical Engineering
Mechanics, Remodeling, and Resilience in Aortopathies
Cheavar Blair, PhD | Assistant Professor, Saha Cardiovascular Research Center
A Dial, Not a Switch: Does the time of day grade calcium release in the human cardiomyocyte?

Break @ 10:15 am

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Round 2 - Lightning Talks: Trainee Research Snapshots @ 10:30 am

Moderator: Preetha Shridas, PhD

Esterline Charles | Undergraduate | Daugherty Lab
Zander Houchens | Undergraduate | Fisher Lab
Sandra Miranda | Graduate Student | Blair Lab
Megan Blair Turner | Postdoc | Mishra Lab
Yugui Wang | Graduate Student | Xiao Lab
Alex Pettey | Postdoc | Daugherty Lab

Alumni Presentation @10:45 am

Moderator: Nate Helsley, PhD

A Phillip Owens III, PhD | Professor, Internal Medicine, University of Cincinnati
The Evolving Role of Platelets in AAA Initiation and Progression

Poster Judging @ 11:15 am

Odd numbered posters judged.

Poster Session @11:30 am

Presenters remain at their boards for collaborative discussions.

Lunch @ 12:15 pm

Moderator: Alan Daugherty, PhD

Andrew Plump, MD, PhD | President, Research & Development, Takeda
Careers and the Making of Medicines: From Your 'Why' to the Real World of Drug Development

Break @ 1:15 pm

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Grand Ballrooms

Trainee Showcase @ 1:30 pm

Moderator: Wally Whiteheart, PhD

Erich Franz - MD, PhD Student | Whiteheart Lab
Combining Volume Electron Microscopy and Computational Fluid Dynamics Analysis to Understand How Arterial Thrombi Become Occlusive

Grant Kelley - Medical Student | Mentor: Sibu Saha, MD
Diagnosis and Treatment of Patent Ductus Arteriosus in Full-Term Infants: A Single-Center Experience

Katie Neglia - Undergraduate | Cooper Lab
Isoflurane Effects on Larval Drosophila Cardiac Tissue

Matt Thomas, PhD - Postdoctoral Fellow | Pendergast Lab
Late Sleep Timing is Associated with Metabolic Risk in Postmenopausal Women

Clairity Voy - Graduate Student | Gordon Lab
Lipoproteins Are Susceptible to Proteolytic Modifications That May Alter Their Atherogenicity

Evan Neary - Undergraduate | Macauley Lab
A High-Fat, High-Sugar Diet Drives Regional Plaque Deposition and Vascular Remodeling in a Model of Amyloid Pathology

Round 3 - Lightning Talks: Trainee Research Snapshots @ 2:30 pm

Moderator: Ila Mishra, PhD

Meredith Campbell | Graduate Student | Graf Lab
Dorottya Gal | Undergraduate | Gensel Lab
Scout Grimes | Undergraduate | Viswanathan Lab
Oyenmwen Abu | Graduate Student | Shridas Lab
Delgama Nishadhi | Postdoc | Wu Lab
Josephine Otuagomah | Graduate Student | Wang Lab

Gatton Student Center
Grand Ballrooms

Poster Judging @ 2:45 pm

Even numbered posters judged.

Poster Session @ 3:00 pm

Presenters remain at their boards for collaborative discussions.

Gill Award for Outstanding Cardiovascular Research @ 3:45 pm

Moderator: Scott Gordon, PhD

Alan Remaley, MD, PhD | Senior Investigator, NHLBI

Lipoprotein Metabolism: A Personal Journey from Bench to Bedside

Networking Reception @ 4:30 pm

An informal gathering to continue conversations sparked during the research day and to foster new connections across research areas and career stages. Trainees are encouraged to participate in the Networking Game at this time.

Awards Ceremony @ 5:15 pm

A special thank you to the sponsors of the 2026 University of Kentucky Cardiovascular Research Day.

Gill Foundation of Texas

The Saha Fund for Cardiovascular Research & Education

Bob and Kathy Allen

The Estate and Family of Mrs. Hager Koostra

Institutional support for the
2026 University of Kentucky Cardiovascular
Research Day is provided by the following units:

Keynote Orator

Andrew Plump, MD, PhD
President, Research & Development
Takeda

Andrew Plump, MD, PhD, is the president of Research and Development at Takeda and serves as a member of the company's board of directors. His career spans nearly 30 years in the pharmaceutical industry and academia.

Dr. Plump has been recognized for his contributions to the health care industry, education, and the arts. He serves on several not-for-profit boards, including the Board of Trustees for the Boston Symphony Orchestra, the Sarnoff Cardiovascular Research Foundation, the Biomedical Science Careers Program, and as Chairman of the Board of Directors for the PhRMA Foundation.

Prior to Takeda, Dr. Plump served as head of Research and Translational Medicine, deputy to the president of R&D at Sanofi, based in Paris, France. Prior to Sanofi, Dr. Plump served as worldwide cardiovascular research head at Merck.

Dr. Plump received his MD from the University of California, San Francisco (UCSF), his PhD in cardiovascular genetics, and his BS from the Massachusetts Institute of Technology (MIT). He completed a residency in internal medicine and a fellowship in medical genetics at UCSF.

Gill Heart and Vascular Institute Outstanding Contributions to Cardiovascular Research Award

Alan Remaley, MD, PhD

Section Chief, Lipoprotein Metabolism Laboratory
NHLBI

Alan T. Remaley, MD, PhD, is the section chief of the Lipoprotein Metabolism Laboratory in the Translational Vascular Medicine Branch of the National Heart, Lung, and Blood Institute (NHLBI) in Bethesda, MD. He is also a senior staff member of the department of laboratory medicine at the National Institutes of Health Clinical Center.

Dr. Remaley received his BS in Biochemistry and Chemistry from the University of Pittsburgh in 1981. He received in 1987 an MD and PhD (Biochemistry) degree from the University of Pittsburgh and, in 1990, completed a residency in Clinical Pathology at the University of Pennsylvania. He did a post-doctoral fellowship with Dr. Bryan Brewer at NHLBI from 1990-1995.

He has been the recipient of many awards for his research and has published over 500 papers in the field of lipoprotein metabolism and cardiovascular disease. He is an inventor on multiple patents related to new therapeutic agents and diagnostic tests for cardiovascular disease. He has made important contributions to the field of High-density lipoprotein metabolism, particularly related to the mechanism of cholesterol efflux by the ABCA1 transporter and has developed apoA-I mimetic peptides and recombinant Lecithin:Cholesterol acyl transferase into therapies, which have been tested in early-stage clinical trials. More recently, he has expanded his research into low-density lipoproteins (LDL), the main driver of atherosclerosis and cardiovascular disease. He has developed an equation, which is widely used for estimating the cholesterol content of LDL, and described the first high-resolution structure of APOB100, the main protein constituent of LDL and the ligand for the removal of LDL from the circulation by its receptor.

Distinguished Alumni Speaker

Phillip Owens, PhD
Associate Professor
University of Cincinnati

Dr. Owens is currently an associate professor at the University of Cincinnati. The primary focus of Dr. Owens's research is to examine the effects of coagulation proteins, proteases, and receptors in the pathogenesis of cardiovascular disease (CVD), specifically atherosclerosis and abdominal aortic aneurysms (AAAs). We utilize genetically modified mice and mouse models of disease to generate data and attempt to verify these results in the human condition, when possible, with retrospective clinical data or human specimens.

Diego Lucero, PhD
Assistant Professor
University of Kentucky

Dr. Lucero is an Assistant Professor in the Saha Cardiovascular Research Center and the Department of Physiology at the University of Kentucky College of Medicine. His research focuses on elucidating the cellular and molecular mechanisms that regulate lipoprotein metabolism and their role in cardiovascular disease. His laboratory integrates functional genomics, molecular and cell biology, and animal models to identify and characterize novel regulators of LDL metabolism. Dr. Lucero's research aims to bridge gene discovery and mechanistic biology to deepen understanding of the pathways that control circulating lipoproteins and cardiovascular risk.

Xu Xiao, PhD
Assistant Professor
University of Kentucky

Dr. Xiao is an Assistant Professor in the Saha Cardiovascular Research Center and Department of Physiology at the University of Kentucky College of Medicine. His laboratory studies how intracellular cholesterol transport and organelle lipid homeostasis regulate metabolic diseases, including MASLD, liver fibrosis, obesity, and vascular inflammation.

Cheavar Blair, PhD
Assistant Professor
University of Kentucky

Dr. Blair is an Assistant Professor in the Department of Physiology at the University of Kentucky. He earned his PhD from the University of Kentucky, where his research investigated the biophysical properties of human myocardial tissue. He subsequently completed postdoctoral training at Stanford University and the University of California, Santa Barbara, where his work focused on quantifying the mechanical responses of stem cell-derived cardiomyocytes to chemotherapeutic agents and disease-associated genetic mutations. His current research program seeks to elucidate how sarcomeric dysfunction contributes to the pathogenesis of heart failure.

Brooks Lane, PhD
Assistant Professor
University of Kentucky

Dr. Lane is an Assistant Professor in the Biomedical Engineering Department at the University of Kentucky. He earned his BS and PhD in Biomedical Engineering from the University of South Carolina prior to postdoctoral trainings at Emory and Drexel Universities supported by the AHA and Hartwell Foundation. Dr. Lane's laboratory integrates experimental biomechanics, vascular biology, advanced imaging, and engineered in vitro models to investigate interactions among vascular cells, extracellular matrix structure, inflammation, and mechanical forces. His work seeks to identify mechanisms governing vascular adaptation, degeneration, and failure and translate these insights toward improved assessment and treatment of cardiovascular disease.

Trainee Showcase

Erich Franz

MD, PhD Student | Whiteheart Lab

"Combining Volume Electron Microscopy and Computational Fluid Dynamics Analysis to Understand How Arterial Thrombi Become Occlusive"

Grant Kelley

Medical Student | Mentor: Sibu Saha, MD

"Diagnosis and Treatment of Patent Ductus Arteriosus in Full-Term Infants: A Single-Center Experience"

Katie Neglia

Undergraduate | Cooper Lab

"Isoflurane Effects on Larval Drosophila Cardiac Tissue"

Matt Thomas, PhD

Postdoctoral Fellow | Pendergast Lab

"Late Sleep Timing is Associated with Metabolic Risk in Postmenopausal Women"

Clairity Voy

Graduate Student | Gordon Lab

"Lipoproteins Are Susceptible to Proteolytic Modifications That May Alter Their Atherogenicity"

Evan Neary

Undergraduate | Macauley Lab

"A High-Fat, High-Sugar Diet Drives Regional Plaque Deposition and Vascular Remodeling in a Model of Amyloid Pathology"

Lightening Talks

McKayla Dinkha | Undergraduate | Graf Lab
Sajeev Kaur | Postdoc | Gensel Lab
Raj Neupane | Postdoc | Gordon Lab
Garrett Anspach | Graduate Student | Helsley Lab
Abhilash Prabhat | Postdoc | Delisle Lab
Esterline Charles | Undergraduate | Daugherty Lab
Zander Houchens | Undergraduate | Fisher Lab
Sandra Miranda | Graduate Student | Blair Lab
Megan Blair Turner | Postdoc | Mishra Lab
Yugui Wang | Graduate Student | Xiao Lab
Alex Pettey | Postdoc | Daugherty Lab
Meredith Campbell | Graduate Student | Graf Lab
Dorottya Gal | Undergraduate | Gensel Lab
Scout Grimes | Undergraduate | Viswanathan Lab
Oyenmwen Abu | Graduate Student | Shridas Lab
Delgama Nishadhi | Postdoc | Wu Lab
Josephine Otuagomah | Graduate Student | Wang Lab

Cardiovascular Research Day 2027
Attendees

Osemwen Abu
University of Kentucky

Toni Adedokun
University of Kentucky

adebola adegboyega
University of Kentucky

Nermin Ahmed
University of Kentucky

Samuel Akyirem
University of Kentucky

Shekinah Alfaro
University of Kentucky

Gavin Alfon
University of Kentucky

Heba Ali
University of Kentucky

Yasir Alsiraj
University of Kentucky

Douglas Andres
University of Kentucky

Oelisoa M Andriankaja
University of Kentucky

Garrett Anspach
University of Kentucky

Skylie Antle
University of Cincinnati

Gokul Anugrah
University of Kentucky

Sandra Bakare
Research Junction Institute

Sara Bakhtiari
University of Kentucky

Ryan Barney
University of Kentucky

Tyler Benson
University of Cincinnati

Olivia Hage
University of Kentucky

Graycen Hall
University of Kentucky

Abir Hamdaoui
University of Kentucky

Mike Harders
University of Kentucky

Evan Haynes
University of Kentucky

Robert Helsley
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Susana Hincapie Gomez
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Thomas Hinneh
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Zander Houchens
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Ava Hubbs
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Yu Hui
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Riley Irmen
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Rahatul Islam
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Sohei Ito
University of Kentucky

Amunet Jacobs
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Delgama Nishadhi
University of Kentucky

Vicky Noffisinger
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Samuel Nwadialo
University of Kentucky

Kendra OoNorasak
University of Kentucky

Sophia Ospina
University of Kentucky

Josephine Otuagomah
University of Kentucky

A Phillip Owens, III
University of Cincinnati

Vivek Pandey
University of Kentucky

Sehyung Park
University of Kentucky

Priya Patel
University of Kentucky

Pranay Patel
University of Kentucky

Julie Pendergast
University of Kentucky

Alex Pettey
University of Kentucky

Andrew Plump
Takeda

Abhilash Prabhat
University of Kentucky

Deb Rateri
University of Kentucky

Lucas Redmond
University of Kentucky

Alan Remaley
NIH

Cardiovascular Research Day 2027
Attendees

Cheavar Blair
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Cristina Bonilla
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Seth Brunner
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Don Burgess
University of Kentucky

Lei Cai
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Meredith Campbell
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Kenneth Campbell
University of Kentucky

Kyler Carl
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Lisa Cassis
University of Kentucky

Sarah Cayton
University of Kentucky

Esterline Charles
Berea College

Leona Ciarlariello
University of Cincinnati

Daniëlle Coenen
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Jonathan Copfer
University of Kentucky

Lindsay Czuba
University of Kentucky

Alan Daugherty
University of Kentucky

Ravichandra Davargaon
University of Kentucky

Luiza de Siqueira Almeida Reis
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Anna Jaimes
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Kelsey Jarrett
UCLA

Ailing Ji
University of Kentucky

Smita Joshi
Eastern Kentucky University

Thomas Kampourakis
University of Kentucky

Alexander Karakashian
University of Kentucky

Sajeev Kaur
University of Kentucky

William Kelley
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Percell Kendrick
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Chase Kiser
University of Kentucky

Deepak Kotiya
University of Kentucky

Tatsushi Kurokawa
University of Kentucky

Brooks Lane
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Ian Leatherman
University of Kentucky

Bryana Levitan
University of Kentucky

Qingxuan Li
University of Kentucky

Hua Li
University of Kentucky

Xiangan Li
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Alexis Rippel
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Darrell Robertson
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Chloe Roth
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Ezekiel Rozmus
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Sierra Rusch
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Susma Sah
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Sibu Saha
University of Kentucky

Rani Saha
Saha Foundation

Jonathan Satin
University of Kentucky

Elizabeth Schroder
University of Kentucky

Ivanka Sevrieva
University of Kentucky

Sydney Sharon
University of Cincinnati

Mary Sheppard
University of Kentucky

Preetha Shridas
University of Kentucky

Ashley Smith
University of Kentucky

Tyler Spear
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Mohammed Srour
University of Kentucky

Ian Stone
Eastern Kentucky University

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Attendees

Brian Delisle
University of Kentucky

McKayla Dinkha
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Renee Donahue
University of Kentucky

Emily Dunn
University of Kentucky

Gabriel Emerson
University of Kentucky

Victoria English
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Marsha Ensor
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Candice Falls
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Guan Fan
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Gregorio Farina
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Victor Ferraris
University of Kentucky

Robin Finch
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Michael Franklin
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Erich Franz
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Dorottya Gal
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Caroline Geisler
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Niloufar Gholamrezaeinejad
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Mina Lin
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Brittany Little
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Wei-Hsien Liu
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Sarisha Lohano
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Analia S Loria Kinsey
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Hong Lu
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Diego Lucero
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Mbombu Lukusa
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Andrew Mallamaci
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Kimberly Massengill
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Garrison McMillian
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Nicholas McVay
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Areli Medina Hernandez
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Austin Minton
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Sandra Miranda
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Edward Teutla Acosta
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Sathya Velmurugan
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Hannah Vorndran
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Sydney Walker
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Yugui Wang
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Austin Wellette-Hunsucker
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Jonathan Wenk
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Elise Westhafer
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Cardiovascular Research Day 2027
Attendees

Rita Gholizadeh Kojidi
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Jason Gill
Gill Foundation of Texas

Ming Gong
University of Kentucky

Scott Gordon
University of Kentucky

Elizabeth Gordon
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David Graf
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Gregory Graf
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Tanya Graf
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Scout Grimes
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Utku Gulbulak
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Zhenheng Guo
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Ling Guo
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Jessica Moorleghen
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Karl Muonio
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Maggie Murphy
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Courtney Murray
University of Kentucky

Shrishti Naidu
University of Kentucky

Evan Neary
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Katie Neglia
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Marzieh Nemati
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Raj Neupane
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Lara Westhafer
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Congqing Wu
University of Kentucky

Xu Xiao
University of Kentucky

Jimmy Xue
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Dien Ye
University of Kentucky

Mindy Yin
University of Kentucky

Qianru Yong
University of Kentucky

Shinichiro Yoshino
University of Kentucky

Kaiwen Zheng
University of Kentucky

Liyuan Zhu
University of Kentucky

Qingzhang Zhu
University of Kentucky

Abstracts

Prenatal fentanyl exposure induces an opioid-deficient state that results in a pro-hypertensive phenotype reverted by fentanyl re-exposure.

Percell T. Kendrick Jr., Mary Christina Obeng, Analia S. Loria

Department of Pharmacology and Nutritional Sciences. University of Kentucky.

Fentanyl is a highly addictive synthetic mu-opioid receptor agonist that exerts cardiorespiratory depression by beta-arrestin signaling. Fentanyl misuse is a severe public health crisis associated with cardiovascular comorbidities such as hypertension, atherosclerosis, vascular stiffness. Increased number of cases of substance use in pregnant women raised concerns about increased risk for cognitive, anxiety and depressive disorders in their offspring. However, little is known about the effect of transplacental fentanyl in the programming of cardiovascular disease. Our lab has shown that offspring with prenatal fentanyl exposure (PFE) display increased sympathetic drive, increased plasma and urinary norepinephrine, reduced heart rate variability (HRV), and endothelial dysfunction, resulting in a pro-hypertensive phenotype. Notably, we showed that PFE also produces persistent dysregulation of the endogenous opioid system, comprised by reduced endogenous opioid peptides. Thus, this study investigated the effect of fentanyl re-exposure in blood pressure and vascular function in females rats with PFE.

Adult female Sprague-Dawley rats with PFE or vehicle exposure were implanted with radiotelemetry and jugular vein catheter for fentanyl self-administration (SA). After recovery, rats were trained to self-administer fentanyl for 2 hours daily under a fixed-ratio 1 schedule. Blood pressure was continuously recorded during the whole experiment. At the endpoint, thoracic aortas were harvested, aortic rings were carefully cleaned from perivascular fat, and ex vivo endothelial function was evaluated using wire myography to perform cumulative concentration-response curves with Acetylcholine (ACh), and endothelium-dependent vasorelaxant.

After 14 days of fentanyl-SA, female PFE rats displayed a significant greater consumption. In addition, there was a significant reduction in blood pressure and HRV. Also, there was a rightward shift in the ACh-induced relaxation curve suggesting a better sensitivity; however, maximal relaxation was similar between PFE and vehicle exposed rats. Yet, the correlation between fentanyl consumption and maximal relaxation was positive only in PFE rats ($p<0.05$).

Changes in fentanyl consumption or endothelial function were not found in male offspring. However, further studies are needed to understand whether there are any sex specific implications of PFE influencing the neurogenic control of blood pressure.

Taken together, this data suggests that PFE may have reprogrammed the endogenous opioid system, so that adult cardiovascular regulation becomes abnormally dependent on opioid signaling, at least in female rats. Thus, testing the use of a non-beta arrestin-biased mu opioid receptor agonist may be a safer and more effective approach to restore the inhibition of the sympathetic-mediated pro-hypertensive phenotype in offspring with PFE.

Cold exposure regulates angiotensin II-induced aortopathies in a region-specific manner

Lisa A. Cassis, Charles M. Ensor, Victoria L. English, Seth Brunner, Shekinah Alfaro, Heba Ali, Seth Gosser, Yasir AlSiraj

Department of Pharmacology and Nutritional Sciences, College of Medicine, University of Kentucky, Lexington, KY

Background/ Objectives

We demonstrated previously that serotonin (5HT) 3 receptors (5HT3R) contribute to the formation and severity of angiotensin II (AngII)-induced aortopathies. Moreover, localization of 5HT and serotonin 3a receptors (*Htr3a*) to periaortic fat (PAF) surrounding the aorta demonstrated marked regional differences between thoracic and abdominal PAF in expression of the serotonergic system. Specifically, abdominal PAF (APAF) exhibited 41-fold higher *Htr3a* mRNA abundance than thoracic PAF (TPAF). This was of interest as APAF is predominately white adipocytes, while TPAF is primarily brown adipocytes, involved in temperature regulation and non-shivering thermogenesis. Lower environmental temperatures have been associated with increased incidence of aortic dissection, and cold exposures activate non-shivering thermogenesis through stimulation of brown fat thermogenesis. In this study, we examined effects of short-term (7 days) versus long-term (18 days) of cold exposures (8°C) on regional AngII-induced aortopathies of Western diet-fed low density lipoprotein receptor (*Ldlr*) deficient male mice.

Methods/Results

AngII-infused male mice housed at thermoneutrality (30°C) conditions were controls. To define the role of 5HT and 5HT3R, we included groups of cold-exposed AngII-infused mice administered Tropisetron (Tropi, 20 mg/kg, subQ). In AngII-infused mice exposed to 8°C versus 30°C for 18 days, mRNA abundance of Htr3a was reduced by cold exposures in APAF, but not in TPAF. Cold exposure (7 or 18 days) resulted in a marked increase in mRNA abundance of uncoupling protein 1 (*UCP1*), integral to non-shivering thermogenesis, in both APAF and TPAF of AngII-infused (750 ng/kg/min) mice. Notably, Tropi reduced cold-induced elevations of *UCP1* mRNA abundance in APAF, but not in TPAF. These effects of Tropi on *UCP1* mRNA abundance of APAF of cold-exposed mice were not present in subcutaneous white adipose tissue. Thoracic aortic maximal external diameters of AngII-infused mice were significantly increased by long-term (18 days), but not short-term (7 days) cold-exposure. Cold-induced increases in thoracic external diameters of AngII-infused mice were abolished by Tropi. Surprisingly, AngII-induced abdominal aortopathies were less prevalent and aortic rupture was reduced in mice exposed to cold (7 or 18 days). Moreover, Tropi markedly reduced AngII-induced abdominal aortopathies and prevented aortic rupture at both temperatures.

Conclusions

These results suggest that regional regulation of TPAF and APAF by cold exposure influences regional AngII-induced aortopathies. Moreover, these regional differences in PAF regulation by cold exposure were influenced by Tropi, a 5HT3R antagonist, which was protective against AngII-induced thoracic and abdominal aortopathies. Our results suggest that cold-induced thermogenic remodeling of TPAF through cold exposures may augment thoracic aortopathies, while potential browning of white APAF may be protective against AngII-induced aortopathies.

Studies were supported through NIH HL168633.

Intraluminal Thrombus Growth, Platelet Activation, and Progression of Abdominal Aortic Aneurysms

Tyler W. Benson, Ph.D., Anthony Spuzzillo, Ph.D., Michelle Nieman, Sydney Sharon, Leona Ciarlariello, Ava Hubbs, Anna Jaimes, Aaron Burton, A. Phillip Owens III, Ph.D.

Division of Cardiovascular Health and Disease, Department of Internal Medicine, University of Cincinnati

Introduction: Abdominal aortic aneurysm (AAA) is a progressive infrarenal aortic dilation accounting for >167,200 deaths annually. In ~80% of AAA patients, a platelet laden intraluminal thrombus (ILT) forms in the aneurysm. While thrombosis may stabilize the aorta initially, progressive ILT accumulation is positively associated with aortic rupture, but a causal relationship has not yet been established. Platelet activation is linked to faster AAA growth and anti-platelet therapy shows pre-clinical benefit, yet no pharmacologic therapies are approved for AAA patients. Here, we characterize the temporal relationship between aneurysm growth and ILT formation in a murine model featuring ILT and investigate the impact on platelet biology.

Methods and Results: Male *C57BL/6J* mice (10-14 weeks) received 0.2% BAPN in drinking water for 1 week before laparotomy, when 10mg/mL elastase (EI+B, n=13) or heat inactivated elastase (HI+B, n=5) was applied to the infrarenal aorta. BAPN water was maintained throughout the study. Bi-weekly 3D color Doppler imaging (VisualSonics Vevo F2) showed severe aortic dilation with disturbed and stagnant blood flow at the elastase-treated segment. ILTs formed distal to perturbed blood flow in 3/13, 9/13 and 12/13 mice at 2-, 4-, and 8-weeks post laparotomy respectively. Aneurysms with ILT were larger and grew faster. After 8 weeks, mice were euthanized and aneurysms were OCT-embedded, serially sectioned, and stained for CD41-platelets and αSMA-VSMCs confirming ILT formation with marked platelet luminal platelet accumulation. Moreover, EI+B mice had significantly elevated circulating glycoprotein VI compared to Hi+B mice consistent with increased platelet activation. Next, in a new cohort of EI+B mice, we collected serial retroorbital blood draws for complete blood counts paired with functional platelet assays and ultrasonic measurements of the ILT containing aneurysms. We identified a significant positive association between ILT expansion and decreased platelet count. Platelets isolated from EI+B mice had significantly diminished activation in response to agonism compared to platelets isolated from Hi+B mice.

Conclusions: In this ILT murine AAA model, progressive thrombus formation parallels accelerated aneurysm growth and precedes rupture. Our results indicate that platelet consumption at the ILT perturbs systemic platelet counts and diminishes platelet function.

GLP-1–Based Incretin Therapies Across HFpEF and HFrEF: A metanalysis of clinical outcomes.

Sandra Bakare MBBS **Affiliation:** Research Junction Institute, Ohio, USA.

Introduction

GLP-1–based incretin therapies are increasingly used in cardiometabolic disease, but their clinical effects may differ across heart failure phenotypes. We assessed cardiovascular death, heart failure hospitalization, and the composite of cardiovascular death or heart failure events in HFpEF and HFrEF, with severe adverse events evaluated as a secondary safety outcome.

Methods

We searched PubMed, Web of Science, the Cochrane Library, and ClinicalTrials.gov for eligible studies evaluating GLP-1–based incretin therapies in patients with heart failure. The review followed the **PRISMA 2020 guidelines** and was prospectively registered with **PROSPERO** [CRD420261412017]. Ten studies were included. Risk ratios were pooled for binary event outcomes and hazard ratios for time-to-event composite outcomes. Random-effects models using restricted maximum-likelihood estimation with Hartung-Knapp confidence intervals were used. Analyses were stratified by HFpEF and HFrEF, with tests for subgroup differences. Sensitivity analyses were performed for the phenotype-specific pooled estimates.

Results

GLP-1–based incretin therapy was associated with a lower risk of cardiovascular death in the overall heart failure population (RR 0.78, 95% CI 0.66–0.93; I^2=0%). The overall effect on heart failure hospitalization was not statistically significant (RR 0.87, 95% CI 0.68–1.10; I^2=51.4%). Likewise, the pooled effect for the composite of cardiovascular death or heart failure events was not statistically significant overall (HR 0.79, 95% CI 0.61–1.03; I^2=55.1%). In phenotype-specific analyses, cardiovascular death did not differ significantly between HFpEF and HFrEF (P for subgroup difference=0.154). Heart failure hospitalization showed a significant difference by phenotype, with a more favorable effect in HFpEF than HFrEF (P for subgroup difference=0.001), and this finding remained significant in sensitivity analysis. The composite of cardiovascular death or heart failure events was significantly reduced in HFpEF (HR 0.68, 95% CI 0.55–0.84) but not in HFrEF (HR 0.98, 95% CI 0.68–1.39), with a significant subgroup difference (P=0.008), which remained significant in sensitivity analysis. Severe adverse events were not significantly different overall (RR 0.78, 95% CI 0.49–1.24), although heterogeneity was substantial (I^2=82.5%).

Conclusion

The clinical effects of GLP-1–based incretin therapies appear to differ across heart failure phenotypes. Cardiovascular death was reduced overall, while the clearest phenotype-specific benefit was seen in HFpEF for heart failure hospitalization and the composite of cardiovascular death or heart failure events. These findings suggest that the effects of GLP-1–based therapies in heart failure may not be uniform across HFpEF and HFrEF and support further phenotype-specific trials, particularly in HFrEF.

Liver-Specific Overexpression of Serum Amyloid A Promotes Chronic Kidney Injury and Fibrosis

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Objectives: Serum amyloid A (SAA) is an acute-phase protein and mediator of kidney injury. Chronic SAA elevation is a hallmark of persistent inflammation, with the liver as its primary source. This study aimed to determine whether sustained liver-derived serum amyloid A (SAA) expression drives renal injury and to define the structural and pathological alterations in the kidney associated with chronic SAA elevation.

Methods: All mice were on an apolipoprotein E-deficient ($ApoE^{-/-}$) background and included three groups: SAA-WT (normal endogenous SAA), SAA-KO (SAA-deficient), and SAA-Liv (SAA-KO with doxycycline-inducible, liver-specific SAA expression). Mice received standard chow and doxycycline (1 mg/mL drinking water) for 8 weeks. Plasma SAA and creatinine were measured at endpoint. Kidney tissues were assessed by H&E, Congo Red, and Masson's trichrome staining, with pathological scoring for injury and chronic inflammation. Renal inflammatory and fibrotic gene expressions were analyzed by qPCR.

Results: Doxycycline induced robust hepatic SAA expression and markedly increased plasma SAA in SAA-Liv mice. Plasma creatinine was significantly higher in SAA-Liv than SAA-KO mice, indicating impaired kidney function. Masson's trichrome staining revealed extensive fibrosis in SAA-Liv mice compared with SAA-WT and SAA-KO controls. H&E staining showed severe tubular dilation and atrophy, Bowman's capsule thickening, and capsular space collapse. Pathological scoring showed mean scores of 2.6 for both tubular dilation/flattening and interstitial chronic inflammation in SAA-Liv mice, corresponding to involvement of approximately 34–66% of renal tubules and interstitium, versus 0 in both control groups. These abnormalities were accompanied by increased expression of fibrotic markers (COL1A1, COL3A1), inflammatory and NLRP3 pathway markers (IL-1β, TGF-β, TNF-α, NLRP3), and mesenchymal markers (α-SMA, vimentin, SNAI1). E-cadherin was also upregulated, suggesting partial epithelial-to-mesenchymal transition (pEMT) rather than complete phenotypic conversion. Congo Red staining demonstrated development of AA amyloid deposits.

Conclusions: Liver-specific SAA overexpression promotes kidney dysfunction and structural injury characterized by AA amyloid deposition, tubular damage, inflammation, and extensive fibrosis with pEMT. These findings identify liver-derived SAA as a potential mediator of chronic kidney injury and suggest that SAA-driven renal fibrosis may involve inflammation and the NLRP3 inflammasome activation.

Funding sources: This work was supported by the National Institutes of Health Grants HL147381 (to PS) and HL173026 (to PS) and the start-up fundings from Barnstable Brown Diabetes Center and Internal Medicine of University of Kentucky (to PS).

Same Platelet, Different Disease: The Circulating Platelet Transcriptome Encodes an Insult-Specific Fingerprint Across Cardiovascular and Metabolic Disease

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Background. Platelets are anucleate yet carry a rich, dynamic transcriptome that is increasingly recognized as a readout of systemic disease. Antiplatelet therapy, however, is applied largely uniformly across indications, and it remains unknown whether mechanistically different systemic insults elicit a shared “stressed-platelet” program or distinct, disease-specific signatures. Resolving this determines whether platelets can serve as a disease-resolving “liquid biopsy” and whether antiplatelet strategy should be matched to the underlying etiology.

Methods. We compared circulating platelet bulk RNA-sequencing across three mechanistically distinct mouse models of systemic stress: PCSK9 gain-of-function/Western-diet hypercholesterolemia (PCSK9), angiotensin II-induced abdominal aortic aneurysm (AngII), and polygenic metabolic syndrome with steatohepatitis (MS-NASH), processed through a single unified pipeline. Differential expression used a nominal $p<0.05$ threshold; baseline comparability of control groups was assessed by principal component analysis (PCA), and cross-experiment batch effects were addressed with a limma-style linear-model correction. Directional concordance was quantified across all conditions, and pathway-level responses were profiled by pre-ranked gene-set enrichment analysis (GSEA) against curated mouse hallmark and platelet-specific gene sets.

Results. All three insults extensively remodeled the platelet transcriptome, to markedly different magnitudes (PCSK9, 1,104 DEGs [547 up/557 down]; AngII, 997 [550/447]; MS-NASH, 4,128 [2,205/1,923]). Despite this, the responses were overwhelmingly insult-specific: across all three conditions no gene was concordantly upregulated and only three were concordantly downregulated (*Hgsnat, Gpm6b, Ap1s2*). Counterintuitively, the two vascular insults, PCSK9 and AngII, shared the fewest concordant genes of any pair (18), whereas the two lipid/metabolic-linked models shared the most (PCSK9∩MS-NASH, 139; AngII∩MS-NASH, 136), showing that the limited cross-disease overlap tracks a metabolic rather than a shared vascular-activation axis. GSEA reinforced this divergence: AngII enriched coagulation, complement, and platelet-activation programs, PCSK9 was defined by lipid-metabolism enrichment, and the models were directionally opposed on oxidative phosphorylation (enriched in AngII, depleted in PCSK9). PCA further confirmed that control groups were transcriptionally non-equivalent, separating by experiment of origin (PC1, 38.4%).

Discussion / Conclusion. The circulating platelet transcriptome is reprogrammed in a disease-specific manner rather than through a generic stress response, with a negligible shared signature across cardiovascular and metabolic insults. These data overturn the intuitive assumption that the two vascular models would converge, and instead reveal a metabolic axis as the dominant source of cross-disease similarity. This reframes platelets as a disease-resolving liquid biopsy and argues that antiplatelet strategy, long applied uniformly, may need to be matched to disease etiology. Our head-to-head framework provides a template for reading systemic disease state from a single, accessible cell.

Title: The Impact of Intestinal Carnitine PalmitoyItransferase 1a (Cpt1a) deficiency and overexpression on the development of Obesity Phenotypes in Response to High Fat Diet

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

Background: Cpt1a esterifies long chain fatty acids to carnitine allowing entry into mitochondria, β-oxidation, and the production of cellular energy. Cpt1a is considered the liver isoform, but transcript abundance is greater throughout the intestinal tract in both mice and humans than that of the liver. 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 and over expression in the development of metabolic phenotypes in response to a high fat diet.

Methods: Eight-week-old male and female Control, intestinal-specific knockout Cpt1a-deficient mice ($Cpt1a^{IKO}$), and intestinal-specific overexpression ($CPT1a^{ITG}$) mice were fed high fat 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 mice in either mouse model, but $Cpt1a^{IKO}$ females were modestly protected from diet-induced obesity. Fasting glucose and HOMA-IR values suggested improved insulin sensitivity irrespective of sex, but glucose tolerance did not differ between genotypes. An increase in fasting triglycerides was observed in $CPT1a^{IKO}$ in males and females, but not in $CPT1a^{ITG}$ mice. Total plasma cholesterol was unaffected in either model. Elevated levels of B-hydroxybutyrate was observed in cpt1a deficient mice.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 phenotypes in female, but not male mice. Improvements in insulin sensitivity and the increased plasma TGs following ingestion of fat suggest that the loss of CPT1a diverts dietary fat from oxidative metabolism in the gut and promotes lipid absorption.

Galactolipids Regulate Systemic Immunity in Plants

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Abstract

Background: Systemic acquired resistance (SAR) is a unique form of long-lasting systemic immunity that provides protection against a broad-spectrum of pathogens in plants. SAR involves the generation of a mobile signal at the primary infected site, which upon translocation to the distal tissues, prepares the plant to better resist future infections. Multiple, chemically diverse SAR inducers that have been identified to-date. The phosphorylated sugar derivative, glycerol-3-phosphate (G3P) and C9 dicarboxylic acid, azelaic acid (AzA) are two such SAR inducers. AzA, which acts upstream of G3P and confers SAR by increasing G3P levels, is generated upon the hydrolysis of C18 unsaturated fatty acids present specifically on the galacatolipids, mono- and di-galacaltosyl-diacylglycerol (MGDG and DGDG). MGDG and DGDG are major components of chloroplast membranes and therefore the most abundant lipids in plants.

Methods and Results: SAR was assayed in *Arabidopsis thaliana* infected with *Pseudomonas syringae* pv. *tomato* (Pst) DC3000, and SAR signals were quantified using high performance liquid chromatography mass spectrometry (UHPLC-MS). The mutants deficient in MGDG (*mgd1*) or DGDG (*dgd1*) synthesis, are impaired in SAR. In addition, the *dgd1* mutant is defective in gene expression responsive to another important SAR activator, salicylic acid (SA). Notably, transgenic expression of a bacterial glucosyltransferase (GT) in the *dgd1* background, which introduces glucose instead of galactose into the membrane lipids partially restores photosynthetic functions, but not SAR functions. Using genetics, biochemical and molecular analyses, my work further elucidates the importance of the DGDG galactolipid in modulating various aspects of the plant's systemic immune response including generation and systemic movement of multiple SAR inducers.

Conclusions: Galactolipids are important for the establishment of SAR. The *dgd1* plants can generate SAR signals but are unable to perceive them. The *dgd1* plants are impaired in transport of SA, which is possibly associated with cuticle.

Funding: This study is funded by National Science Foundation and conducted in collaboration with the Center for Agricultural and Life Sciences Metabolomics (CALM).

Spontaneous Inferior Mesenteric Artery Occlusion after Endovascular Aneurysm Repair for Abdominal Aortic Aneurysm and its Impact on Clinical Outcomes

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Abstract

Background: The incidence, associated factors, and clinical implications of spontaneous occlusion of a patent inferior mesenteric artery (IMA) after endovascular aneurysm repair (EVAR) for abdominal aortic aneurysm (AAA), in the absence of pre-emptive embolization, remain unclear. This study aimed to determine the incidence, clinical implications, and predictors of spontaneous IMA occlusion after EVAR.

Methods: This single-center, retrospective cohort study included patients who underwent elective EVAR between 2007 and 2022. Patients were categorized into three groups: group 1, spontaneous IMA occlusion; group 2, patent IMA without type II endoleak (T2EL) from the IMA; and group 3, T2EL from the IMA. Endpoints included the incidence of spontaneous IMA occlusion, sac enlargement, freedom from re-intervention, and overall survival after EVAR.

Results: Among 372 elective EVAR cases for AAA, 230 patients with a patent preoperative IMA were analyzed after excluding 127 patients with pre-occluded IMA and 15 who underwent pre-emptive IMA embolization. Spontaneous IMA occlusion occurred in 101 patients (44%). The rate of sac enlargement was lower in group 1 than in groups 2 and 3. Freedom from re-intervention was higher in group 1 than in group 3 but did not differ between groups 1 and 2. Multivariable analysis identified absence of antiplatelet therapy, higher preoperative hematocrit, absence of concomitant iliac artery aneurysm, posterior thrombus in the aneurysm sac, and use of the Endurant device as predictors associated with spontaneous IMA occlusion. Spontaneous IMA occlusion occurred in 7% of patients with no predictors and in 78% of patients with four or five predictors.

Conclusions: Spontaneous IMA occlusion occurred in nearly half of patients and was associated with favorable clinical outcomes. In patients with a high predicted likelihood of spontaneous IMA occlusion, pre-emptive IMA embolization may be omitted.

Keywords: Abdominal aortic aneurysm, endovascular aneurysm repair, pre-emptive inferior mesenteric artery embolization, spontaneous inferior mesenteric artery occlusion, type II endoleak.

The Effect of Poncirin on Platelet Function

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Background: Cardiovascular diseases (CVDs) including myocardial infarction (MI) and stroke claim one in four lives, making CVDs the leading cause of death worldwide. Platelets are central to the pathology of these events; their activation and the subsequent release of their granule cargo is essential for thrombosis and vascular inflammation. While current anti-platelet therapies prevent clotting, they carry a high risk of bleeding. Poncirin (PCN), a potent flavonoid found in oranges, is known for its anti-inflammatory properties and may offer a safer alternative.

Objective: This study investigates the effects of PCN on platelet function in vitro.

Methods: Platelet aggregation, ATP secretion, clot retraction and intracellular signaling were monitored to evaluate the impact of PCN on platelet function and understand their underlying mechanism.

Results: In washed platelets, preincubation with PCN (100 uM) aggregation decreased by approximately 20%, and reduced ATP secretion by 50%. Similar trends were observed at lower concentrations of PCN treatment. Interestingly, PCN did not affect aggregation or ATP release post stimulation by thrombin (0.1U/mL), suggesting PCN preferentially affects GPVI-mediated pathways over GPCR-mediated signaling. These results were further validated by ATP—luminescence-based assays. Additionally, clot contraction was delayed upon treatment with PCN (200 uM), suggesting potential platelet surface integrin or cytoskeletal defects.

Conclusion and Future Directions: Our results suggest that PCN inhibits platelet aggregation and ATP secretion through GPVI-mediated pathway. It is not yet clear if the secretion from alpha granules is also affected. Studies will be conducted to understand mechanistic details of PCN's effects on platelets and how it affects in vivo hemostasis.

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Asprosin regulates autonomic tone via oxytocinergic-Ptprd signalling

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Metabolic syndrome is a major driver of cardiovascular disease, characterized in part by dysregulated autonomic balance. Asprosin, a newly identified fasting-induced adipokine, is elevated in metabolic syndrome and has been implicated in the central regulation of autonomic tone. We have recently demonstrated that oxytocin neurons in the paraventricular nucleus of the hypothalamus (PVN) express Protein Tyrosine Phosphatase Receptor Type D (Ptprd), the high-affinity neural receptor for asprosin. Mice that are deficient in asprosin display hypotension as measured by tail cuff, and genetic ablation of Ptprd in oxytocin neurons (*Oxytocin* cre^{+}; *Ptprd*$^{fl/fl}$; OT-Ptprd KO) also decreases blood pressure. Therefore, we evaluated asprosin's effects on autonomic regulation of heart rate and blood pressure by administering autonomic tone-targeting drugs in the OT-Ptprd KO mouse model.
OT-Ptprd KO and oxytocin-cre^{+} control mice were implanted with radiotelemeters in order to monitor blood pressure and heart rate in conscious, freely-moving mice. Low blood pressure in OT-Ptprd KO mice was confirmed using this gold-standard measure. Additionally, spectral analysis of heart rate variability revealed that OT-Ptprd KO mice have altered autonomic tone (iwith increased LFSP (low Frequency Systolic Pressure), with increased parasympathetic tone [HFSP (High frequency Systolic Pressure) and root mean square of successive differences (rMSSD)] and decreased sympathetic tone (decreased urinary norepinephrine). No differences were found in sympathetic or parasympathetic regulation of heart rate, or intrinsic heart rate as determined after IP injection with propanalol, atropine or propanalol + atropine. However, the magnitude of dip in blood pressure with Prazosin was significantly lower in OT-Ptprd KO, than in the littermate controls, indicating decreased alpha-adrenergic receptor mediated sympathetic tone in mice with OT specific ablation of Ptprd. Taken together, this data indicates the autonomic tone regulatory function of the oxytocin specific asprosin-Ptprd signaling.

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NBEAL2 Deficiency Drives Early Abdominal Aortic Aneurysm Rupture in Males and Overrides Female Protection

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Background: Platelets form aneurysmal thrombi at the diseased aortic wall, where activation-dependent α-granule release is proposed to affect abdominal aortic aneurysm (AAA). NBEAL2 is required for α-granule biogenesis; mutations in *NBEAL2* cause Grey Platelet Syndrome (GPS; OMIM 139090), in which platelet α-granules lack ~90% of their soluble cargo but retain their membrane proteins, resulting in attenuated hemostasis. We tested whether NBEAL2 loss alters AAA outcomes in a hypercholesterolemic/angiotensin II (AngII) induced model.

Methods and Results: Male and female wildtype (WT) and *Nbeal2*$^{-/-}$ mice received a PCSK9 gain-of-function AAV injection followed by Western diet feeding and were infused with AngII for 28 days. Body weight and plasma cholesterol were comparable between genotypes in both sexes, with cholesterol was markedly elevated throughout the study. Hematology confirmed the predicted macrothrombocytopenia in *Nbeal2*$^{-/-}$ mice, regardless of treatment. Loss of NBEAL2 profoundly augmented aneurysm pathology, manifesting as a marked increase in rupture. Male *Nbeal2*$^{-/-}$ mice exhibited ~90% mortality (10/11), with most deaths occurring within the first 10 days of AngII infusion, indicating early aneurysm destabilization. In contrast, WT males showed substantially lower mortality over the same period (4/13). Notably, NBEAL2 deficiency unmasked susceptibility in females, a group typically protected from AAA. While WT females maintained complete survival, *Nbeal2*$^{-/-}$ females showed ~40% mortality (5/12) by day 28 and a delayed onset of rupture events, relative to males. This shift to vulnerability indicates that loss of platelet α-granule cargo directly negates sex-based protection. Under basal saline infused conditions, *ex vivo* aortic diameters were similar between genotypes, demonstrating that loss of NBEAL2 may amplify pathological instability.

Conclusion: NBEAL2-dependent platelet α-granule biogenesis protects against AngII-induced aneurysm rupture in both sexes, supporting a stabilizing role for platelet granule cargo in aneurysm containment.

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Title: The Role of Metabolic Plasticity and Mitochondrial Bioenergetics in Platelet Clot Contraction

Background: Platelets appear reliant on bioenergetic flexibility for their hemostatic functions; however, the extent of this flexibility and the sources of the relevant fuels are unclear. Historic, *in vitro* experimental data suggest resting platelets use both glycolysis and oxidative phosphorylation (OxPhos), both of which are increased upon activation. Initial activation steps (adhesion, spreading, and aggregation) appear to be fueled predominantly by glycolysis, while later steps (clot contraction) are more reliant on OxPhos for sustained ATP levels. Diseases with defective platelet energy metabolism, *e.g.,* Glycogen Storage Diseases, do affect thrombosis. Additionally, anucleate platelets can be affected by mitochondrial health. The objective of this project is to assess the impact of compromised mitochondrial function on platelet bioenergetics and function and to define the contributions of various energy fuel sources and their metabolic pathways to clot contraction.
Methods: Platelets were prepared from wildtype (WT) and platelet-specific mice with deletions in Transcription Factor A (TFAM; PF4-Cre^{+}::TFAM$^{flox/flox}$), a key regulator of mtDNA, and Q-binding protein (QPC; PF4-Cre^{+}::QPC$^{flox/flox}$), a subunit of mitochondrial Complex III. Their ability to form and contract clots in response to 5 or 20 μU/mL thrombin was measured by digital recording; contraction curves were generated and analyzed to assess kinetic parameters (*i.e.,* lag time, maximal contraction rate, and extent). Specific inhibitors (*i.e.,* 2-Deoxy-D-glucose (2DG); OxPhos cocktail (OxPC): rotenone, antimycin, and oligomycin; etomoxir) and discrete metabolic fuels (*i.e.,* pyruvate, glucose, and palmitate) were also added to the reactions to assess the specific roles of different pathways. Hemostatic potential was assessed in these mice using standard assays: tail-bleeding and $FeCl_3$ injury.
Results: Inhibiting glycolysis/OxPhos with 2DG robustly impaired contraction, reducing the average velocity and the overall extent of contraction. This was reversed with added pyruvate or excess glucose. OxPC impaired clot contraction to a lesser extent but could only be reversed by the addition of glucose. When used together, 2DG and OxPC completely inhibited contraction and could not be reversed with either glucose or pyruvate. These data show the dual importance of glycolysis and OxPhos to platelet function. Blocking fatty acid β-oxidation with etomoxir had a modest effect on contraction regardless of glucose addition. When glucose is limited, palmitate restored platelet clot contraction, indicating that platelet can use exogenous fatty acids as an alternative energy source to support clot contraction. Platelets from both mitochondria defective models showed impaired clot contraction *in vitro*. *In vivo*, mice from both strains displayed impaired hemostasis in the tail-bleeding injury and impaired occlusive thrombosis in the carotid $FeCl_3$-injury model.
Conclusion: Our data highlights the importance of functional mitochondria to platelet function by demonstrating the metabolic flexibility of platelet fuel usage and the role that mitochondria play to sustain the more energy-requiring aspects of thrombosis, *e.g.,* clot contraction.

TTN truncating variants show reduced transcript expression with limited phenotypic separation in human non-ischemic cardiomyopathy

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Background

Truncating variants in *TTN* (TTNtvs) are a major genetic contributor to non-ischemic cardiomyopathy (NICM), but their molecular and functional consequences in end-stage disease remain incompletely defined. TTNtvs may be processed at the RNA level, potentially limiting proteostatic burden. Leveraging a cardiac biobank containing more than 30,000 specimens from 800 patients, we tested whether TTNtv-associated changes in transcript expression extend to titin abundance, myocardial remodeling, mechanics, and clinical function in human NICM.

Methods

The study included myocardium from three experimental groups (n=8/group): (i) organ donors, (ii) patients with NICM who do not harbor a TTNtv, and (iii) patients with NICM who harbor a TTNtv. Whole-exome sequencing was used to identify TTNtvs, which were annotated by exon, amino acid position, and sarcomeric region. Bulk RNA sequencing and proteomics were used to quantify *TTN* transcript expression and titin abundance. Sudan black B and picrosirius red staining were used to quantify lipofuscin and fibrosis accumulation, respectively. Agarose gel electrophoresis was used to assess titin isoform composition. Global troponin I and site-specific myosin-binding protein C phosphorylation were evaluated with Western blotting. Multicellular mechanics were used to measure force-pCa relationships. Echocardiographic and invasive hemodynamic measurements from matched clinical data were used to evaluate cardiac structure and function.

Results

TTN transcript expression differed among groups ($p<0.05$), with lower expression in NICM TTNtv myocardium compared with both donor and NICM myocardium (both $p<0.05$). In contrast, titin protein abundance was preserved across groups ($p=0.39$). Titin isoform composition and global troponin I phosphorylation did not differ among groups (both $p>0.10$). Myosin-binding protein C phosphorylation at Ser273 was lower in both NICM groups compared with donors (both $p<0.01$), whereas phosphorylation at Ser282 and Ser302 was similar (all $p>0.06$). Lipofuscin accumulation was higher in NICM myocardium compared with donors ($p<0.01$), but fibrosis was comparable ($p=0.26$). Multicellular mechanics demonstrated a sarcomere length-dependent shift in calcium sensitivity in donor myocardium ($p<0.01$), which was absent in both NICM groups (both $p>0.05$). Myofilament cooperativity and maximum active tension showed no group- or sarcomere length-dependent differences (all $p>0.06$). Clinically, ejection fraction was lower in both NICM groups compared with donors (both $p<0.01$), while left-ventricular mass index was highest in NICM myocardium (both $p<0.05$).

Conclusions

TTNtvs were associated with lower *TTN* transcript expression without a concordant shift in titin protein abundance, suggesting buffering between transcript and protein levels. Despite this molecular difference, myocardium from patients with NICM harboring a TTNtv did not exhibit a distinct downstream profile in titin isoform composition, myofilament phosphorylation, fibrosis, or multicellular mechanics. Lower myosin-binding protein C Ser273 phosphorylation and loss of sarcomere length-dependent calcium sensitization were shared across NICM groups, suggesting these alterations reflect disease state rather than TTNtv-carrier status. Together, these findings indicate that TTNtvs produce a detectable transcript-level signature but limited phenotypic separation within end-stage NICM.

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Adipocyte deletion of tryptophan hydroxylase 1 augments angiotensin II-induced abdominal aortopathies of male mice.

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Background/Objectives

Tryptophan hydroxylase 1 (Tph1) is the rate-limiting step in the synthesis of peripheral serotonin (5HT). 5HT exerts wide-spread physiological and pathophysiological functions through seven receptors, of which one, the serotonin 3 receptor (5HT3R), is an ion channel. Several peripheral cell types express Tph1 and thus can synthesize 5HT, including adipocytes, where adipocyte-derived 5HT has been linked to the regulation of energy expenditure and body weight. We demonstrated previously that antagonism of 5HT3R markedly reduced angiotensin II (AngII)-induced abdominal aortopathies (AAs). Moreover, results demonstrated immunolocalization of 5HT to abdominal periaortic adipose tissue surrounding an established AA, suggesting that adipocytes provide 5HT to act at local 5HT3R in the regulation of aortopathy formation and severity.

Methods/Results

To define the role of adipocyte-derived 5HT in AngII-induced AAs, we used transgenic mice with genetic deletion of *Tph1* in adipocytes. Male control (*Tph1*$^{fl/fl}$) and adipocyte (adiponectin-Cre) *Tph1* deleted (*Tph1*$^{Cre+}$) mice were administered tamoxifen (75 mg/kg/day for 5 days) to induce adiponectin-driven*Tph1* deletion in white and brown adipocytes, and then two weeks later administered adeno-associated virus PCSK9 to induce hypercholesterolemia. One week later, mice were fed Western diet for 7 days and throughout infusion of AngII (1,000 ng/kg/min for 28 days). Expression of *iCre* was detected in adipose tissue from *Tph1*$^{Cre+}$, but not *Tph1*$^{fl/fl}$ mice, and *Tph1* mRNA abundance was significantly reduced in epididymal white adipose tissue of *Tph1*$^{Cre+}$ mice. Body weight, systolic blood pressure, and total serum cholesterol concentrations were not significantly different between genotypes. Abdominal aortic internal lumen diameters, measured by ultrasound, were significantly increased over time of AngII infusion in both genotypes. However, abdominal aortic lumen diameters were significantly increased in adipocyte *Tph1* deficient mice compared to control (day 27: *Tph1*$^{fl/fl}$, 1.31 ± 0.07; *Tph1*$^{Cre+}$, 1.76 ± 0.23 mm; p = 0.03). Maximal abdominal aortic external diameters of *Tph1*$^{Cre+}$ mice were modestly, but not significantly increased compared to controls (*Tph1*$^{fl/fl}$: 1.31 ± 0.09; *Tph1*$^{Cre+}$, 1.70 ± 0.20; p = 0.08).

Conclusions

These results suggest that adipocyte-derived 5HT is protective against AngII-induced AAs. Future studies will define 5HT receptors responsible for protective, versus potential detrimental effects of 5HT on AngII-induced abdominal aortopathies.

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High-Fat Diet Triggers Fatal Immunothrombosis via IL-6–STAT3 Signaling in Mice with Impaired Reverse Cholesterol Transport

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Abstract:

Hypercholesterolemia is traditionally viewed as a chronic risk factor for cardiovascular disease. Whether disrupted cholesterol homeostasis can also trigger acute thromboinflammatory events remains unclear. We investigated this question using hepatocyte-specific scavenger receptor class B type I (SR-BI)-deficient mice, a model of impaired reverse cholesterol transport (RCT).

Following the Paigen diet challenge, liver SR-BI-KO mice developed hypercholesterolemia, and exhibited approximately 50% mortality within 7 days. Disease progression was characterized by dramatically decreased body temperature and physical activity. These were associated with hemolysis, thrombocytopenia, elevated levels of IL-6 and d-dimer. Histological analysis revealed extensive neutrophil accumulation, and occlusive thrombi within the pulmonary vasculature. Transcriptomic and western blot analyses identified robust activation of the IL-6–STAT3 signaling pathway in both lung and liver. This response promoted acute-phase protein production and increased circulating prothrombotic mediators, including fibrinogen. Importantly, blockade of IL-6 signaling, pharmacologic inhibition of STAT3, or neutralization of tissue factor markedly reduced thrombosis and prevented mortality.

These findings demonstrate that impaired RCT creates a previously unrecognized vulnerability in which acute dietary lipid overload triggers hemolysis, pulmonary immunothrombosis, and circulatory collapse. Our results identify the RCT–IL-6–STAT3 axis as a critical regulator of cholesterol-induced thromboinflammatory injury and a potential therapeutic target for preventing catastrophic vascular complications associated with disrupted cholesterol homeostasis.

Severe high-thoracic spinal cord injury disrupts acute autonomic function in female rats: cardiovascular, temperature, and activity implications

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Abstract

Spinal cord injury (SCI) disrupts supraspinal regulation of autonomic function, leading to cardiovascular and physiological dysregulation that may contribute to long-term morbidity. However, acute autonomic alterations following severe high- thoracic SCI remain poorly characterized. This study investigated changes in cardiovascular parameters, temperature, and activity during the first week after SCI. Telemetric probes were implanted in the descending aorta of adult female Wistar rats prior to SCI, and baseline recording of systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), heart rate (HR), temperature, and activity were collected for 48 h before injury. Two weeks after telemetry surgery, the rats received a T3 contusion SCI (400 kdyn, 5s dwell time), followed by continuous telemetric monitoring from 1 to 7 days post-injury. SCI significantly increased SBP, DBP, and MAP from 1 to 4 days post-injury. It also markedly reduced activity, induced tachycardia, altered heart rate variability, and caused temperature instability. In addition, SCI disrupted normal diurnal rhythms, particularly in activity, DBP, MAP, HR, and body temperature. These findings provide a comprehensive characterization of acute autonomic dysfunction following severe thoracic SCI and may help define therapeutic windows for future interventions aimed at improving long-term SCI outcomes.

Title: CD47 knockdown reprograms NK-Cell Immunometabolism under MASH-like conditions

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Abstract Body

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the hepatic component of a systemic cardiometabolic syndrome, affecting more than one-third of the global population and as many as 70% of individuals with type 2 diabetes. Its presence is associated with adverse cardiovascular outcomes. The progressive form, metabolic dysfunction-associated steatohepatitis (MASH), promotes fibrosis, cirrhosis, and hepatocellular carcinoma through interconnected metabolic, inflammatory, and fibrogenic pathways that share key features with those implicated in atherosclerotic cardiovascular disease. Natural killer (NK) cells make up as much as 50% of hepatic lymphocytes and constrain fibrosis by eliminating activated hepatic stellate cells, yet they become functionally exhausted under sustained metabolic stress. CD47, an immune checkpoint receptor upregulated on NK cells in MASH, is a candidate mediator of this dysfunction and a potential lever for restoring immune surveillance in the metabolically injured liver.

To test this hypothesis, we investigated whether CD47 regulates NK cell metabolism under MASH conditions and whether metabolic reprogramming restores its effector function. Splenic NK cells isolated from C57BL/6 mice were treated with control or CD47 antisense oligonucleotide to knock down CD47. These cells were exposed to MASH-mimicking media containing high concentrations of glucose, palmitate, and LPS. Cellular metabolism, gene expression, and effector function were assessed using Seahorse analysis, qPCR, flow cytometry, and cytotoxicity assays. We found that MASH conditions induced a hyperglycolytic shift in NK cells. CD47 knockdown suppressed this aberrant glycolysis while promoting a shift toward fatty acid oxidation. Using Hepa1-6 hepatoma cells as a target to gauge restored NK effector capacity, a flow cytometry-based killing assay further suggested enhanced cytotoxicity by CD47 ASO-treated NK cells under MASH conditions. Together, these findings identify CD47 as a regulator of NK cell immunometabolism and support its potential as a therapeutic target for restoring NK cell function in MASH. Ongoing studies aim to define the signaling pathways and molecular mechanisms underlying CD47-mediated metabolic reprogramming and enhanced cytotoxicity.

Platelet TGF-β1 and Fibroblast TGF-βRII Signaling Promote Aneurysm Stabilization in Experimental Abdominal Aortic Aneurysm

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BACKGROUND: Systemic inhibition of transforming growth factor beta (TGFβ) **signaling** via accelerates abdominal aortic aneurysm (AAA) progression and rupture in rodent models. However, the primary sources of protective TGFβ and the cell types mediating this effect remain unclear. As such, we hypothesized that platelets are an important source of protective TGFβ1 and that fibroblast activation via TGFβ receptor II (TGFβRII) is **required** for **aneurysm stabilization**.

METHODS AND RESULTS: Male 8-12 week-old *Ldlr*$^{-/-}$ mice with platelet-specific *Tgfβ1* deletion (Pf4-Cre) or littermate controls underwent angiotensin II (AngII)-induced AAA. After 28 days, ex vivo aortic diameter was significantly greater in mice lacking platelet *Tgfβ1*. These mice also exhibited reduced type I collagen deposition and increased macrophage infiltration compared to Cre- mice.

To investigate fibroblast-specific *Tgfβ* signaling, we generated Ldlr$^{-/-}$ mice with a tamoxifen-inducible *Pdgfrα* Cre-driven deletion of *TgfβRII* and a tdTomato reporter (*Ldlr*$^{-/-}$/*TgfβRII*$^{flox/flox}$*ROSA26-STOP*$^{flox/flox}$ *tdTomato+ PDGFRα*^{CreERT2}). Following tamoxifen feeding for two weeks, 8-12 week old *TgfβRII*$^{flox/flox}$ Cre+ and *TgfβRII*$^{wt/wt}$ Cre+ male mice underwent laparotomy and topical elastase application on their infrarenal aorta (10mg/mL for 5 minutes) and were followed for 4 weeks. Fibroblast-specific TGFβRII deficiency resulted in greater abdominal aortic dilation (P<0.001) and increased rupture (50% [6/12] vs. 0% [0/18] in TGFβRII-proficient mice). In healthy aortas, fibroblasts were primarily confined to the adventitia; after aneurysm induction, TGF-βRII–proficient mice exhibited fibroblast expansion and migration into the media, whereas TGF-βRII–deficient mice had significantly reduced fibroblast area. RNA sequencing 2 weeks after elastase treatment revealed that when compared with TGF-βRII–proficient mice, TGF-βRII–deficient mice had increased expression of oxidative phosphorylation and adipogenesis-associated genes and reduced expression of core matrisome genes.

CONCLUSIONS: Our results support prior literature showing that TGFβ signaling is protective in preclinical models of AAA. Platelet-derived TGFβ1 appears to account for a significant portion of protective TGFβ ligand and fibroblasts expansion and migration mediated by activation of TGFβRII appears to be an important step for aneurysm stabilization in the elastase model. The overall downregulation of core matrisome genes suggests a compromised extracellular matrix and vascular protection in TGFβRII deficient mice.

Title: Patients with Heart Failure and Impella Support Show Reduced Phosphorylation of Troponin I

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Background
Heart failure is a condition where the heart cannot meet the demand of the body's circulatory needs. In advanced heart failure, left-ventricular assist devices (LVADs) can be surgically implanted to guide blood from the left ventricle into the aorta, which helps maintain cardiac output. Our lab has previously demonstrated that the phosphorylation of sarcomeric regulatory proteins increased after LVAD implantation. In a similar way to LVADs, Impella devices allow for mechanical unloading but in a shorter-term setting. The effect of Impella devices on sarcomeric protein phosphorylation has yet to be investigated. The goal of this study was to test the hypothesis that patients with Impella implants have higher troponin I (TnI) phosphorylation than organ donors.

Methods
Through the University of Kentucky Gill Cardiovascular Biorepository (CVBR), we collected left-ventricular mid-myocardium from patients with Impella implants (n=26) and organ donors (n=37). Impellas were explanted at the time of sample procurement. The University of Kentucky Institutional Review Board approved the collection procedure from patients who undergo cardiovascular surgery and/or clinical procedure (IRB 46103). Patients at the University of Kentucky were consented by a member of the research team through informed consent and authorization to participate in a research study. CVBR has a research agreement with the Organ Procurement Office, Network for Hope, to obtain tissue and blood samples from organ donors whose heart doesn't qualify for transplant. To evaluate TnI phosphorylation, protein lysates were subjected to Phos-Tag gel electrophoresis and immunoblotting. Immunoblot images were quantified using GelBox software, and results for both patients with Impella and organ donors were compared to existing data of TnI phosphorylation for patients with heart failure without any mechanical support.

Results
Samples from patients with Impella implants showed lower TnI phosphorylation relative to organ donors ($p < 0.001$). TnI phosphorylation of myocardium from patients with Impella was not significantly different from patients without mechanical support.

Conclusion
TnI phosphorylation levels were comparable between failing myocardium with and without Impella implants. Nonetheless, Impella devices remain a valuable source of circulatory support in patients with advanced heart failure.

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Impact of human apolipoprotein E isoforms on abdominal aortic aneurysm

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Abdominal aortic aneurysm (AAA) is clinically defined as a permanent dilation of the normal diameter of the abdominal aorta and is pathologically characterized by the thinning and weakening of the vessel wall. AAA rupture results in 60-80% mortality and annually kills ~10,000 people in the US. Our previously published meta-analysis of 17 individual genome-wide association studies from 14 discovery cohorts in the AAAgen Consortium resulted in the discovery 121 loci that were associated with AAA. Greater than a third of the loci (42/121) were associated with circulating lipid levels, including common protein coding variants at the *APOE* locus. We have therefore determined the effects of *APOE* haplotypes on AAA risk using data from 434,361 veterans genetically similar to European reference populations in the Veteran Affairs Million Veterans Program. With the ε3/3 haplotype as the reference, ε2/2 and ε4/4 carriers had significantly increased AAA risk (OR=1.43, 95% CI: 1.22-1.66; OR=1.34, 95% CI: 1.22-1.47). Lipid traits associated with the ε2/2 and ε4/4 haplotypes were distinct. ε2/2 carriers had reduced plasma LDL cholesterol concentrations but increased triglycerides and total cholesterol, indicating lipid accumulation in triglyceride-rich lipoproteins. The increased total cholesterol concentrations in ε4/4 carriers were associated with increased LDL cholesterol. We also studied angiotensin II-induced AAA development in male (n=10/genotype) and female (n=10/genotype) mice that have the mouse *Apoe* coding exons replaced with those of the human ε3 or ε4 alleles. External diameter of the suprarenal aorta, which is the region of AAA development in AngII-infused mice, was similar in APOE3/3 and APOE4/4 mice. AAA incidence was also not significantly different between genotypes. Analysis of plasma total cholesterol showed no differences between APOE3/3 and APOE4/4 mice. In conclusion, humans but not mice expressing ApoE4 have increased AAA risk. The difference in AAA risk between the species could be due to ApoE4 expressing humans but not mice having increased total and LDL cholesterol.

Vascular Smooth Muscle Cell PERK Mediates Microbiome-Derived TMAO–Associated Abdominal Aortic Aneurysm

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BACKGROUND: The microbiome-derived metabolite trimethylamine N-oxide (TMAO) has been linked to AAA in both human and mechanistic mouse studies. TMAO binds protein kinase R-like endoplasmic reticulum kinase (PERK), leading to selective activation of the unfolded protein response (UPR), and our prior RNA sequencing demonstrated this PERK-mediated UPR activation in the aneurysmal aortic wall. We have previously shown that global-inducible PERK deletion is lethal in our models. As such, we generated vascular smooth muscle cell (VSMC)-specific PERK knockout mice using the Transgelin (Tagln) promoter, which attenuated AAA formation and rupture in both AngII and elastase models. However, Tagln lacks full VSMC specificity, raising the possibility that these effects were not VSMC-specific. Thus, the current study utilizes the more specific Myh11 promoter to define the functional and mechanistic role of VSMC-specific TMAO-mediated PERK activation in AAA pathogenesis.

METHODS & RESULTS: Mice with VSMC-specific deletion of PERK were produced by breeding hemizygous male mice expressing both Cre and a floxed tdTomato reporter gene under control of the Myh11 promoter (Myh11-CreERT2-RAD-ROSA floxed-STOP-tdTomato) to female PERK floxed mice. Deletion of PERK was validated by western blot and lineage tracing. Male mice ($Perk^{WT/WT}$ *Cre*+: N=9; $Perk^{Flox/Flox}$ *Cre*+: N=4; $Perk^{Flox/Flox}$ *Cre*-: N=2) were fed a tamoxifen diet for 2 weeks (250mg/kg tamoxifen/chow) to induce VSMC deletion followed by a high choline diet (1.2% choline supplementation) for one week prior to and throughout the study to augment circulating TMAO. Mice then underwent laparotomy and application of topical elastase (10 mg/mL porcine pancreatic elastase, 5 minutes) to induce AAA. Mice with VSMC-specific PERK knockout demonstrated a trend toward reduced aortic diameter growth over time compared to control ($Perk^{WT/WT}$ *Cre*+: 1.540 ± 0.1906 mm; $Perk^{Flox/Flox}$ *Cre*+: 1.278 ± 0.1307 mm; $Perk^{Flox/Flox}$ *Cre*-: 5.131 ± 0.3198 mm; P = 0.0511).

CONCLUSIONS: These results indicate that VSMC-specific knockout of PERK using a more specific Cre driver blunts AAA formation in the elastase murine model, corroborating our previous findings with the Tagln-Cre model. Further studies are needed to elucidate the mechanism of PERK-mediated ER stress in AAA, which may reveal novel therapeutic targets for AAA treatment.

RRAD Links L-Type Calcium Channel Function to STAT3 Signaling

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RRAD is a monomeric GTP-binding protein that restrains cardiac L-type Ca^{2+} channel (LTCC) activity. Cardiomyocyte interleukin-11 receptor alpha (IL11RA) couples IL-11 stimulation to JAK/STAT3 signaling. Prior work showed that IL11 reduces cardiac performance via transcriptional changes including up-regulated RRAD mRNA; however, whether IL11 changes RRAD protein level, and how RRAD links calcium handling to STAT3 signaling is unknown. *We hypothesized that RRAD links LTCC regulation to signal transducer and activator of transcription 3 (STAT3), thereby shaping the acute cardiac response to IL-11.* Adult inducible cardiomyocyte-specific RRAD knockout (cRADKO) mice and controls received IL-11 or saline. Left ventricular function was assessed by echocardiography before treatment and at 2 and 4 h, and cardiac cytosolic and nuclear fractions were analyzed by immunoblotting. Calcineurin sensitivity was evaluated using cyclosporin A (CsA). Complementary guide RNA-mediated RRAD-knockout human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) and RRAD/MLP double-knockout hearts were used to examine total STAT3 abundance. IL-11 reduced left ventricular ejection fraction (LVEF) in control mice at 2 and 4 h, whereas cRADKO hearts maintained systolic function. RRAD protein increased at 4 h and was inversely associated with LVEF ($p = 0.0003$), extending prior transcript-level observations to the protein level. IL-11 increased phosphorylated STAT3 (pSTAT3) in controls; this response was blunted by RRAD loss, and CsA restored pSTAT3 in cRADKO hearts, supporting calcineurin-sensitive regulation. Total STAT3 was not significantly reduced in healthy cRADKO hearts but was lower following RRAD loss in guide RNA-mediated RRAD-knockout hiPSC-CMs and RRAD/MLP double-knockout hearts, suggesting context-dependent regulation of STAT3 abundance. Together, these findings support a model in which RRAD connects its established control of LTCC-dependent calcium signaling to STAT3 activation and protein abundance, thereby contributing to acute IL-11-induced cardiac dysfunction. Moreover, RRAD abundance changes heart function on an hour's timescale. Funding: Supported by NIH HL166280; HL147921; and USARMC (DoD) PR22074.

CETP, Cholesterol, and SAA Are Associated with Survival in Sepsis-Associated ARDS

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Background: The ARDSNet-SAILS trial evaluated rosuvastatin treatment (RT) for improving outcomes in sepsis-associated acute respiratory distress syndrome (ARDS). In addition to their cholesterol-lowering effects, statins have pleiotropic anti-inflammatory properties. However, the trial showed no clinical benefit of RT in improving survival in sepsis and suggested a potential increase in renal and hepatic dysfunction. We investigated plasma total cholesterol (TC), free cholesterol (FC), HDL cholesterol (HDL-C), serum amyloid A (SAA), and cholesteryl ester transfer protein (CETP) levels in plasma samples from patients enrolled in the trial and determined their associations with survival and clinical outcomes.

Methods: Plasma samples from 471 participants randomized to rosuvastatin treatment (RT) or placebo treatment (PT) were analyzed for TC, FC, HDL-C, SAA, and CETP at enrollment (Day 0, n=466), Day 3 (n=425), and Day 6 (n=342). Linear mixed-effects models were used to assess treatment effects over time, and Cox regression models were used to evaluate associations with survival outcomes.

Results: RT significantly decreased plasma TC and FC levels but had no effect on HDL-C, SAA, or CETP levels. Survivors in the PT group showed significantly higher TC and HDL-C levels on Days 3 and 6 compared to non-survivors, while in the RT group, significant increases in TC and HDL-C among survivors were observed only on Day 6. CETP activities were significantly higher among survivors than non-survivors on all three days in both treatment groups. SAA levels decreased significantly over time in both PT and RT groups, and Day 0 SAA levels were significantly higher in PT survivors than in non-survivors ($p=0.005$). In the PT group, higher TC and HDL-C levels were strongly associated with lower APACHE scores and more hepatic dysfunction-free days ($p<0.001$). In the RT group, higher HDL-C was associated with more hepatic dysfunction-free days ($p\leq0.001$), however, TC and HDL-C levels were not strongly associated with APACHE scores. Higher plasma SAA levels, particularly on Days 3 and 6, were significantly associated with more renal and hepatic dysfunction-free days in the RT group ($p=0.001$). Fast protein liquid chromatography (FPLC) profiles demonstrated higher VLDL-C, LDL-C, and HDL-C levels among sepsis survivors, particularly on Day 3, compared to non-survivors. Sepsis survivors also showed a trend toward greater distribution of SAA on small, dense HDL particles compared with non-survivors in both treatment groups.

Conclusion: Higher TC and HDL-C levels, particularly during the early course of sepsis, were associated with improved survival, lower disease severity, and more organ dysfunction-free days. Plasma CETP activity and early SAA levels also emerged as potential prognostic biomarkers associated with improved sepsis survival. Although RT did not affect CETP activity or SAA levels, it appeared to modify the association between SAA, mortality, and organ dysfunction outcomes. The distribution of SAA among lipoprotein particles, particularly small, dense HDL, may contribute to its association with improved survival in sepsis.

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Title: Identification of Novel Repressor Elements in the Human *GJA1* Promoter

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Background: Connexin 43 (Cx43), encoded by *GJA1*, is the primary gap junction protein in the ventricular myocardium. In ischemic heart disease and heart failure, ventricular *GJA1*/Cx43 expression decreases and remodeling occurs, with impaired intercellular coupling contributing to ventricular arrhythmias and sudden cardiac death. We previously found that *GJA1* mRNA exhibits circadian oscillations in mouse ventricular tissue.

Hypothesis: Conserved regulatory sequences in the proximal *GJA1* region regulate its circadian promoter activity.

Methods: We constructed a luciferase promoter reporter vector containing the human (*h*) *GJA1* promoter sequence (6987 bp; pGL3 basic vector + proximal promoter) and assessed its circadian promoter activity using real-time bioluminescence monitoring (LumiCycle, Actimetrics) in C2C12 myotubes. Cells were transfected and continuously monitored for 7-10 days at 10-minute intervals. Two targeted promoter deletion constructs were generated to map functional regulatory regions (Δ*hGJA1* (-10 to -393 bp; mutant 1 (M1)) and Δ*hGJA1* (-444 to -1973 bp; mutant 2 (M2)) upstream of the transcription start site). Site-directed mutagenesis was used to introduce mutations in conserved promoter regions suspected of regulating circadian promoter activity (E-box elements). Statistical significance was assessed using ANOVA with Bonferroni post hoc correction. Data are reported as mean ± SD.

Results: All three constructs, full-length h*GJA1* (n=22), M1 (n=23), and M2 (n=18), exhibited clear circadian oscillations in bioluminescence, with periods of 24.1 ± 0.3, 24.5 ± 0.3, and 23.9 ± 0.1 hours, respectively. Removing the proximal promoter segment (M1) caused a roughly 20-fold increase in rhythm amplitude relative to the full-length construct (62.9 ± 13.9 vs. 3.0 ± 1.5 counts/sec; $p<0.0001$), whereas M2 showed no such change. M1 also exhibited a marked phase delay compared to full-length (9.4 ± 2.8 vs. 3.0 ± 3.4 hours; $p<0.0001$), with no phase shift observed in M2. Early mutagenesis data targeting a single E-box within the M1 region produced effects comparable to the full M1 deletion.

Conclusions: We identified a previously underrecognized repressive regulatory region within the proximal promoter (-10 to -393 bp) that dampens and phase-shifts *GJA1* promoter activity. Preliminary findings implicate a conserved E-box in this region in regulating circadian *GJA1* transcription. Defining this regulatory mechanism may help explain how ischemia and heart failure disrupt *Gja1* expression.

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The adrenal stress response is an essential host response against hemolysis through metabolic reprogramming

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Hemolysis accompanies severe diseases such as malaria, sepsis, and sickle cell disease, leading to the release of large amounts of hemoglobin and heme that drive oxidative injury and mortality. Glucocorticoids are frequently used in hemolytic disorders because of their potent immunomodulatory effects. However, the role and underlying mechanisms of endogenous glucocorticoids produced by the adrenal stress response during hemolysis remain poorly understood, leaving the efficacy of glucocorticoid therapy in non-immune hemolytic diseases unresolved.

Loss of iGC signaling, either through impaired iGC production (SF1CreSR-BI$^{fl/fl}$ mice) or pharmacological glucocorticoid receptor blockade (RU486), markedly increased mortality during hemolytic stress, whereas glucocorticoid supplementation restored survival. Mechanistically, iGCs promoted adaptive metabolic reprogramming through dual activation of nuclear- and mitochondrial-encoded oxidative phosphorylation (OXPHOS) programs. Inhibition of OXPHOS with rotenone worsened survival, while enhancement of mitochondrial oxidative metabolism with dichloroacetate (DCA) improved survival, establishing OXPHOS as a downstream effector of glucocorticoid-mediated protection. Importantly, this mechanism was validated in transgenic sickle cell disease mice expressing human sickle hemoglobin, supporting the clinical relevance of the SR-BI-iGC-OXPHOS axis.

In conclusion, these findings identify the adrenal stress response as a critical host defense against hemolysis, dependent on SR-BI–mediated inducible glucocorticoid (iGC) production and metabolic reprogramming, and provide mechanistic insight into glucocorticoid therapy in hemolytic diseases.

Knockdown of angiopoietin-like protein 3 partially corrects the type III hyperlipoproteinemia in APOE*2 mice

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Type III hyperlipoproteinemia (HLP) is characterized by an elevation in chylomicrons and VLDL, resulting in a significant increase in the plasma concentrations of cholesterol and triglycerides. This lipid disorder affects 1-5,000 to 1-10,000 people and increases the risk of atherosclerotic cardiovascular disease (ASCVD) by 5-10 times. Individuals who have an ε2/2 *APOE* haplotype are largely normolipidemic or hypolipidemic. However, 10-15% of ε2/2 carriers have Type III HLP. Angiopoietin-like protein 3 (ANGPTL3), which is secreted into circulation by the liver, increases circulating levels of chylomicron and VLDL remnants by inhibiting lipoprotein lipase and endothelial lipase. Individuals with loss-of-functions mutations in ANGPTL3 have significant reductions in triglycerides, LDL cholesterol and HDL cholesterol and are at lower risk of ASCVD. Therapies that block ANGPLT3 function or expression are either approved or in development for the treatment of familial hypercholesterolemia, but there are no reports on the effectiveness of these therapies for Type III HLP. Therefore, we conducted an ANGPTL3 inhibitor study in a Type III HLP mouse model. Male and female APOE*2 mice, which have the *Apoe* coding exons replaced with that of the human ε2 allele, were fed Western-type diet and treated for 6 weeks with either saline or a GalNAc conjugated ASO targeting hepatic Angptl3 mRNA. Plasma ANGPTL3 concentration was reduced by >90% in both male and female mice treated with Angptl3 ASO. Knockdown of ANGPTL3 resulted in a significant decrease in the plasma particle number of triglyceride-rich lipoprotein remnants and HDL. Plasma triglyceride and total cholesterol were both significantly reduced with Angptl3 ASO, but the percentage decrease from saline-treated, sex matched values were greater for triglyceride (93% males, 78% females) than total cholesterol (59% males, 31% females). In addition, for plasma lipids and lipoprotein levels, the percentage drop between Angptl3 ASO and saline treatment was greater in males compared to females. In conclusion, our results indicate that ANGPTL3 inhibitors could be an effective therapy for patients with Type III HLP.

Environmental Exposure to Fine Particulate Matter Contributes to Cardiovascular Risk in Children with Elevated Blood Pressure

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Background: There is a growing evidence that exposure to particulate matter ($PM_{2.5}$) is linked to cardiovascular morbidity and mortality in adults; however, few studies have assessed indoor air quality including exposure to $PM_{2.5}$ in the development of early cardiovascular disease in children.

Objective: Investigate relationships among indoor $PM_{2.5}$ exposure and cardiovascular risk including BMI, 24-hr blood pressure (BP) status, and left ventricular mass in children ages 8-18 years referred to pediatric nephrology division for BP evaluation.

Methods: With IRB approval, we recruited patients ages 8-18 years who are referred to KCH Pediatric Nephrology clinic for evaluation of elevated BP. Exclusion criteria includes conditions that could affect BP, such as chronic kidney disease, congenital heart disease, and use of antihypertensive medications. Obesity was defined by BMI and weight-for-length percentiles according to CDC guidelines. LV mass was assessed by standard echocardiography. Blood and urine samples were collected. For indoor air sampling, Personal Environmental Monitors attached to sampling pumps were set up in the family's living room for a 3-day period and Environmental Survey Questionnaires were completed.

Results: We have recruited 90 patients and completed 50 in-home air sampling visits with 58% of these in Appalachian counties. The mean age is 13.6 ± 2.7, 70% male, 80% Caucasian, 84% (n=76) obese, 53% (n=48) having stage 1 or stage 2 hypertension with 25% displaying a non-dipping status, and 49% prevalence of left ventricular hypertrophy (LVH). Mean $PM_{2.5}$ exposure level was 25.5 $\mu g/m^3$ ± 3.7 with 10 homes above EPA standards (>35 $\mu g/m^3$); PM_{10}: 31.8 $\mu g/m^3$ ± 4.4. Patients in Appalachian homes have lower exposures to $C0_2$ (945.2 ppm ± 68 vs 1282.9 ppm ± 85.8, p=0.001) and radon (1.5 pCi/L ± 0.3 vs. 2.8 pCi/L ± 0.8, p=0.009). Additionally, 47% (n=42) report second-hand smoke exposure and 21% (n=19) food insecurity.

Conclusions: These findings confirm the high incidence of early cardiovascular risk evidenced by obesity, hypertension, and LVH in patients referred for elevated BP. Furthermore, our findings demonstrate differences in exposure levels depending on geographical location. . Ongoing efforts from our team include the use of advanced statistics (Multivariable Poisson regression modeling) to report percentage of CV risk outcomes for each unit increase of PM exposure as well as interaction variables for CV risk. This study may serve as a model to examine the role of environmental exposures and associated health outcomes in other high-risk patient groups including premature infants.

Funding: This study is funded by NIEHS K23ES034462.

Title: Astrocyte-Derived EV-Mito Treatment Improves Brain Metabolic Deficits Associated with Diabetic Capillary Mitochondrial Dysfunction

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Individuals with diabetic hyperglycemia are at increased risk for vascular cognitive impairment (VCI), with glycemic variability associated with up to a six-fold greater risk of cognitive decline in older adults. However, no FDA-approved therapies currently target the underlying neurovascular dysfunction. Brain capillary endothelial cells maintain blood-brain barrier (BBB) integrity and regulate cerebral nutrient transport, functions that depend on mitochondrial homeostasis and ATP production. How diabetes-induced capillary mitochondrial dysfunction alters brain glucose and lactate transport and metabolism in VCI remains poorly understood.

Using a rat model of insulin-deficient diabetic hyperglycemia, we identified profound brain capillary mitochondrial dysfunction, characterized by reduced oxygen consumption rate (OCR) measured by Seahorse analysis, decreased mitochondrial content by confocal imaging, and remodeling of mitochondrial and capillary transporter proteins by proteomics. Stable isotope-resolved metabolomics (SIRM) using ^{13}C-labeled glucose during glycemic clamps further demonstrated reduced glucose transport from the peripheral circulation into the brain and impaired cerebral glucose metabolism in diabetic rats compared with controls. Untargeted brain metabolomics revealed broad deficits in fatty acid, amino acid, and tricarboxylic acid (TCA) cycle metabolites, including reduced N-acetylaspartate (NAA), a marker associated with neuronal mitochondrial integrity. In contrast, diabetes increased expression of monocarboxylate transporter 1 (MCT1) at the BBB, brain lactate accumulation, and neuronal histone lactylation. Together, these findings indicate that diabetic capillary mitochondrial dysfunction is associated with neurovascular remodeling, impaired glucose metabolism, and a shift toward lactate-associated metabolic reprogramming.

We hypothesized that mitochondrial transplantation using astrocyte-derived mitochondria-containing extracellular vesicles (mtEVs) would restore brain capillary mitochondrial content and function and improve brain metabolic abnormalities caused by diabetes. Control, diabetic, and diabetic mtEV-treated rats were studied. Diabetic rats received 1.5×10^7 mtEVs at 4-day intervals for a total of three administrations (intravenous), followed by brain metabolomic and capillary proteomic analyses.

Astrocyte-derived mtEV treatment partially restored brain TCA cycle, amino acid, and fatty acid metabolism and increased brain capillary mitochondrial content. These findings support mitochondrial transplantation as a potential mitochondria-targeted therapeutic strategy for diabetes-associated VCI. However, persistent lactate-associated metabolic reprogramming suggests that targeting abnormal lactate metabolism may provide an additional therapeutic opportunity and potentially complement mitochondrial restoration.

WIF-B9 Cells: a polarized hepatocyte model for studying the endogenous ABCG5 ABCG8 sterol transporter

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The ABCG5 ABCG8 (G5G8) sterol transporter is expressed in the liver and accounts for greater than 70% of biliary cholesterol secretion. Few hepatocyte cell lines express endogenous G5G8. Those that do fail to polarize under a variety of culture conditions. Primary hepatocytes polarize in vitro but rapidly downregulate G5G8, and its expression cannot be restored with established transcriptional regulators of Abcg5/Abcg8 expression. WIF-B9 cells are a fusion line generated from rat FAO hepatocytes and human WI-38 fibroblasts. These cells undergo polarization over a 10-day culture period and have been used to study the intracellular trafficking and function of a host of proteins, including ABC transporters. First, we confirmed that WIF-B9 cells express endogenous Abcg5 and Abcg8 at the transcriptional level. Expression of each transporter increased following treatment with a liver X receptor (LXR) agonist. This was confirmed in a dose-dependent manner for Abcg8 at the protein level. Next, we examined the subcellular localization of Abcg8 by indirect immunofluorescence microscopy. G8G8 is thought to function at the apical, canalicular surface. However, endogenous Abcg8 was localized to a subapical compartment that co-stained with markers of the Golgi apparatus. The apical domain is known to be detergent resistant. We confirmed that Abcg8 resides in a detergent soluble domain, consistent with intracellular localization. Treatment of cells with the lysosomal inhibitor, chloroquine, resulted in the accumulation of Abcg8 in cells. Localization of Abcg8 following chloroquine treatment revealed accumulation in subapical lysosomes, consistent with the trafficking of other apical transport proteins to the apical surface before internalization and degradation. Finally, we treated WIF-B9 cells with known stimuli of biliary cholesterol secretion. However, none promoted apical accumulation of Abcg8. We conclude that G5G8 matures in the Golgi apparatus and is transported to the apical surface, but is rapidly internalized and degraded in lysosomes. Further investigation will be required to determine what promotes apical transport and stabilizes G5G8 at the canalicular surface to promote biliary cholesterol secretion.

Title: Incidence of Cardiac Dysfunction Among Patients with Intracerebral Hemorrhage: A Retrospective Cohort Study

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Abstract: Intracerebral hemorrhage (ICH) is a severe form of stroke with high mortality and long-term morbidity. Growing evidence indicates that ICH triggers acute and chronic cardiac dysfunction through sympathetic overactivation, neurohormonal dysregulation, and autonomic-driven inflammation. Atrial fibrillation and hypertension are common comorbidities in ICH patients, yet their role in post-ICH cardiac outcomes remains poorly defined, and the incidence and phenotype of post-stroke cardiovascular dysfunction have been more thoroughly characterized in ischemic stroke and subarachnoid hemorrhage than in ICH specifically.

This study aimed to characterize the incidence and phenotype of cardiac dysfunction among ICH patients, determine whether patients had pre-existing cardiovascular risk factors, and assess whether those risk factors were exacerbated by ICH or represented new-onset dysfunction.

Using deidentified data from UK HealthCare (2014-2025), we identified 3,660 confirmed ICH patients and assessed cardiovascular comorbidity burden before and after ICH using diagnosis codes. To minimize ascertainment bias from the index hospitalization's dense inpatient coding, post-ICH windows were anchored to hospital discharge rather than admission, and analyses were restricted to patients surviving the full follow-up window to account for mortality as a competing risk. Changes in diagnosis prevalence from pre- to post-ICH were assessed using McNemar's test.

Pre-existing hypertension was present in 21.4% of patients, diabetes in 10.5%, myocardial infarction in 8.9%, atrial fibrillation in 6.6%, and heart failure in 5.4%, with 25.7% having at least one pre-existing cardiovascular comorbidity. Among patients surviving at least one-year post-discharge (n=2,612), diagnosed hypertension prevalence increased by 19.9 percentage points (95% CI 17.7-22.2, $p<0.001$) from pre- to post-ICH, with significant increases also observed for hyperlipidemia (+8.2%, $p<0.001$), atrial fibrillation (+5.7%, $p<0.001$), diabetes (+4.8%, $p<0.001$), and heart failure (+2.4%, $p<0.001$).

These findings suggest that ICH survivors experience a substantial increase in diagnosed cardiovascular and metabolic comorbidity burden over the first year following discharge. Subsequent analyses will incorporate demographic, procedural, pharmacologic, and anatomic covariates to identify predictors of this increased burden and its association with morbidity and mortality. Ultimately, these findings may inform new cardiocerebral therapeutic strategies aimed at preventing secondary cardiac injury and reducing the burden of stroke.

DENND5B Couples Microtubule-Based Trafficking to Post-Golgi Lipoprotein Secretion

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Background: Hypertriglyceridemia is an atherosclerosis risk factor that is impacted by dietary triacylglyceride (TAG) absorption via the small intestine and endogenous hepatic production. Our laboratory reported that *Dennd5b*-deficient mice have impaired intestinal triglyceride-rich lipoprotein (TRL) secretion and resist diet-induced hepatic steatosis, hyperlipidemia, and atherosclerosis. Electron microscopy imaging of intestinal epithelium of *Dennd5b*$^{-/-}$ mice revealed significant accumulation of intracellular chylomicron secretory vesicles, suggesting a post-Golgi secretion defect. Since *DENND5B* is expressed by both TRL-secreting organs (liver and small intestine) in Humans, we hypothesized that *DENND5B* disruption attenuates TRL secretion in both tissues.

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 and verified lack of DENND5B protein via western blot. *DENND5B* disruption significantly reduced TAG secretion compared to *DENND5B*$^{+/+}$ controls (Caco-2: -67.8%, $p<0.0001$; HepG2: -91.0%, $p<0.0001$) without affecting cellular TAG content. To define the mechanism by which DENND5B influences TRL secretion, tagged DENND5B plasmids were transfected into HepG2 and Caco-2 cells for subcellular visualization and proximity-ligation based identification of DENND5B binding partners. Super-resolution microscopy of HepG2 cells revealed distinctive localization of DENND5B and APOB-positive puncta along microtubules. Caco-2 cell imaging shows similar APOB and DENND5B distributions. Co-Immunoprecipitation experiments in both cell types reveal that DENND5B interacts with proteins involved in motor protein-binding, membrane remodeling, and microtubule-membrane tethering, indicating a potential role for DENND5B in vesicular trafficking or fusion with the plasma membrane. Ongoing studies are using siRNA-mediated knockdown of DENND5B-interacting proteins to identify new proteins that participate in TRL secretion. Additionally, we are using mutagenesis experiments to identify functional protein domains in DENND5B that are important for TRL secretion.

Conclusions: These data support the hypothesis that DENND5B is involved in TRL secretion from human enterocytes and hepatocytes. Mechanistically, these studies associate DENND5B physically to the cytoskeleton and functionally to membrane docking and secretion. These findings also introduce new potential players in TRL exocytosis. Understanding the mechanistic details of this process may provide novel targets for regulation of plasma TAG and APOB concentrations.

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Pathological Heterogeneity of Aortopathy in Angiotensin II-infused Mice with Smooth Muscle Cell Fibrillin-1 Deficiency

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Abstract

Background: Fibrillin-1 (FBN1) is an essential microfibrillar component required for elastic fiber formation and maturation in the aortic wall. Previous work from our group demonstrated that FBN1 deficiency in smooth muscle cells (SMCs) causes progressive disruption of the aortic wall. This study aimed to characterize the pathological features of aortopathy in mice with SMC-FBN1 deficiency infused with angiotensin II (AngII), a critical contributor to hypertension.

Methods: Male SMC-FBN1-deficient mice (SMC-FBN1-/-) and wild-type littermates (SMC-FBN1+/+) were infused with AngII (0.5 μg/kg/min) for 28 days beginning at 7–8 weeks of age. At study termination, micro-computed tomography (micro-CT) was performed to assess aortic pathology. Histological analyses included hematoxylin and eosin (H&E), Verhoeff's, and Picrosirius Red staining.

Results: AngII infusion resulted in approximately 40% mortality due to abdominal aortic rupture or non-aortic rupture-related death. Micro-CT revealed aortic elongation and prominent dilation from the distal ascending to suprarenal regions, with no apparent pathology in the proximal ascending or infrarenal aorta. The most pronounced pathological dilation occurred in the suprarenal aortic region. Histological analyses demonstrated elastic fiber fragmentation, wall thickening, and collagen deposition, with region-specific features in the aortic root/proximal ascending aorta, distal ascending/arch region, descending thoracic aorta, suprarenal aorta, and infrarenal aorta.

Conclusions: AngII infusion accelerates aortopathy in SMC-FBN1-deficient mice and induces heterogeneous pathological patterns across distinct aortic regions.

Keywords: smooth muscle cell, fibrillin-1, extracellular matrix, aneurysm, angiotensin II, mouse, micro-CT, histology

Hepatic Overexpression of Serum Amyloid A Promotes Liver Fibrosis in in Apolipoprotein E-deficient Mice

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Abstract

Objectives: Chronic elevation of acute-phase serum amyloid A (SAA) is a hallmark of persistent inflammation and has been implicated in the pathogenesis of multiple chronic inflammatory diseases. The liver is the primary site of SAA expression and a major contributor to circulating SAA levels. We previously demonstrated that liver-specific overexpression of SAA promotes atherosclerosis in mice. Here, we sought to characterize the hepatic consequences of sustained liver-specific SAA overexpression and identify molecular pathways altered by chronic SAA elevation.

Approach and Results: Twelve-week old male and female mice of the following background were used for the study: 1. apolipoprotein E-deficient (apoE-/-) mice with intact endogenous SAA (SAA WT), apoE-/- mice also deficient in all three acute-phase isoforms of SAA but carrying either a liver-specific, doxycycline-inducible SAA1.1 transgene (SAA-Liv) or a liver-specific reverse tetracycline transactivator without the SAA gene (SAA KO). Mice were maintained on a standard chow diet and treated with doxycycline (1 mg/mL in drinking water) for eight weeks for SAA expression (in SAA-Liv mice). Histological analyses indicated marked hepatic fibrosis, confirmed by Picrosirius Red and α-SMA staining, along with amyloid deposition in SAA-Liv mice, accompanied by increased expression of NLRP3 inflammasome components and profibrotic genes at both transcript and protein levels. In vitro analysis in LX-2 human hepatic stellate cells, treatment with recombinant SAA showed an upregulation of profibrotic and inflammatory pathways including NLRP3 inflammasome signaling. Consistently, immunofluorescence analysis of human liver specimens across a spectrum of liver disease demonstrated elevated SAA expression.

Conclusions: These findings identify SAA1.1 as a previously unrecognized driver of hepatic fibrosis. Using complementary in vivo, in vitro, and human clinical studies, we demonstrate that sustained SAA elevation promotes hepatic fibrosis, potentially through activation of NLRP3 inflammasome pathways. These results expand the role of SAA beyond an acute-phase inflammatory marker and suggest SAA as a potential therapeutic target for inflammation-associated liver fibrosis.

Noninvasive Spinal Neuromodulation to Stabilize the Blood Pressure Pendulum After Spinal Cord Injury

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BACKGROUND

Spinal cord injury (SCI) disrupts sympathetic cardiovascular control, causing severe blood pressure (BP) instability. Systolic BP can drop to ≤50 mmHg during orthostatic hypotension (OH) or surge above 300 mmHg during autonomic dysreflexia (AD) triggered by noxious stimuli below the lesion, often recurring within the same individual and severely impairing quality of life. No current therapies effectively address both conditions.

OBJECTIVE

Evaluating translational potential of transcutaneous spinal cord stimulation (tSCS) to mitigate OH and AD in SCI.

METHODS

In adult male Wistar rats (n=11) with a severe T3 contusion injury, tSCS (30 Hz) was delivered via skin electrodes at T6/7. Wireless BP telemeters were implanted 8 weeks later. Beat-by-beat BP and heart rate were recorded at rest, during OH induced by lower body negative pressure, and during AD triggered by colorectal distension.

Three individuals with chronic motor-complete cervical SCI (AIS A/B) received tSCS (10 kHz carrier, 30 Hz) at T7/9 or T11/L1. Beat-by-beat BP measured via finger photoplethysmography during head-up tilt induced OH and anorectal stimulation induced AD.

RESULTS

In rats, tSCS reduced OH severity by +26.7 ± 14.1 mmHg and AD severity by -39.3 ± 17.5 mmHg. In humans, tSCS reduced OH by +47.9 ± 23.2mmHg, while AD was reduced by -37.1 ± 17.1 mmHg. Wavelet analysis of BP suggested that tSCS modulates sympathetic activity by altering low-frequency power in both animals and humans.

CONCLUSIONS

tSCS mitigates opposing extreme BP events in animals and humans with SCI, supporting its clinical viability for treating BP dysregulation after SCI.

Title: Pharmacologic stimulation of calcium signaling upregulates the core circadian protein BMAL1 in human induced pluripotent stem cell-derived cardiomyocytes

Authors
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Background: Circadian rhythms govern physiology and behavior across the 24-hour light-dark cycle. Rhythm disruption is associated with higher incidence of arrhythmia, which can cause sudden cardiac death. Shift work and other lifestyles that deviate from this cycle are risk factors for arrhythmia in humans. Additionally, knockout models of core circadian proteins in rodents result in arrhythmia. Arrhythmias are primarily caused by altered calcium signaling as calcium flux through cardiomyocytes controls cardiac contraction and relaxation. Despite a known association between circadian disruption and arrhythmia incidence, the mechanisms behind circadian regulation of cardiac calcium dynamics and contraction remain elusive.

Objective: Utilize a small molecule (ISX9) that alters calcium signaling and increases expression of the core clock protein BMAL1 to determine if calcium channels are under circadian regulation.

Hypothesis: The cardiomyocyte molecular clock regulates cardiac calcium handling by modulating expression of calcium channels and/or their modulators.

Methods: Treated human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) with ISX9 (10 μM) for 24-hours. Isolated proteins and performed Western blots to assess changes in circadian and cardiac calcium channel protein expression. Observed calcium dynamics via live-cell microscopy using the calcium reporter GCaMP to investigate functional effects.

Results: Our data show that in hiPSC-CMs, ISX9 treatment significantly shortens calcium time-to-peak and decay time (p<1e-4), reflecting accelerated cardiac calcium handling dynamics. ISX9-treated hiPSC-CMs also showed a 2-fold increase in expression of the core circadian transcription factor BMAL1 (p<1e-3), accompanied by a 4-fold decrease in RRAD protein expression (p<1e-4). RRAD slows calcium entry into cardiomyocytes by inhibiting L-type calcium channels. Interestingly, the relationship between BMAL1 and RRAD showed reciprocity, as BMAL1 protein expression was increased 1.5-fold in RRAD knockout hiPSC-CMs (p<1e-3).

Conclusion: This study identified a reciprocal link between the circadian clock protein BMAL1 and the L-type calcium channel regulator RRAD. These data suggest that calcium dynamics in cardiomyocytes are, in part, regulated by circadian clock proteins. Future work aims to identify the mechanisms through which circadian proteins regulate calcium ion channel function.

Retinoic acid-mediated differentiation of adventitial progenitor cells and de novo elastic fiber synthesis following aortic dissection

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Background: Elastic fibers are crucial for aortic wall integrity and are severely disrupted in aortic dissection (AD). We demonstrated previously that new elastic fibers are generated in the false lumen wall of AD in humans and mice after long-term β-aminopropionitrile (BAPN) administration. The false lumen wall was predominantly populated by cells expressing smooth muscle cell (SMC) markers. However, the mechanisms by which these elastogenic cells emerge and expand after AD remain unclear.

Methods and Results:
To explore the molecular basis underlying the expansion of elastogenic cells following AD, we first performed bulk RNA sequencing in normal aortas of control mice and AD samples of mice administered BAPN for 12 weeks. Transcriptomic analyses revealed upregulation of genes implicated in SMC differentiation, along with increased expression of elastic fiber-component genes. Genes associated with TGFβ signaling, including *Tgfb1*, *Tgfbr2*, and *Smad3*, as well as Notch signaling-related genes, such as *Notch3*, *Jag1*, and *Hey1*, were increased in AD samples. In addition, retinoic acid (RA) signaling-related genes, including *Aldh1a1*, *Rara*, and *Cyp26a1*, were upregulated. These data indicated active SMC differentiation accompanied by de novo elastic fiber synthesis in AD. Next, spatial transcriptome was performed to determine the molecular features of elastogenic cells. Although cells composing the false lumen wall were enriched for SMC marker genes, *Acta2* and *Myh11*, their abundance was lower than that observed in resident SMCs. Instead, immature and progenitor cell signatures, such as *Cd34* and *Thy1*, were prominent in the false lumen wall. Consistent with the bulk RNA sequencing, RA signaling-related genes, *Aldh1a1* and *Rbp1*, were highly abundant in cells composed of the false lumen. Interestingly, lineage tracing studies found that elastogenic cells did not originate from the resident SMC lineage, supporting differentiation from a non-SMC lineage after AD. Immunostaining revealed that these cells were positive for stem cell antigen 1, markers for adventitial progenitor cells of the aorta. In vitro experiments demonstrated that adventitial progenitor cells spontaneously initiated expression of SMC markers and *Eln* mRNA. RA stimulation enhanced SMC marker expression and elastic fiber synthesis in cultured progenitor cells.

Conclusion: RA mediates the differentiation of adventitial progenitor cells into SMC-like cells and de novo elastic fiber synthesis following AD.

Title: Cpt1a-dependent transcriptional responses to SREBP2 induction and suppression in primary hepatocytes

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Background: EWAS and GWAS have linked methylation status within the proximal promoter and rare and common variants in carnitine palmitoyltransferase 1a (CPT1a) with plasma cholesterol levels. CPT1a is the rate-limiting step of fatty acid oxidation (FAO) in mitochondria. *However, the mechanisms linking CPT1a activity to cholesterol metabolism are poorly understood.* Our lab recently demonstrated that liver-specific deletion (LKO) of Cpt1a in a humanized ApoB100 mouse model lowers LDL-C. We also observed increased protein expression of nuclear sterol regulatory element-binding protein 2 (SREBP2) and LDL-receptor (LDLR) from livers of $Cpt1a^{LKO}$ mice.

Methods: Primary hepatocytes were isolated from wildtype (WT) and Cpt1aLKO mice. The cells were seeded on collagen-coated 6-well plates. After 3 hours, the cells were treated overnight with either a control, SREBP2 induction (50μM Mevastain + 50μM Mevalonate), or SREBP2 suppression (25μM cholesterol + 25μM 25-OH cholesterol). RNA was isolated from the cells and the expression of SREBP2 target genes analyzed by rtPCR and RNA-sequencing. From a second cohort of hepatocytes, protein lysates were analyzed by SDS-PAGE and immunoblotted for SREBP2 target genes.

Results: In primary hepatocytes isolated from WT mice, statin treatment significantly increased *Cpt1a* and SREBP2-target genes. Primary hepatocytes isolated from $Cpt1a^{LKO}$ mice, however, had elevated basal levels of SREBP2 targets, but were unresponsive to statin treatment. Taken together, these data support a role for CPT1a in the regulation of hepatic sterol balance.

Spatially distinct fibroblast subsets exhibit unique features during atherosclerosis progression

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Background: Vascular fibroblasts are increasingly recognized as active participants in atherogenesis. Fibroblasts and exhibit heterogeneity, but whether distinct subsets have transcriptional differences during atherosclerosis progression remains unclear. The purpose of this study was to determine the spatial distribution of specific fibroblast subsets during atherosclerotic progression.

Methods: Atherosclerosis was induced in male C57BL/6J mice administered a *Ldlr* antisense oligonucleotide combined with Western diet feeding and angiotensin II (0.5 μg/kg/min) infusion for 4 or 12 weeks to model concomitant hypercholesterolemia and high blood pressure. Age-matched male C57BL/6J mice without these interventions were used as for comparison. Single-cell RNA sequencing (scRNA-seq) was performed on the ascending aorta, and spatial transcriptomic was performed on tissue sections of aortic root. scRNA-seq data were Harmony-integrated, clustered, and visualized by UMAP. Fibroblasts and smooth muscle cells (SMCs) were subclustered in scRNA-seq, and their identities were transferred onto Visium HD sections by anchor-based label transfer.

Results: Re-clustering of aortic fibroblasts and SMCs resolved 6 subsets: 5 fibroblast-like (FB) and 1 contractile SMCs. Three FB subsets were progressively increased in abundance during atherosclerosis. A *Cpxm2*$^{+}$/*Hsd11b1*$^{+}$/*F3*$^{+}$ subset (FB1) was enriched for extracellular matrix secretion. A *Pi16*$^{+}$/*Cd248*$^{+}$/*Ly6c1*$^{+}$ subset (FB2) displayed a marked type-I interferon signature (Ifi204, Ifi205, Ifi207), consistent with cytosolic nucleic acid sensing. A *Spp1*$^{+}$/*Lgals3*$^{+}$/*Acan*$^{+}$ subset (FB3) exhibited features of SMC differentiation and osteochondrogenesis and expanded during atherosclerosis progression. Visium HD mapping revealed their distinct spatial distributions: FB1 subset distributed broadly across the adventitia and outer media; FB2 subset localized to the outer adventitia; and FB3 subset accumulated within lesions at 4 and 12 weeks. These data demonstrate that fibroblasts expand and exhibit spatially distinct subsets in atherogenesis: an interstitial *Cpxm2*$^{+}$/*Hsd11b1*$^{+}$ subset, an adventitia-restricted *Pi16*$^{+}$ subset undergoing adventitial expansion, and a lesion-restricted *Spp1*$^{+}$/*Lgals3*$^{+}$ remodeling subset.

Conclusion: These findings reveal the spatial heterogeneity of fibroblasts during atherosclerosis progression that provide a foundation for the determination of these subsets in the disease process.

Key Words: Atherosclerosis, Vascular Fibroblasts, *Dcn*, *Pi16*, Adventitial heterogeneity, Spatial transcriptomics

Asters mediated cholesterol trafficking regulates hepatic stellate cell activation and liver fibrosis

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) affects nearly 30% of population worldwide and is the most prevalent liver disease. Although hepatic lipid accumulation is common in MASLD, progression to fibrosis generally occurs in patients with persistent liver injury and inflammation. Fibrosis is the strongest histologic predictor of liver-related morbidity and mortality. Nowadays lifestyle changes have induced weight loss and metabolic therapies such as GLP-1 receptor agonists or THR-β agonists can reduce hepatic steatosis and improve steatohepatitis. Although these recently FDA-approved therapies improve MASH histology, fibrosis regression remains incomplete, with fibrosis improvement reported in only ~24–37% of treated patients in phase 3 trials. Thus, effective treatments that efficiently reverse liver fibrosis remain limited. The fibrosis progression highly depends on hepatic stellate cells (HSCs) activation, which produce collagen rich extracellular matrix, remodel the fibrotic niche, and amplify profibrotic inflammatory and contractile signaling. Therefore, understanding the mechanisms that drive HSCs activation, is essential for identifying therapeutic targets in MASLD.

Recent studies suggested that free cholesterol (FC) level are dramatically increased in activated HSCs. In addition, FC distribution is unevenly distributed between different organelles with 60%-80% in the plasma membrane (PM) and only 0.5-1% in ER. Our previous studies have observed that Aster/GRAMD1 proteins (Aster-A, -B and -C) regulate non-vesicular transport of free cholesterol from PM to ER. In this study, we found that Aster-A and -B are highly expressed in HSCs, and knockdown of Aster-A in LX-2 cells, a human HSCs cell line, leading to the activation of HSCs and increasing the fibrosis markers, indicating that Asters may play an extrinsic role in HSCs activation by regulating cholesterol levels in the cells.

Based on the previous literature and our preliminary data, we hypothesize that Asters regulate intracellular cholesterol distribution in HSCs to restrain pro-fibrotic signaling and protect against liver fibrosis. We will test this hypothesis by two aims. Aim 1 will be to investigate the role of Aster-A and Aster-B in HSCs activation and fibrosis by using in vitro si-RNA knockdown and in vivo HSCs knockout mouse model. Aim 2 will be to determine whether Asters dependent PM to ER cholesterol transport regulates HSCs activation by testing Hedgehog (Hh) and Liver X receptor (LXR) signaling. Successful completion of this study will further strengthen our understanding of how Asters mediated intracellular cholesterol trafficking in HSCs activation and fibrogenesis and reveal a previously unrecognized role for HSCs cholesterol homeostasis in controlling liver fibrosis.

Title: Crosslinking mass spectrometry reveals details of protein-protein interactions on HDL

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Introduction: HDL is a compositionally complex lipoprotein, carrying a wide variety of proteins that may mediate its observed functional heterogeneity. Studies over the last 2 decades have identified over 250 proteins as being associated with HDL. However, many of these studies have stopped short of investigating the structural interactions of these proteins with lipoproteins and their functional implications. The objective of this study was to examine protein-protein interactions on HDL using mass spec cleavable crosslinkers.

Hypothesis: We investigated the hypothesis that peripheral HDL-associated proteins form stable physical interactions with the core structural proteins of HDL, apolipoprotein A-I and/or apolipoprotein A-II.

Methods: Peripheral blood was collected from 4 healthy adult male volunteers and plasma was immediately isolated and divided into both individual and pooled aliquots. HDL was isolated by density gradient ultracentrifugation, and incubated for 1 hour with a 50- or 200-fold molar excess of the MS-cleavable molecular crosslinker disuccinimidyl sulfoxide (DSSO) (molar ratio DSSO:total HDL protein). Samples were trypsinized, de-salted, and dried, and analyzed via LC-MS/MS on an Orbitrap Ascend Tribrid mass spectrometer. MS/MS data were analyzed for protein and crosslink identification using XlinkX in ThermoFisher Proteome Discoverer. Protein-protein interaction networks were generated using xiVIEW.

Results: Numerous crosslinks between both core apolipoproteins and non-apolipoprotein proteins were detected in isolated HDL samples, indicating structural interactions between these proteins. Filtered to an estimated false detection rate (FDR) of 1%, 200:1 DSSO-treated HDL samples yielded 229 unique crosslinks among 23 different proteins. All 23 detected crosslinked proteins have been reported as HDL-associated in the literature. XiVIEW structural analysis revealed 3 distinct regions of ApoA-I that are consistently involved in interactions with several peripheral proteins. Intramolecular crosslinks identified in ApoA-I also aligned well with the accepted trefoil model of ApoA-I on spherical HDL.

Conclusions: Binding of peripheral proteins to HDL is mediated at least in part through protein-protein interactions with ApoA-I and ApoA-II. We identified 3 distinct regions of A-I that consistently interact with peripheral proteins. Crosslinking-MS with cleavable crosslinkers is a promising new method for examination of the HDL protein interactome.

Cardiovascular-Kidney-Metabolic Syndrome in Children with Obesity: Impact of Semaglutide Therapy

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Background: There is growing evidence that childhood obesity increases the risk of early cardiovascular, renal, and metabolic disease. The American Heart Association's recently introduced construct of Cardiovascular-Kidney-Metabolic (CKM) syndrome captures the interconnected, multi-organ nature of this risk; however, few prospective studies have characterized CKM-related morbidity, including left ventricular mass, in children with severe obesity or examined how these measures change with pharmacologic weight-loss therapy. Emerging retrospective data suggests that left ventricular hypertrophy (LVH), an early marker of pathologic cardiac remodeling, may be more prevalent among children with severe obesity than has been clinically appreciated.

Objective: To characterize CKM syndrome-related morbidity and its relationship to weight loss, body composition, and inflammatory status in adolescents with severe obesity undergoing GLP-1 receptor agonist therapy, with particular attention to left ventricular mass

Methods: MODERN (Mitigation of Cardiovascular Disease Risks in Pediatric Extreme Obesity) is an ongoing, IRB-approved prospective observational study characterizing baseline cardiovascular risk profiles and longitudinal cardiometabolic phenotypes in adolescents with Class II–III obesity undergoing clinically indicated semaglutide therapy. The protocol is designed to align research and clinical workflow through the UK Golisano Children's Pediatric High BMI Clinic; enrollment target is 50 adolescents ages 12–18 years. Research visits are scheduled alongside routine clinical care through 16 months of treatment and include cardiac MRI, body composition analysis, cardiopulmonary exercise testing (CPET), and metabolic and inflammatory laboratory panels.

Results: We have enrolled 31 adolescents to date (median age 15–16 years, 59% female, 69% Class III obesity). At baseline, mean BMI was 43.6 ± 1.3 kg/m² (n=30), mean systolic/diastolic blood pressure was 123.5 ± 2.1 / 75.3 ± 1.5 mmHg, and mean body fat was 48.9 ± 0.9%. Low HDL (39.7 ± 1.4 mg/dL, n=27), elevated ALT (34.5 ± 5.8 U/L, n=29), and prediabetic-range HbA1c (5.45 ± 0.06%, n=27) were each present in a subset of patients. Cardiorespiratory fitness was reduced, with mean percent-predicted VO_2max of 59.2 ± 2.7% (n=31); 29 of 31 patients (93.5%) measured below 80% of predicted. Among patients with paired baseline and 6-month data (n=22), BMI decreased from 44.5 ± 1.6 to 40.1 ± 1.8 kg/m² ($p<0.0001$), systolic BP from 124.9 ± 2.5 to 115.9 ± 2.7 mmHg ($p=0.008$), diastolic BP from 77.4 ± 1.7 to 72.6 ± 1.3 mmHg ($p=0.026$), and body fat from 49.8 ± 1.1% to 46.8 ± 1.4% ($p=0.0001$). HbA1c improved from 5.41 ± 0.08% to 5.20 ± 0.09% (n=12, $p=0.001$) and ALT from 40.5 ± 10.5 to 29.9 ± 6.3 U/L (n=15, $p=0.042$); triglycerides, HDL, LDL, and AST did not change significantly (all $p>0.05$). Weight loss was driven predominantly by fat mass (138.8 ± 6.9 to 118.9 ± 8.0 lb, $p<0.0001$) with relative preservation of lean body mass (138.1 ± 4.8 to 131.7 ± 5.0 lb, $p=0.001$); a median of 72% of total weight lost was attributable to fat mass. Cardiac MRI-based assessment of left ventricular mass is ongoing, with baseline imaging completed and longitudinal analyses forthcoming.

Conclusions: These findings confirm a burden of CKM-related morbidity in adolescents with severe obesity, including reduced cardiorespiratory fitness. Semaglutide therapy produced significant early improvements in BMI, blood pressure, adiposity, HbA1c, and ALT, with weight loss favoring fat mass over lean tissue. Left ventricular mass analyses by cardiac MRI are ongoing and will address whether these early metabolic gains translate into measurable reverse cardiac remodeling. By embedding this level of mechanistic assessment within routine clinical care, MODERN offers a scalable, clinic-integrated model for characterizing and monitoring CKM syndrome in pediatric obesity.

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Liver-specific Deletion of Carnitine Palmitoyltransferase 1a Promotes Tumorigenesis in a Mouse Model of Obesity-driven Hepatocellular Carcinoma

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Background: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the fastest-growing etiology of hepatocellular carcinoma (HCC). The primary objective of this project is to determine the contribution of carnitine palmitoyltransferase 1a (CPT1a)-mediated fatty acid oxidation (FAO) in MASLD-driven HCC.

Methods: Paired tumor (n=8) and adjacent non-tumor tissue (n=8) were collected from patients with suspected MASLD-driven HCC at the University of Kentucky Markey Cancer Center. Hematoxylin and eosin (H&E) staining was used for pathological determination of tumor and adjacent nontumor tissue. Lipids were extracted via methyl-tert-butyl ether and subjected to lipidomics by the West Coast Metabolomics Center. Bulk RNA-sequencing was employed to assess gene expression changes across paired samples. For murine studies, four-to-five-day old $CPT1a^{F/F}$ and liver-specific CPT1a KO (LKO) pups were treated with a single application of 50uL 0.5% DMBA (7,12-dimethylbenz[a]anthracene) in acetone and fed the Gubra Amylin-NASH diet (GAN; 40% kcal fat; Research Diets) until 34 weeks of age. Mice were necropsied after a 24-hour fast. Livers were excised, and total tumor number was calculated.

Results: H&E staining showed significant lipid vacuole accumulation in human HCC tumors relative to nontumor tissue. Lipidomic analysis 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 HCC. On the contrary, MUFA-enriched acylcarnitines (C14:1, C18:1) were collectively reduced in human tumors. Consistent with this lipid profile, fatty acid oxidation genes (*CPT1A*, *CPT2*, *ACADL*, *ACADM*, *ACADS, HADHA*) were significantly decreased which associated with increased proliferation markers (*KI67, TERT*) in tumor versus nontumor tissue. In mice, CPT1a deletion increased liver to body weight ratios by ~44% (*6.4% vs. 9.2% liver weight as % of body weight*; *P*=0.0003) and increased overall tumor number by ~3.4X (*3.7 vs. 12.4 average nodules per mouse; P*=0.0055). H&E analysis suggests that tumors in mice replicate the histopathology of human HCC.

Conclusions: These results suggest human 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. Complimentary mouse studies show that CPT1a deletion in hepatocytes accelerates HCC in mice. Future studies are underway to identify mechanisms governing these differences. These findings identify mitochondrial FAO as a potential therapeutic target for MASLD-HCC prevention and treatment.

Hepatic Aster-C Deficiency Protects Against Diet-induced Hepatic Steatosis in Mice

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Objective

Aster family of proteins (Aster-A, -B, -C), which are involved in non-vesicular cholesterol transport from the plasma membrane (PM) to the endoplasmic reticulum (ER). Disruption of Aster protein function impairs PM-to-ER cholesterol transport in the liver, ovary and adrenal gland. Notably, Aster-C is expressed selectively in hepatocytes, the primary cell type responsible for lipid accumulation in metabolic dysfunction-associated steatotic liver disease (MASLD). In this study, we investigated the effects of Aster-C on lipid accumulation in hepatocytes in a mouse model of diet-induced hepatic steatosis.

Approach and Result

AAV8-Cre and AAV8-Control were injected in Aster-$C^{Flox/Flox}$ (F/F) mice, then fed 3 weeks of Western diet. Result shows that hepatic Aster-C gene expression was significantly abolished in AAV-Cre injected mice. Perform liver lipid assay, we found that liver cholesterol and triglyceride levels were significantly decreased in AAV-Cre injected mice compared to AAV-Control group (liver cholesterol, 81.86 ± 4.0 vs. 177.38 ± 24.1 μg/mg, $P<0.01$; liver triglyceride, 149.18 ± 1.9 vs. 292.28 ± 32.6, $P<0.01$). We also generated Aster-C hepatocytes specific knockout mice (LKO) by crossing Aster-$C^{Flox/Flox}$ mice with albumin-Cre mice. There is no significant difference on the levels of liver cholesterol and triglyceride when mice were fed Chow diet (LKO vs. F/F: liver cholesterol, 12.21 ± 1.6 vs. 10.96 ± 1.4 μg/mg, $P=0.562$; liver triglyceride, 386.06 ± 21.8 vs. 432.48 ± 36.6 μg/mg, $P=0.301$). However, with 10 weeks of Western diet feeding, hepatic Aster-C deficient mice show significant decrease in liver cholesterol (31.7 ± 4.1 vs. 70.4 ± 10.1 μg/mg, $P<0.01$) and triglyceride (1407.0 ± 433.7 vs. 2913.2 ± 404.2 μg/mg, $P<0.05$) compared to control mice. Liver histology images show less lipid drops and macrophages accumulation in hepatic Aster-C deficient mice. What's more RNA sequencing result show that ablation of Aster-C improves nonalcoholic steatohepatitis (NASH) relative gene expression in liver.

Conclusion

Loss of Aster-C in hepatocytes protects against steatosis by reducing hepatic cholesterol and triglyceride accumulation, ameliorating liver damage, and decreasing expression of inflammatory genes following Western diet feeding.

Asprosin as a novel target for treating metabolic hypertension

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Metabolic dysregulation is a major driver of hypertension, accounting for more than 60% of hypertension risk. Clinical data heavily associates metabolic conditions including obesity, type 2 diabetes and hypertension with increased level of a newly-discovered adipokine – asprosin. First identified in 2016-2017, asprosin's roles in hepatic gluconeogenesis and appetite regulation have been well-characterized thus far, however a direct mechanistic link to blood pressure has never been studied. In a mouse model of asprosin deficiency (*Fbn1*$^{NPS/+}$), we observed a baseline hypotension, which was restored by intranasal administration of recombinant human asprosin. Because Protein Tyrosine Phosphatase Receptor Delta (Ptprd) is a known neural receptor of asprosin, and the paraventricular nucleus of the hypothalamus (PVN) is the seat of neurogenic hypertension, we identified Ptprd-expressing neurons in this brain region and found heavy co-localization with oxytocin-expressing (OT) neurons. Thus, we hypothesized that oxytocinergic Ptprd modulates asprosin's blood pressure effects.

To test this, we generated an OT-specific Ptprd knockout mouse model (OT$^{Ptprd\text{-}KO}$), and found that blood pressure was significantly decreased by OT-Ptprd ablation. Importantly, OT$^{Ptprd\text{-}KO}$ did not induce changes in appetite, indicating a distinct physiology from asprosin's orexigenic function. In addition, PVN administration of asprosin increased blood pressure in OTcre control mice, but not in mice with receptor ablation. Next, we wanted to know how asprosin-Ptprd signaling affects OT neurons, so we generated mice with TdTomato labeling in OT neurons and found that asprosin administration decreased firing rate in slice culture. In OT$^{Ptprd\text{-}KO}$ TdTomato-labelled mice, asprosin did not affect OT neuronal activity, recapitulating our *in vivo* data.

Finally, we wanted to test if targeting asprosin-Ptprd signaling offered therapeutic potential for the treatment of metabolic hypertension. First, slice culture preps were pre-treated with Ptprd antagonist 7-Butoxy Illudalic Acid (7-BIA), which completely prevented the inhibitory effects of asprosin on PVN-OT neurons. Then, we looked at an in vivo model of metabolic hypertension by treating hypertensive Db/Db mice with 7-BIA. Ptprd-inhibition reduced blood pressure in our model up to 6 hours post treatment.

Overall, this work indicates that asprosin decreases the activity of OT neurons in the PVN which causes autonomic-driven increases in blood pressure, and positions asprosin-Ptprd signaling as a promising therapeutic for metabolic hypertension.

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Aged *db/db* Female Mice Show Evidence of Diabetic-induced Cardiac Autonomic Neuropathy and Sinus Node Dysfunction

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Introduction: Cardiovascular autonomic neuropathy (CAN) is a common complication of diabetes and is associated with increased mortality, particularly among women with type 2 diabetes. Housing young female *db/db* mice at thermoneutrality unmasked the CAN phenotype. Aging exacerbates diabetic CAN. We investigated whether aging female *db/db* mice would exacerbate the CAN phenotype using continuous electrocardiography (ECG).
Hypothesis: Aged female *db/db* mice will exhibit an exacerbated CAN-related phenotype under thermoneutral conditions.
Methods: Eight- to nine-month-old control (*db/+*) and *db/db* female mice (n=5/genotype) were implanted with telemetry devices to record continuous ECG, core temperature, and activity, and were housed under a 12-hour light:12-hour dark cycle with ad libitum access to food and water. Mice were housed at room temperature (25°C) and then at thermoneutral temperature (30°C). We quantified changes in autonomic input to the heart by administering the autonomic receptor inhibitors propranolol (10 mg/kg) and methylatropine (1 mg/kg) at zeitgeber times 23.5 and 11.5, bracketing the night-to-day and day-to-night transitions.
Results: At room temperature and thermoneutrality, control mice had faster heart rates, higher core temperatures, and greater activity during the dark (active) cycle than during the light (rest) cycle. At room temperature, *db/db* mice had slower heart rates and lower core body temperatures than controls during both the light and dark cycles. Thermoneutrality eliminated the genotype differences in heart rate and core body temperature. Autonomic inhibition studies showed that *db/db* mice had slower heart rates at both room temperature and thermoneutrality. The differences at thermoneutrality did not appear to be due to differences in core body temperature or activity.
Summary: These findings demonstrate that aged female *db/db* mice housed in thermoneutrality do not have CAN-related tachycardia. However, older female *db/db* mice have heart rates that are relatively insensitive to autonomic input and an abnormally slower intrinsic heart rate than control mice, indicating sinus node dysfunction. These studies suggest that aged female *db/db* mice may be a good model for understanding the progression of diabetes-induced CAN and sinus node dysfunction.

X-Linked *Kdm5c* Gene Dosage Protects Females from Aortopathy

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Background: Aortopathies, including thoracic and abdominal aortic aneurysms, occur at greater prevalence and severity in males than females. We recently demonstrated that an XX sex chromosome complement protects female mice from AngII-induced aortopathies and that *Kdm5c*, an X-linked gene that escapes X-inactivation in mice and humans, is more highly expressed in aortas of XX than XO female mice. Here, we hypothesized that increased *Kdm5c* gene dosage contributes to female protection against aortopathy.

Methods: Female *Kdm5c+/+* and *Kdm5c+/-* mice (C57BL/6 background) were generated by crossing Kdm5c floxed heterozygotes with CMV-Cre transgenic males. *Kdm5c*+/- females have one functional copy of Kdm5c, analogous in gene dosage to XY males. At two months of age, female mice were injected intraperitoneally with AAV-PCSK9 to induce hypercholesterolemia and fed a 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. Aortic disease progression was monitored by ultrasound. To further characterize the role of *Kdm5c*, aortic smooth muscle cells (SMCs) were treated with PDGF to induce a synthetic phenotype, and single-cell genomic data from normal and aneurysmal human aortas were analyzed for SMC Kdm5c expression.

Results: At baseline, prior to AngII infusion, *Kdm5c*+/- females exhibited significantly greater ascending and abdominal aortic lumen diameters than *Kdm5c*+/+ females. Following AngII infusion, ascending and abdominal aortic lumen diameters increased in *Kdm5c*+/- females, whereas this effect was not observed in Kdm5c+/+ females. At study endpoint, maximal external diameters of ascending and abdominal aortas were also significantly greater in AngII-infused *Kdm5c*+/- females. Consistent with increased aortic pathology, aortic weight and aortic arch area were significantly greater in *Kdm5c*+/- than *Kdm5c*+/+ females. Induction of a synthetic SMC phenotype with PDGF reduced *Kdm5c* mRNA and protein expression by approximately 50%. In human single-cell genomic data, SMCs from normal female aortas exhibited higher *Kdm5c* expression than those from males, while aneurysmal aortas from both sexes showed lower SMC *Kdm5c* expression than normal aortas.

Conclusion: These findings identify Kdm5c gene dosage as a potential X-linked mechanism contributing to female protection from aortopathy and link reduced Kdm5c expression to a disease-associated SMC phenotype in both experimental and human aortic disease. Future studies will define the downstream targets and pathways regulated by Kdm5c that mediate these protective effects.

Title: Long-read sequencing reveals conserved splicing mechanisms underlying isoform-level cardiac remodeling in response to mistimed eating

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Introduction: The time of day when calories are consumed affects gene expression and metabolism. For nocturnal mice, mistimed eating means eating during the light cycle, their inactive cycle. Here, we used long-read RNA sequencing to test whether restricting food intake to the inactive cycle reorganizes both gene expression and alternative splicing, an important regulatory layer of functional protein expression.
Hypothesis: Light-restricted feeding induces differential alternative splicing patterns in cardiac mRNA from male and female mice.
Methods: Male and female mice were maintained on 12h:12h light-dark cycles and assigned to ad libitum feeding (ALF) or light-restricted feeding (LRF, ZT2-9) for two weeks. Ventricular tissue was collected at four time points (ZT1, ZT7, ZT13, ZT19). Oxford Nanopore long-read RNA sequencing captured full-length transcripts. Differential gene expression, differential transcript expression (DTE), and differential transcript usage (DTU) were assessed using DESeq2 and DRIMSeq. Splicing event enrichment in DTU genes was assessed using IsoformSwitchAnalyzeR.
Results: Approximately 1 in 4 cardiac transcripts represented novel isoforms of known genes. LRF shifted the phase of circadian clock genes. LRF induced largely sex-specific changes at gene and transcript levels and in isoform usage. Most DTE (70%) and DTU (93%) changes occurred independently of gene-level expression changes. Despite this sex-specificity, both sexes showed shared directional splicing-event patterns in response to LRF. Alternative 5' splice site was enriched in isoforms gaining proportion, while alternative 3' splice site was enriched in isoforms losing proportion under LRF. Similar trends were observed for transcription start and termination site and exon or multi-exon skipping.
Conclusion: We identified isoform-level regulation as a fundamental feature of heart's response to feeding behavior, regardless of sex. Meal timing reorganized the cardiac transcriptome through isoform-level remodeling, independent of gene-level expression changes. The shared directionality of splicing events across sexes suggests conserved post-transcriptional mechanisms underlie the cardiac response to mistimed eating.

Title: Endothelial mineralocorticoid receptor mediates aortic aneurysms in female mice.

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- **Objective** – Women experience more aggressive progression once they develop aortic aneurysm (AA) though they are at a lower risk than men. The underlying mechanisms for such sex dimorphism remain to be fully understood. Our lab reported aldosterone and high salt (Aldo-salt) induced aortic aneurysms in a sex-dependent manner in mice but the mechanism for female protection against Aldo-salt-induced AA is unknown. The current study tested the hypothesis that estrogen mediates the protection in the Aldo-salt-induced aortic aneurysms in female mice and investigated the potential mechanisms.

- **Approaches and Results** – 12- or 3-month-old female mice were used in experiments. We first demonstrated the effect of ovariectomy (OVX) in abolishing the protection in 12-month-old mice with a 61.9% incidence in OVX mice (13/21) compared to sham mice (15.8%, 3/19). Moreover, such ovariectomy effect was not seen in 3-month-old mice with comparable aortic dilation and incidences in sham and OVX mice [10% (1/10) vs 0% (0/14)]. To dissect the mechanism, we examined the mRNA and protein level of mineralocorticoid receptor (MR), the receptor for aldosterone, in the aortic tissue and found that MR mRNA expression increased with age. More importantly, MR was specifically upregulated and condensed in the nuclei of endothelial cells only in OVX mice administered with Aldo-salt. Furthermore, estrogen effectively inhibited the MR translocation upon aldosterone stimulation in human umbilical vein endothelial cells. Next, to establish the pathogenic role of endothelial MR, we ovariectomized endothelial specific MR knockout mice (Ec-MR$^{-/-}$) and MR flox littermates and administered Aldo-salt for four weeks. Results showed that Ec-MR knockout effectively suppressed the aortic dilation and mitigated the AA formation (30%, 5/15) compared to flox mice (73.7%, 14/19). We further investigated the pathways regulated by Ec-MR by RNA sequencing and surprisingly found circadian rhythm played an important role.

- **Conclusions** – Ovariectomy abolishes the female protection against Aldo-salt-induced aortic aneurysms in an age-dependent manner. Estrogen mediates such protection via inhibiting the expression and translocation of endothelial mineralocorticoid receptor. Circadian rhythm pathways are involved in Ec-MR-mediated aortic aneurysm.

Title: Development of a Living Myocardial Slice Platform: A Proof-of-Concept Study Across Rat, Piglet, and Human Heart Tissue

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Background
Cardiac muscle generates more force when it is stretched, a property known as the Frank-Starling relationship. This behavior has been characterized in trabeculae, whole hearts, and more recently, living myocardial slices. Living myocardial slices, thin sections of the left ventricle, retain native myocardial architecture, while allowing force to be measured under controlled changes in length. The goal of this work is to establish and validate this platform in our laboratory as the foundation for future Frank-Starling studies in human myocardium.

Methods
Left ventricular slices (300 μm) were prepared from rat, piglet, and human (left ventricular assist device) heart tissue. For mechanical testing, slices were mounted between a force transducer and a length controller and super fused with Tyrode's in tissue chambers. Representative twitch contractions were recorded and characterized (developed force normalized to cross-sectional area, times to 50% and 90% peak and relaxation, and rates of force development and relaxation). Length-tension behavior was assessed in a rat slice, sustained contraction was tracked over 24 hours in a rat slice, and multi-day viability was followed in a piglet slice across days 1–3. This is a technical feasibility study; n = 1-2 hearts per species, with representative recordings shown.

Results
The preparation produced measurable active twitch contractions in rat, piglet, and human LVAD tissue, demonstrating contractile output across species and in failing human myocardium. A rat slice maintained paced contraction for 24 hours, and a piglet slice retained function across days 1-3, supporting sustained viability and functional stability.

Conclusion
This data establishes the technical feasibility of a living myocardial slice platform that preserves contractile behavior across rat, piglet, and human tissue. A validated preparation provides the foundation for planned studies integrating tissue level mechanics with biochemical measurement of regulatory contractile proteins.

Spatially Derived Hepatocytes Maintain Metabolic Characteristics in Cell Culture

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BACKGROUND: Hepatocytes exhibit remarkable functional heterogeneity along the radial axis of the liver lobule. Perturbations in this spatial compartmentalization contribute to metabolic dysfunction-associated steatotic liver disease (MASLD). Herein, we aim to define hepatocyte zone-specific functions using proteomic, lipidomic, and click chemistry-based approaches.

METHODS: Zone-specific hepatocytes were identified by single nuclei RNA-sequencing and were isolated using a lineage-tracing approach by crossing glutaminase2 (*Gls2*; a periportal marker)-*CreER* with *TdTomato*-reporter mice. Periportal (PPHs) and pericentral hepatocytes (PCHs) were isolated from collagenase-perfused livers using fluorescence-activated cell sorting (FACS). Sorted hepatocytes were then used for bulk RNA-sequencing, proteomics, lipidomics, and for functional readouts of fatty acid uptake and oxidation.

RESULTS: FACS analysis revealed *Gls2*(+) PPHs represent ~40% of the total hepatocyte pool in mouse liver. Zonal-specific markers *Acly*, *Cps2*, *Cyp2f2*, *Gls2*, and *Pck1* were enriched in PPHs while *Oat*, *Cyp1a2*, *Cyp7a1*, *Cyp2e1*, and *Glul* were enriched in PCHs, indicative of successful zone-specific hepatocyte isolations. Integration of bulk RNA-sequencing data from post-sorted hepatocytes with snRNA-sequencing data from mouse liver revealed significant overlap (R^2=0.71) of genes differentially expressed across the PP-PC axis. Consistent with gene expression patterns, proteomic analysis revealed PCHs were enriched in proteins related to xenobiotic metabolism (Cyp3a11, Cyp2e1), bile acid synthesis (Cyp7a1, Cyp27a1), and fatty acid metabolism (Acot1, Cd36). Functionally, PCHs exhibited greater fatty acid uptake (BODIPY-FL-C16 & C18-azido) which coincided with greater PLIN2-associated lipid droplet accumulation as compared to PPHs. Meanwhile, PPHs were largely protected from lipid droplet accumulation which coincided with increased acylcarnitine lipids and enrichment of proteins related to amino acid metabolism (Ass1, Ftcd), glutathione detoxification pathways (Gsta1, Sqor), and mitochondrial energy and redox metabolism (Ndufv3, Bdh2).

CONCLUSION: Taken together, our study shows PCHs exhibit a greater capacity for fatty acid uptake and storage into lipid droplets thereby providing a mechanistic explanation as to why early-onset MASLD often presents as a pericentral disease.

Aspirin Partially Preserves Cardiac Function During Recurrent Hypoglycemia in Streptozotocin-Induced Diabetic Rats

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Introduction: Recurrent hypoglycemia (RH) remains a major barrier to intensive insulin therapy and is associated with increased cardiovascular morbidity and mortality[1]. Emerging evidence suggests that RH promotes endothelial dysfunction, platelet activation, and a prothrombotic state, potentially contributing to cardiac electrical and mechanical dysfunction[2,3]. Aspirin may mitigate these effects through irreversible inhibition of platelet cyclooxygenase-1 and thromboxane A_2 synthesis[4].

Hypothesis: RH promotes progressive cardiac electrical and mechanical dysfunction in diabetes, whereas aspirin treatment attenuates these pathological changes.

Methods: Adult male Sprague Dawley rats were assigned to control (Ctl; n=6), streptozotocin-induced diabetes with recurrent insulin treatment without hypoglycemia (STZ-RI; n=5), STZ with RH (STZ-RH; n=8), or STZ-RH with aspirin (STZ-RHA; n=8). All STZ-treated rats received Linplant insulin pellets (approximately 0.5-1 U/24 h). RH was induced 3 times per week for 5 weeks by insulin administration to lower blood glucose to <70 mg/dL, after which food was returned. STZ-RHA rats received aspirin (6 mg/kg) in drinking water, provided morning and evening for 5 weeks. Body composition (EchoMRI), electrocardiography, echocardiography, heart-rate variability (HRV), and hematologic parameters were assessed at baseline and after 5 weeks. Statistical analysis included one-way ANOVA, two-way repeated-measures ANOVA, or mixed-effects models, as appropriate. Statistical significance was defined as $P<0.05$.

Results: After 5 weeks, aspirin partially preserved lean body composition during RH. Mean platelet volume was increased in the STZ-RI and STZ-RH groups and reduced by aspirin treatment, suggesting attenuation of diabetes- and RH-associated platelet activation. STZ-RH increased cardiac autonomic variability, with only limited modification by aspirin. RH also increased T-wave amplitude and ST-segment height, while aspirin significantly reduced T-wave amplitude. Aspirin partially preserved left ventricular systolic function: ejection fraction and fractional shortening were significantly higher in STZ-RHA rats compared to STZ-RH rats. Cardiac output was reduced in the STZ-RI and STZ-RH groups but preserved in the aspirin-treated group.

Conclusion: Aspirin partially attenuates electrical abnormalities and preserves left ventricular systolic function and cardiac output in diabetic rats subjected to RH. These findings support further investigation of antiplatelet therapy as a strategy to reduce cardiovascular dysfunction associated with RH in diabetes.

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A platelet conundrum: the dispensability of hemostasis in wound healing

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Background: As first-line responders and vascular guardians, platelets are essential for preserving the vascular barrier by maintaining hemostasis with a more crucial role for dense granule compared to α-granule cargo release. We showed that release of α-granule proteins is important for normal wound healing, beyond the initial hemostasis phase (Coenen *et al.*, 2026). Others have shown that increased vascular permeability accelerates wound healing. In this study we investigate the specific roles of platelet α- and dense granule release in sustaining vascular integrity and its subsequent effects on skin wound healing.
Methods: Two dorsal 4-mm full-thickness circular excisions were made on 8-to-10-week-old male and female mice with defects in dense granule release (Munc13-4^{Jinx} mice), α-granule cargo packaging (Nbeal2$^{-/-}$ mice), and C57BL/6J wildtype mice. Wounds were measured daily with calipers to assess healing progression. Laser Speckle Contrast Imaging (LSCI) was used to assess blood flow in the (healing) wounds and surrounding tissue over time. On days 3 and 7, mice were retro-orbitally injected with Evans Blue dye to assess vascular leakage, after which wound sites were harvested for histology.
Results: In line with our previous study, wound healing in Nbeal2$^{-/-}$ mice, which have defective platelet α-granule biogenesis and cargo packaging, was severely impaired. Strikingly, Munc13-4^{Jinx} mice, lacking dense granule release and having attenuated α-granule release, exhibited similar or even slightly faster wound healing compared to wild-type mice, despite their extreme bleeding phenotype. Using LSCI, we defined explicit areas to identify flow patterns in **1)** the center of the wound (“wound”; includes the center and one to two mm distance from the center in every cardinal direction averaged along the y-axis [North-South] and x-axis [West-East]), **2)** the near direct surroundings of the wound (“edge”; three to four mm distance from the center), and **3)** the healthy skin away from the wound (“periphery”; five to seven mm distance from the center). Preliminary data (n = 3 per strain) show an overall increased blood flow in the center of the wounds directly after wounding, with significant higher values in Munc13-4^{Jinx} mice compared to wild-type. Interestingly, the perfusion rates before injury were already increased in both Munc13-4^{Jinx} and Nbeal2$^{-/-}$ mice compared to wild-type, particularly in areas considered to be the wound edge and periphery of the wounds. Hours after wounding, the edge and peripheral blood flow in Nbeal2$^{-/-}$ mice lowered, returning to perfusion rates as seen in wild-type mice. However, in Munc13-4^{Jinx} mice, flow was continuously and significantly increased until five days post-wounding, when it quickly reduced, even dropping below wild-type levels. Colorimetric analysis of skin-extracted Evans Blue dye corroborated this with significantly increased extravasation in Munc13-4^{Jinx} compared to Nbeal2$^{-/-}$ mice three days after wounding ($p = 0.0084$). Seven days post-wounding, the extent of Evans Blue in and around the skin tissue of Munc13-4^{Jinx} mice decreased, returning to similar levels as wild-type mice, suggesting the progression of angiogenesis and subsequent tissue repair.
Conclusion: Together, our data show that release of platelet α-granule cargo is important for normal wound healing, while dense granules, and thus platelets’ hemostatic functions, are less so, or even prejudicial. Ablation of dense granule release was accompanied by increased vascular leakage and (peripheral) perfusion, but whether this advances wound healing by extravascular platelet migration, leakage of circulatory proteins in the tissue, or removal of harmful components from the tissue into the circulation, still needs to be further investigated.

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Intranasal Tin Exposure Exacerbates Allergic Rhinitis Through Mucin-Mediated Retention

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Abstract

Background: Information on the health effects of airborne particle components is limited. Recent studies have shown that intranasal exposure to lead (Pb) exacerbates allergic rhinitis symptoms. However, the effect of intranasal tin (Sn) exposure, a homolog of Pb with similar chemical properties, on AR remains unexplored.

Objective: This study integrated clinical and experimental approaches to clarify the airway distribution of Sn after intranasal exposure and its impact on AR symptoms.

Methods: During the pollen season, the symptom scores and Sn levels in the nasal epithelial lining fluid (nELF) and serum were determined in patients with Japanese cedar pollinosis (JCP), a representative form of seasonal AR, and healthy controls. The effect of intranasal exposure to a putative human-equivalent dose, of Sn on allergic symptoms and airway Sn distribution was examined in an allergen-induced AR mouse model.

Results: Univariate analysis showed 3.4- and 1.4-fold higher Sn levels in the nELF and serum of the in JCP patients than in the controls. Multivariate analysis revealed significant positive associations between symptom scores and Sn levels in the nELF, but not in the serum. In AR model mice, intranasal Sn exposure via nasal drops resulted in sustained elevations of Sn in nELF, serum and nasal mucosa, along with increased mucin density. Mucin-Sn colocalization suggests that mucin contributes to prolonged intranasal Sn retention. Correspondingly, AR mice with elevated mucin levels exposed to Sn-containing PM2.5-like aerosols exhibited higher intranasal Sn and lower intrapulmonary Sn levels.

Conclusion: PM2.5-derived intranasal Sn may exacerbate AR symptoms via mucin-mediated retention.

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Low phosphorylation of myofilament proteins predicts diastolic deceleration time in a cohort of 90 patients

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Diastolic dysfunction is an important clinical problem that remains poorly understood. It is characterized in clinical settings through detailed analysis of echocardiograms. Relatively few studies have investigated potential relationships between patients' diastolic function and the biochemical status of their myocardium.

Myocardial samples were procured from the middle transmural region of the left ventricular free wall from a cohort of 90 patients who had advanced heart failure or were organ donors. 80% of the patients had an ejection fraction below 40%. 67% of the cohort had an elevated left atrial pressure consistent with diastolic function. Experiments performed with the samples included histological staining for collagen, gel electrophoresis to quantify titin isoforms and the relative phosphorylations of troponin I and regulatory light chain, and western blotting to determine the relative phosphorylation of myosin binding protein-C.

In myocardium from patients with heart failure, fibrosis was elevated and regulatory proteins were less phosphorylated than in samples from organ donors. No strong relationships were observed between any biochemical measure and the E to A, E to e', or isovolumic relaxation time indices of diastolic function.

The deceleration time of flow through the mitral valve quantifies how long pressure takes to equilibrate across the valve during diastole. This clinical parameter was negatively correlated with myocardial collagen content and positively correlated with the relative phosphorylation of troponin I and myosin binding protein-C. A composite metric quantifying the combined phosphorylations of troponin I and myosin binding protein-C predicted the deceleration time with $p < 0.001$.

Ongoing studies are using multiscaled computer modeling to investigate the physiological and pathophysiological significance of these new findings.

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Title: *KCNH2* Promoter Activity is Temperature Dependent

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Background: The *KCNH2* gene encodes the Kv11.1 K^+ channel, which conducts the rapidly activating delayed rectifier K^+ current (I_{Kr}). Drug block of I_{Kr} or loss-of-function *KCNH2* mutations are associated with long QT syndrome and an increased risk of sudden cardiac death. Several studies suggest that fever can exacerbate QT interval prolongation and the risk of Torsades de Pointes, implicating temperature-sensitive regulation of *KCNH2* expression.

Objective: To determine whether *KCNH2* promoter activity is temperature sensitive.

Methods**:** We cloned the conserved human *KCNH2* promoter upstream of a luciferase reporter and expressed constructs in C2C12 myoblasts. Bioluminescence was recorded at 10-minute intervals for 7–10 days under a static 37°C or oscillating temperature (36.5–38.5°C, 24-hour period). Promoter variants were generated via restriction enzyme digestion, targeted deletions, and site-directed mutagenesis to introduce naturally occurring single-nucleotide polymorphisms (SNPs) within a highly conserved tandem E-box element. Cross-correlation analysis was used to identify the relationship between temperature and promoter activity.

Results: Compared with cells cultured at 37°C, cells exposed to oscillating temperature exhibited a large increase in overall human *KCNH2* promoter activity (37.66±16.78 counts/sec vs 15.39±6.08 counts/sec, $p < 0.0001$, $n = 19$ and $n = 14$, respectively). Cross-correlation analysis between incubator temperature and *KCNH2* promoter activity demonstrated a moderate positive correlation ($r = 0.498$, $n = 14$). We identified a highly conserved putative cis-regulatory region in the *KCNH2* promoter consisting of tandem E-box elements upstream of the exon 1 start site. Deleting the tandem E-box element decreased overall *KCNH2* promoter activity by 91.6% under oscillating temperature conditions ($p < 0.0001$, $n = 5$). We also engineered 4 naturally occurring variants identified within the tandem E-box element. 2 of the 4 variants did not alter overall *KCNH2* promoter activity ($p > 0.05$, $n = 15$ and $n = 18$). However, one variant increased overall activity by 40.2% ($p <0.001$, $n = 15$), and another variant decreased overall activity by 68.8% ($p <0.01$, $n = 12$).

Conclusion: *KCNH2* promoter activity is modified by physiological temperature changes that mimic daily oscillations in core body temperature. A conserved tandem E-box element in the *KCNH2* promoter is critical for this regulation. Together, these data suggest that physiological temperature modifies *KCNH2* promoter activity via a conserved cis-regulatory mechanism.

Direct Cardiomyocyte Effects of Interleukin-11 in Wild-Type

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Aims: Inflammatory signals mediate progression to heart failure. The RRAD-L-type calcium channel signalosome is a potential therapeutic target for reversing heart failure progression. Recent studies have shown that interleukin-11 (IL-11) mediates cardiac remodeling and left ventricular dysfunction. Research has also shown that IL-11 increases RAD transcription, which is expected to reduce cardiac performance. We sought to determine whether acute IL-11 exposure directly alters calcium handling and contractile function in isolated cardiomyocytes and whether IL-11-induced dysfunction requires transcription and translation.

Methods and results: Adult cardiomyocytes were isolated and incubated at 37°C with IL-11. Using Fura-2 AM fluorescence imaging with the IonOptix system, calcium transients and sarcomere dynamics were assessed. IL-11 impaired isolated cardiomyocyte calcium handling and contractility after 4 hours of exposure. In vivo experiments further demonstrated that IL-11 reduction of cardiac performance requires RAD. To investigate the mechanisms underlying this dysfunction, cardiomyocytes were treated with actinomycin D (ActD), a transcription inhibitor, or cycloheximide (CHX), a translation inhibitor, during IL-11 exposure. Inhibition of transcription with ActD significantly preserved aspects of cardiomyocyte function, indicating that transcription contributes to IL-11-induced dysfunction. However, inhibition of translation with CHX produced greater preservation of calcium handling and contractile function, suggesting that translation is more strongly required than transcription for IL-11-induced cardiomyocyte dysfunction.

Conclusion: IL-11 directly impairs cardiomyocyte calcium handling and contractile function. Both transcription and translation contribute to the development of this dysfunction; however, our findings indicate that new protein synthesis through translation plays a greater role in mediating the functional effects of IL-11. Transcription inhibition partially preserved cardiomyocyte function, while translation inhibition produced greater protection, suggesting that IL-11-induced dysfunction is more dependent on translation than transcription. Future studies will investigate the effects of IL-1β, a cytokine rapidly induced following myocardial injury, in isolated cardiomyocytes and may further explore the specific proteins synthesized through translation that contribute to cytokine-induced cardiac dysfunction.

Asprosin Deficiency Impairs β-Cell Secretory Function in *Fbn1*$^{NPS/+}$ Mice

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Background: Neonatal Progeroid Syndrome (NPS) is a rare genetic disorder caused by pathogenic variants in *FBN1*, resulting in deficiency of novel metabolic hormone, Asprosin. Although asprosin is recognized as an important regulator of systemic glucose metabolism and appetite regulation, its physiological role in pancreatic β-cell function remains poorly understood. Whether chronic endogenous asprosin deficiency alters β-cell secretory function or glucose-regulated islet-derived asprosin secretion has not been investigated. Therefore, we examined pancreatic β-cell function and islet-derived asprosin secretion in *Fbn1*$^{NPS/+}$ mice.

Methods: To investigate the effect of asprosin deficiency on β-cell function, *Fbn1*$^{NPS/+}$ mice and wild-type (*Fbn1*$^{+/+}$) littermate controls were studied at 8 weeks of age. In vivo glucose-stimulated insulin secretion (GSIS) was assessed following intraperitoneal glucose administration (3g/kg), and plasma insulin concentrations were measured before and after glucose challenge. In a separate experiment, pancreatic islets were isolated and ex vivo GSIS analysis under low- (2 mM) and high-glucose (16.7 mM) conditions was also performed.

Results: Compared with wild-type mice, *Fbn1*$^{NPS/+}$ mice exhibited reduced body weight, lower fasting insulin concentrations, impaired insulin secretion during in vivo GSIS. Isolated islets from Fbn1$^{NPS/+}$ mice also showed reduced stimulation index and significantly lower pancreatic islet count, indicating impaired β-cell secretory function. Interestingly, high glucose significantly increased islet-derived asprosin secretion in wild-type islets, whereas this glucose-responsive increase was absent in *Fbn1*$^{NPS/+}$ islets. Intracellular asprosin levels in islet lysates were not significantly different between groups.

Conclusions: Chronic endogenous asprosin deficiency is associated with impaired β-cell secretory function. These findings identify pancreatic islet dysfunction as a previously unrecognized feature of NPS and provide new mechanistic insight into the role of endogenous asprosin in maintaining β-cell function.

Keywords: Asprosin; β-cell function; Glucose-stimulated insulin secretion; Pancreatic islets

MyoProfiler: Open-source software to analyze patterns of fluorescently labeled sarcomeric proteins

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MyoProfiler is open-source software that was developed to extract and analyze striated patterns of fluorescently labeled contractile proteins. It combines image analysis and segmentation to quantify sarcomere length, localization, and structural metrics within the user selected region of interest. The software calculates metrics automatically and accelerated the team's previous workflow by a factor of 10. The investigators have used the software to analyze fluorescence patterns associated with myosin, regulatory light chain, myosin binding proteins, and myomesin.

MyoProfiler extracts intensity profiles from experimental images. Sarcomere length is calculated from the distance between adjacent Z lines. The full-width of A-band patterns can also be measured. The straightness of Z lines can be quantified via the tortuosity metric which is defined as the ratio of a curve's length to the straight-line distance between the curve's endpoints. In this case, the curves are smoothing splines fitted to each Z-line. The Z-lines are identified by segmenting the raw image to obtain binary objects and then linking adjacent objects appropriately through an iterative process.

MyoProfiler analyzes each channel in multi-layered images to better support colocalization studies. Raw data, metadata, and analysis workflows are combined into a single open-format file to improve reproducibility. The software has a user-friendly interface and works with proprietary microscope image formats, including ND2 files, as well as open-formats, such as PNG and TIF.

Title: Impacts of chronic thoracic spinal cord injury on cardiometabolic pathology in a porcine model

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While cardiometabolic disease is a leading cause of morbidity and mortality globally, disease risk is significantly elevated in people living with spinal cord injury (SCI). Compared to the able-bodied population, the SCI population experiences higher rates of cardiovascular and metabolic disease including atrial fibrillation, coronary artery disease, heart failure, stroke, insulin resistance, diabetes, dyslipidemia, and metabolic dysfunction-associated liver disease. This may be explained by secondary effects of SCI, such as rapid and sustained body composition remodeling and changes to metabolic profile, including skeletal muscle atrophy, increased intramuscular adipose tissue, increased whole-body adiposity, and changes to lipid panel and glucose transport. Despite the prevalence and severity of cardiometabolic disease in the SCI population, chronic, post-SCI cardiovascular and hepatic pathology has yet to be captured in a large-animal model. Our porcine model (adult Yucatan pigs) offers a novel and reliable approach to investigating chronic, post-SCI cardiometabolic pathology pre-clinically because pigs have lipid profiles, overall body size, cardiovascular anatomy, and metabolic and immune responses that are highly comparable to humans. Pigs were uninjured or received thoracic SCI (T8 to T9 level, weight-drop contusion compression model), were euthanized 12 weeks post-surgery date, and hearts and livers were collected. First, we investigated hearts with micro-CT scanning to observe whether SCI induced left ventricular remodeling. Next, we seek to analyze the livers and hearts of our sample from three histological perspectives—inflammation, lipid accumulation, and fibrosis—which are hallmarks of cardiometabolic pathology. We predict left ventricular atrophy to be present in the SCI hearts because this has been shown to occur post-SCI in humans due to cardiac deconditioning and lower total blood volume. We predict inflammation, lipid accumulation, and fibrosis to be greater in the SCI heart and liver tissue given the systemic immune response and metabolic remodeling that occurs post-SCI. Experiments are ongoing to test these hypotheses. The overall goal of this project is to lay the foundation for translational mechanistic studies and preclinical testing of therapies targeting SCI-associated cardiometabolic disease.

Angiotensin II Exacerbates Aortopathies in Mice with Fibrillin-1 Deficiency in Smooth Muscle Cells

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Abstract

Background: Fibrillin-1 (FBN1) is essential for the structural integrity of the aortic wall. Our recent study revealed that FBN1 deficiency in smooth muscle cells (SMCs) induces aortic aneurysms, predominantly affecting the distal portion of the ascending aorta and aortic arch. This study aimed to determine whether angiotensin II (AngII) augments aortic pathologies in mice with FBN1 deficiency in SMCs.

Methods: Male *Sm22α*-Cre$^{+/0}$;*Fbn1* floxed mice were bred with female *Sm22α*-Cre$^{0/0}$; *Fbn1* floxed mice to generate SMC-FBN1-deficient mice (SMC-FBN1 -/-: *Sm22α*-Cre$^{+/0}$; *Fbn1*$^{fl/fl}$) and their wild-type littermates (SMC-FBN1 +/+: *Sm22α*-Cre$^{0/0}$;*Fbn1*$^{fl/fl}$). Vehicle (saline) or AngII (1 or 0.5 μg/kg/min) was infused into 7-8-week-old mice for 28 days. At study termination, micro-computed tomography was used to visualize aortic pathologies and quantify aortic diameters, lengths, and volumes. Both male and female mice were studied.

Results: Infusion of AngII at a rate of 1 μg/kg/min resulted in >70% death due to aortic rupture in either the thoracic or abdominal region of SMC-FBN1 -/- mice of both sexes. Infusion at 0.5 μg/kg/min led to mortality rates of 27% in males and 50% in females. In addition to pathologies in the ascending aorta and aortic arch, prominent pathological features were observed in surviving AngII-infused SMC-FBN1 -/- mice, including: (1) increased total aortic length; (2) pronounced pathological changes in the descending and suprarenal aortic regions, with the infrarenal aorta relatively spared; (3) branch aneurysms involving the celiac and superior mesenteric arteries; and (4) cardiac enlargement, including enlarged hearts or left atria, observed in approximately 64% of males and 25% of females.

Conclusions: AngII infusion exacerbates aortic pathology in SMC-FBN1 -/- mice, manifested by aortic rupture, increased aortic tortuosity, widespread aortic aneurysms, aneurysms of the celiac and superior mesenteric arteries, and cardiac enlargement.

Keywords: smooth muscle cell; FBN1; extracellular matrix; aortopathy; AngII; mouse

Tranexamic Acid Reduces Angiotensin II-induced Cardiac Injury in PAI-1 Deficient Mice

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Background

Deficiency of plasminogen activator inhibitor-1 (PAI-1) leads to a bleeding disorder and is associated with cardiac fibrosis in humans. This phenotype is recapitulated in aged or angiotensin II (AngII)-infused PAI-1 deficient (PAI-1-/-) mice. PAI-1 is the primary inhibitor of the plasminogen activation system (PAS), thereby promoting hemostasis through its reactive center loop (RCL) domain. In contrast to the heart, PAI-1 deficiency protects against fibrosis in the lung through its non-inhibitory somatomedin B (SMB)-binding domain. Notably, the mechanism underlying augmented cardiac fibrosis in PAI-1 deficiency remains unclear, and no effective therapy has been identified.

Methods and Results

AngII was infused for 4 weeks into PAI-1-/- mice and wild-type littermates (PAI-1+/+) to induce cardiac fibrosis. PAI-1-/- mice displayed marked replacement fibrosis predominantly within the epicardium and posterior septum. Ferric iron, indicative of prior hemorrhage, was colocalized with fibrosis. Similar phenotypes were observed in PAI-1-/- mice infused with norepinephrine for 4 weeks. To define the pathological events preceding cardiac fibrosis, either AngII or norepinephrine was infused for 1 week in PAI-1+/+ or -/- mice. Both infusions induced extensive epicardial hemorrhage and posterior septal fibrosis in PAI-1-/- mice. To explore the initiation of cardiac pathology, AngII was infused for approximately 1 day. PAI-1-/- mice developed diffuse hemorrhage and cardiomyocyte injury localized to the posterior septum, pathologic changes that preceded overt fibrosis. To determine the protective domain of PAI-1, saline or AngII was administered to mice harboring loss-of-function point mutations in the RCL (PAI-$1^{Ala/Ala}$) or SMB-binding domains (PAI-$1^{AK/AK}$). Compared to saline infusion, 1 week of AngII induced hemorrhage and heterogeneous fibrosis in PAI-$1^{Ala/Ala}$, but not PAI-$1^{AK/AK}$ mice. To evaluate the therapeutic potential of tranexamic acid (TXA), an FDA-approved PAS inhibitor, against cardiac injury, PAI-1+/+ and -/- mice received vehicle or TXA (50 mg/mL) in drinking water beginning 5 days before and continuing throughout 1 week of AngII infusion. Plasma plasminogen activation was reduced by approximately 75% in TXA-administered PAI-1-/- mice. Vehicle-administered PAI-1-/- mice displayed gross and histologically evident cardiac hemorrhage, ferric iron deposition, and fibrosis. In contrast, TXA-administered PAI-1-/- mice had minimal cardiac hemorrhage and reduced ferric iron deposition and fibrosis.

Conclusion

These findings support the notion that under hemodynamic stress, PAI-1 deficiency promotes early cardiac hemorrhage and cardiomyocyte injury that leads to fibrosis. Studies in loss-of-function PAI-1 mutants and PAI-1-/- mice administered TXA implicate dysregulated plasmin generation as an initiator of cardiac injury and fibrosis. Furthermore, these studies indicate that TXA may mitigate cardiac injury in human PAI-1 deficiency.

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RAD Deletion Enhances L-Type Calcium Channel Activity and Promotes Sarcoplasmic Reticulum Calcium-Handling Adaptations

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Introduction: RAD is a small GTPase that tonically suppresses cardiac L-type calcium channel CaV1.2 activity. Consistent with this role, cardiomyocyte-specific RAD knockout markedly increases basal L-type calcium current in mouse ventricular cardiomyocytes. Increased calcium entry may alter downstream calcium-dependent signaling and sarcoplasmic reticulum (SR) calcium cycling. Because phosphorylation-dependent signaling is sensitive to tissue collection and processing conditions, we generated a human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) model of RAD deletion. We hypothesized that RAD deletion would accelerate calcium handling and remodel molecular regulators of SR calcium uptake and buffering.

Methods: hiPSC-CMs were transduced with a MyoAAV vector encoding Cas9 and a RAD-targeting single-guide RNA. RAD loss was confirmed by western blotting. Calcium transients were recorded using GCaMP6f and analyzed for amplitude, time-to-peak, 50% decay time (T50 off), and calcium-transient duration at 90% recovery (CTD90). Protein abundance and phosphorylation were assessed by western blotting. CaMKIIδ activation was evaluated by autophosphorylation at Thr287, while phospholamban (PLN) phosphorylation at Thr17 was measured as a CaMKII-sensitive downstream readout. Calsequestrin abundance was assessed as a potential indicator of SR calcium-buffering adaptation.
Results: Cardiomyocyte-specific RAD deletion markedly increased basal L-type calcium current density in mouse ventricular cardiomyocytes. RAD-knockout hiPSC-CMs also exhibited faster calcium-transient kinetics than wild-type cardiomyocytes, with shorter time-to-peak ($p = 0.0052$), T50 off ($p = 1 \times 10^{-4}$), and CTD90 ($p = 1 \times 10^{-4}$). Calcium-transient amplitude was unchanged, indicating that RAD deletion primarily affected calcium kinetics rather than transient magnitude. These findings recapitulate the accelerated calcium-handling phenotype observed in murine RAD-knockout cardiomyocytes and support a conserved effect of RAD loss across mouse and human models. RAD deletion also increased PLN-Thr17 phosphorylation without altering steady-state CaMKIIδ-Thr287 autophosphorylation. Calsequestrin abundance was significantly increased in RAD-knockout hiPSC-CMs ($p = 0.0127$), suggesting a potential adaptation in SR calcium-buffering capacity.

Conclusion: RAD deletion accelerates calcium-transient kinetics and remodels molecular regulators of SR calcium uptake and buffering in hiPSC-CMs. Increased PLN-Thr17 phosphorylation and calsequestrin abundance occurred without detectable changes in steady-state CaMKIIδ autophosphorylation. Whether transient or compartment-specific CaMKIIδ activity contributes to these adaptations remains unresolved.

Evaluating Circadian Synchronization Approaches in Human iPSC-Derived Cardiomyocytes

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ABSTRACT:

Circadian rhythms govern fundamental aspects of cardiomyocyte biology, including metabolism, ion channel expression, and contractility. Disruption of these rhythms is increasingly associated with cardiac disease. At the cellular level, these rhythms are sustained by cell-autonomous transcriptional-translational feedback loops (TTFLs), which allow peripheral tissues, including the heart, to maintain robust circadian oscillations independent of the central pacemaker in the suprachiasmatic nucleus (SCN). Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) offer a reliable and clinically relevant platform to investigate the molecular mechanisms underlying circadian regulation of cardiac function *in vitro*. However, to do this, cells must first be synchronized to induce measurable rhythms across the culture. Without synchronization, individual cells may oscillate at different phases, making the overall rhythm difficult to detect. Synchronization approaches, including serum shock and drugs such as dexamethasone and forskolin, are commonly used to synchronize different cell types. However, these approaches can activate cellular signaling pathways and alter the cellular mechanisms or processes being studied. Hence, there is a need to establish a reliable synchronization method for hiPSC-CMs that minimizes pathway-specific effects. Therefore, we evaluated the effects of electrical pulse stimulation and temperature cycling as possible approaches to synchronize circadian rhythms in hiPSC-CMs.

The Role of Vascular Smooth Muscle Cell-specific PAR2 Deletion in AAA Pathogenesis

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Abdominal aortic aneurysm (AAA) is a progressive vascular disease characterized by chronic inflammation, extracellular matrix degradation, and phenotypic switching of vascular smooth muscle cells (VSMCs), ultimately leading to weakening and dilation of the aortic wall. Previous work from the Owens laboratory demonstrated that global deletion of protease-activated receptor 2 (PAR2) attenuates AAA development. Using bone marrow transplantation, we demonstrate this effect is via non-hematopoietic cell populations, with RNA sequencing suggesting VSMCs as a major mediator of PAR2-induced AAA. As such, we hypothesized that VSMC-specific deletion of PAR2 would reduce AAA progression by limiting VSMC phenotypic switching and vascular inflammation. To test this, $Par2^{flox/flox}$ Myh11-$RAD^{CreERT2}$ Cre^{+} (VSMC deletion of PAR2) and Cre^{-} littermate controls were fed a tamoxifen diet (250mg/kg) for two weeks and then underwent topical elastase-induced AAA formation (10mg/mL elastase) on their infrarenal aortas. Aneurysm progression was monitored by serial ultrasound imaging (Vevo F2, Visual Sonics) over four weeks, followed by histological (Verhoeff Van Gieon and Picrosirius Red) and immunofluorescence analyses (αCD68 and αSMA) to evaluate collagen deposition, macrophage accumulation, and VSMC integrity. While both genotypes developed significant aneurysm expansion after elastase, VSMC-specific PAR2 deletion produced only a modest reduction in absolute aortic diameter growth and did not significantly change overall aneurysm progression. Collagen deposition, macrophage infiltration, and VSMC structural integrity were similar between Cre^{+} and Cre^{-} mice. These data indicate that VSMC-specific PAR2 deletion alone is insufficient to attenuate AAA in the topical elastase model, implicating other PAR2-expressing cell types in disease progression. These findings refine the cell-specific role of PAR2 in AAA pathogenesis and will help direct future studies to identify the vascular cell populations mediating PAR2-dependent effects.

An integrative CRISPR/Cas9 and GWAS approach identifies novel regulators of LDL uptake

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Sustained high levels of LDL cholesterol (LDL-C) in plasma are a major driver of atherosclerotic cardiovascular disease (ASCVD). Thus, patients with genetically elevated LDL-C are at the highest risk for ASCVD due to lifelong exposure to elevated LDL-C. Prompt diagnosis and treatment of genetic hypercholesterolemia are key to reducing ASCVD risk. However, our incomplete knowledge of all the contributors to LDL-C challenges the diagnosis of genetically elevated LDL-C. In recent years, genome-wide association studies (GWAS) have identified hundreds of candidate genes, but the function of most remains unknown. We previously applied a whole-genome CRISPR/Cas9 knockout (KO) screen and identified TAGLN as a new gene involved in LDL metabolism, validating it through in vitro and in vivo studies as well as in GWAS datasets. Nonetheless, CRISPR screens can be noisy, and identifying human-relevant targets among the high-scoring hits remains challenging. To address these limitations, we implemented a gene-scoring system that integrates CRISPR screen–derived parameters with genomic associations from GWAS databases to prioritize hits from a whole-genome CRISPR screen comparing cells with high versus low LDL uptake. Our goal is to identify novel regulators of LDL uptake with clinical relevance.

Methods: Cas9-expressing HepG2 cells were transduced with a lentiviral library containing 76,441 guide RNAs (gRNAs; 4 per 19,114 protein-coding genes) at an MOI of 0.4, then incubated with fluorescently labeled human LDL and sorted by FACS. The top 5% (brightest) and bottom 5% (dimmest) cells were collected for DNA extraction. The integrated lentiviral genome was PCR-amplified and deep-sequenced, and gRNA and gene enrichment were analyzed using MAGeCK. Associations with lipid traits (LDL-C, total cholesterol [TC], apolipoprotein B [apoB], triglycerides [TG], and HDL-C) were obtained from public databases including GRASP (NHLBI), the GWAS Catalog, and the Common Metabolic Disease Knowledge Portal.

Results: Genes with negative Log Fold Change (LFC) were considered essential for cellular LDL uptake, whereas those with positive LFC were negative regulators of LDL uptake. Notably, LDLR and MYLIP (IDOL) are at opposite extremes, with LFCs of -3.79 and 3.26, respectively. Genes were assigned a screen score based on (1) the number of gRNAs with a significant effect on LDL uptake and (2) the magnitude of the absolute LFC. The LFC contribution was scored as follows: 0 if LFC<2.0; +1 if 2.0=LFC<2.50; +2 if 2.5=LFC<3.0; +3 if 3.0=LFC<3.5; or +4 if LFC=3.50. For example, LDLR had four significant gRNAs and an absolute LFC >3.5, receiving the maximum score of 8.

A total of 932 genes with screen scores =3 were evaluated in GWAS datasets. Genes received additional points based on significant associations with lipid traits: +5 for LDL-C, +3 for total cholesterol (TC), +3 for apoB, +1 for triglycerides (TG), and -1 for HDL-C. Points were cumulative across traits. Aligned with its key role in LDL uptake, LDLR received the highest combined score. With

a total score >15, 27 genes were identified as high-confidence hits. These included the LDLR and multiple genes with known roles in LDLR stability or LDL internalization (e.g., MYLIP, ASGR1, STAB1, and TAGLN), as well as less-characterized candidates such as DMTN, TFPI, BAIAP2L1, TRAFD1, and RRBP1, suggesting potential novel regulators of LDL uptake.

Conclusion: This integrative approach, combining CRISPR screening with GWAS data, improves candidate selection from functional screens by prioritizing genes supported by both experimental and human genetic evidence, thereby increasing the likelihood of identifying clinically relevant regulators of LDL metabolism. This approach captured key components of LDL metabolism, including LDLR, and also identified novel candidate regulators of LDL uptake. The resulting high-confidence candidates are promising targets for further functional and therapeutic investigation.

Elevated Aldosterone Concentrations are Associated with Blood Pressure and Left Ventricular Hypertrophy in Children at Cardiovascular Risk

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Background: Aldosterone excess is increasingly recognized as a continuum extending beyond overt primary aldosteronism, and is associated with obesity, hypertension, and increased cardiovascular risk in adults. Whether relative aldosterone excess is present in children at cardiovascular risk or associated with early target-organ damage, such as left ventricular hypertrophy (LVH), is unknown.

Methods: We conducted a retrospective, cross-sectional analysis of children aged 6 to 18 years referred for ambulatory blood pressure monitoring (ABPM) through the Pediatric Nephrology Clinic at Golisano Children's at the University of Kentucky. Clinical and laboratory data were abstracted from medical records. Among 449 patients who underwent echocardiography following ABPM (excluded for secondary causes of hypertension, antihypertensive medications, and ABPM readings under 65%) there were 189 with available aldosterone measurements. The cohort was stratified into three groups according to concentration of aldosterone: less than 11 ng/dL (bottom 75%, 138), between 11 – 20 ng/dL (75^{th} – 92^{nd} percentile, 36), and greater than 20 ng/dL (greater than the 92^{nd} percentile, 15), and clinical characteristics compared between groups using one-way ANOVA.

Results: The median aldosterone concentration was 5 (IQR: 2 – 11) ng/dL. Children with the highest concentrations of aldosterone (> 20 ng/dL) had the highest systolic and diastolic pressures, and a graded effect on blood pressure (median and interquartile range) was observed across highest to lowest aldosterone groups: SBP, Day: 145 (137 – 152) vs 141 (132 – 147) vs 139 (132 – 147) mmHg; DBP, Day: 84 (74 – 92) vs 80 (73 – 87) vs 76 (72 – 82) mmHg; $p<0.05$). Lack of nocturnal dipping trended higher with aldosterone group (SBP: 60% vs 47% vs 40%; DBP: 20% vs 19% vs 18%). The incidence of LVH was greater in patients with aldosterone concentrations above the 75th percentile (53% vs 52.3% vs 34%). Median left ventricular mass index was 42.0 (IQR: 33.0 – 52.0) vs 40.0 (32.0

– 49.0) vs 36.0 (30.0 – 43.2) g/m$^{2.7}$, and median BMI was 31.8 (IQR: 23.8 – 33.7) vs 29.2 (23.4 – 36.9) vs 26.4 (22.4 – 31.7) kg/m^2, and trended higher across aldosterone groups.

Conclusions: Elevated concentrations of aldosterone in children are associated with higher blood pressure and incidence of LVH. These findings provide preliminary evidence that the spectrum of relative aldosterone excess increasingly recognized as a cardiovascular risk phenotype in adults may be present in childhood and associated with early target-organ damage. Aldosterone-related biomarkers may therefore identify a mechanistically distinct, potentially treatable cardiovascular risk phenotype in children with obesity and elevated blood pressure.

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Impact of A1c on Postoperative Complications after CABG

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Abstract

Background: Hemoglobin A1c (HbA1c) is a marker to test for chronic glycemia.[1] Recently, Halkos et al published a research paper that established HbA1c usage as being a predicative value for adverse outcomes after CABG.[2] This showed the shift of using HbA1c as diabetes diagnosis and management to be recognized as a preoperative risk stratification tool. With that breakthrough, more research commenced to see the implications of HbA1c levels to complications after CABG. There is a growing interest in assessing HbA1c in relation to coronary artery bypass surgery.

Objective: The objective of this study is to assess the impact of HbA1c on CABG at the University of Kentucky HealthCare (UKHC).

Methods & Materials**:** A retrospective cohort study was conducted using the data from the Society of Thoracic Surgeons (STS) database from 2020 to 2025 under an Institutional Review Board (IRB)-approved protocol. Patients underwent CABG were categorized according to preoperative HbA1c levels as normal (<5.7%), pre-diabetic (5.7%–7.0%, inclusive), and diabetic (>7.0%). Postoperative outcomes (renal failure, respiratory failure, cardiac arrest, mortality, deep sternal wound infection, atrial fibrillation, stroke, and bleeding) were compared across HbA1c groups. Binary variables were compared using a modified Fisher's exact test. Multivariable logistic regression was used to estimate adjusted odds ratios for postoperative outcomes, adjusting for age and sex.

Results: Among 1,509 patients who underwent isolated CABG, the mean age was 62.4 years (S.D. 10.1), 1,139 (75.5%) were male, and 1,344 (89.0%) were White. Of the total cohort, 404 (26.8%) had normal HbA1c levels, 572 (37.9%) had pre-diabetic HbA1c levels, and 533 (35.3%) had diabetic HbA1c levels. The proportions of postoperative respiratory failure (normal, 3.7%; pre-diabetic, 4.7%; diabetic, 8.3%) and stroke (normal, 0.7%; pre-diabetic,1.4%; diabetic, 2.8%) differed significantly across HbA1c groups, whereas no significant differences were observed for the other postoperative outcomes including death. After adjustment for age and sex, patients in the diabetic group had higher odds of respiratory failure (OR, 2.43; 95% CI, 1.36 - 4.59; p= 0.005), and stroke (OR, 4.20; 95% CI, 1.36 - 18.3; p = 0.041) compared with those in the normal HbA1c group.

Conclusion: Stroke and respiratory failure rates after isolated CABG procedures are higher in patients with preoperative A1c >7%.

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Ambient temperature reshapes cardiac autonomic control in *Scn5a*$^{+/-}$ Brugada mice and the ventricular ion channel transcriptome

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Introduction: Brugada syndrome (BrS) is an inherited arrhythmia syndrome caused by loss-of-function *SCN5A* mutations, with expressivity that varies widely among carriers of the same mutation, suggesting non-genetic modifiers of disease penetrance. Ambient temperature is an experimentally tractable environmental variable in mice. Here, we used a *Scn5a*$^{+/-}$ mouse model recapitulating the loss of cardiac sodium current (INa) seen in BrS to test its effect on phenotype.
Hypothesis: We hypothesized that thermoneutral housing, by reducing sympathetic drive and slowing heart rate, would exacerbate the bradycardic phenotype of *Scn5a*$^{+/-}$ mice and produce concordant changes in ventricular autonomic and ion channel transcript expression.
Methods: Male wild-type (WT) and *Scn5a*$^{+/-}$ SV129 mice (n = 5/genotype) were implanted with *in vivo* telemetry devices to record electrocardiogram (ECG) and core body temperature, housed under 12:12-hour light-dark cycles at room temperature (RT; 25 °C), then transferred to thermoneutrality (TN; 30 °C). Cardiac ion channel and autonomic signaling genes show circadian expression rhythms linked to the day-night pattern of arrhythmia risk, so we asked whether ambient temperature also alters this rhythmicity in the ventricle. WT male mice housed at RT (n = 26) or TN (n = 27) were released into constant darkness to remove light-driven masking of endogenous cardiac circadian rhythms in gene expression. Ventricles were collected at 12 time points over 48 hours for bulk RNA sequencing. DESeq2-normalized counts were tested for differential expression and circadian rhythmicity at $p<0.05$.
Results: *Scn5a*$^{+/-}$ mice had lower heart rate (HR) at both RT and TN. Under autonomic inhibition (methylatropine and propranolol), HR was lower in *Scn5a*$^{+/-}$ mice at RT but not at TN. When HR was adjusted for core body temperature (Q_{10} = 2), intrinsic HR was lower in *Scn5a*$^{+/-}$ mice at RT (WT: 520 ± 20; *Scn5a*$^{+/-}$: 483 ± 32), but TN abolished the genotype difference (WT: 435 ± 35; *Scn5a*$^{+/-}$: 440 ± 51). Isolating the temperature-dependent and autonomic components of HR regulation showed that the temperature-dependent component (HR-Tc) was higher in WT than *Scn5a*$^{+/-}$at RT, with no genotype difference in the autonomic component of HR regulation (ΔHR); TN inverted this pattern, unmasking a lower ΔHR in *Scn5a*$^{+/-}$ mice. Relative to RT, TN reduced expression of adrenergic receptor genes (*Adra1a*, *Adra2a*) and cardiac ion channel genes (*Scn5a*, *Kcnj2*, *Ryr2*, *Atp2a2*) and increased autonomic and G-protein signaling genes (*Adcy1*, *Gnaq*, *Gnb3*, *Creb5*, *Ache*). Unlike the differential expression, cosinor regression fits a 24-hour sinusoid to expression values across all 12 time points to test for circadian rhythmicity; applying this to 381 ion channel, ion channel regulatory, and autonomic genes showed a three-fold increase in rhythmically expressed genes at TN (136 vs. 51; 38 shared), with peak expression time clustering at the activity-rest transitions in mice (circadian time 8-12 and 20-24). *Scn5a* transcript was rhythmic only at TN.
Conclusion: Ambient temperature reshapes the phenotype of *Scn5a* haploinsufficiency, shifting genotype differences from a channel-related component of heart rate at RT to an autonomic component at TN. Similarly, a shift from ion channel to autonomic and G-protein signaling transcript changes at thermoneutrality indicates that environmental factors modify arrhythmia expressivity in this Brugada mouse model.

Multi-Omics Analysis of Postprandial Mesenteric Lymph Reveals Role for SAA in Systemic Postprandial Inflammation

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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 the circulation via intestinal lymph. The protein components of CM's can impact their metabolism in the circulation, however, the composition of CM's is difficult to study because these particles are rapidly metabolized upon entry to the circulation.

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

Methods and Results: A conscious lymph fistula procedure was used to collect mesenteric lymph from mice before and after infusion of a mixed lipid bolus into the duodenum. Wild type mice were compared to *Dennd5b*$^{-/-}$ mice (n=5-8 mice/group), a model of impaired chylomicron secretion. This study revealed that, compared to wild type mice, *Dennd5b*$^{-/-}$ mice exhibit significantly reduced TAG content in the lymph (-94%, $p<0.001$). Electron micrographs revealed that the lymph of wild type mice had both greater number and larger sized lipid particles compared to *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 lymph proteome of wild type mice after lipid bolus (14 proteins increased and 18 decreased; $p<0.01$). Key protein changes were confirmed by western blotting. One notable finding was an increase in lymph Saa1 after the lipid bolus (+200%, $p<0.001$). Size-exclusion chromatography was used to determine the distribution of Saa1 across lipoprotein fractions in lymph and demonstrated that Saa1 was associated primarily with fractions that contain chylomicrons and to a lesser extent with HDL-containing fractions. To understand the functional role of Saa association with lipoproteins under postprandial conditions intralipid gavage studies were performed in wild type and Saa$^{-/-}$ mice and plasma triglycerides and plasma IL6 levels were measured over 6 hours. While Saa$^{-/-}$ mice had no significant difference in postprandial plasma triglyceride concentrations compared to

wild type mice, Saa$^{-/-}$ mice have significantly higher postprandial plasma IL6 concentrations compared to wild type mice.

Conclusions: We identified several proteins in intestinal lymph that respond to dietary lipid ingestion. In wildtype mice, lipid feeding increases lymphatic Saa1, which appears to be predominantly associated with chylomicrons. This response was not observed in *Dennd5b*$^{-/-}$ mice, indicating that it is dependent on successful CM secretion from intestinal epithelium. Our studies reveal that Saa attenuates postprandial inflammation, but may not significantly impact postprandial chylomicron metabolism in the circulation.

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The Absence of Rad Elevates Calcium Dependent Facilitation in Adult Cardiomyocytes via CaMKII

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Background: The L-type calcium channel functions as the primary mode of calcium entry during the cardiac action potential. Rad, an RGK protein, confers beta-adrenergic regulation on the channel, providing tonic inhibition that is relieved upon phosphorylation by PKA; resulting in channel modulation. Cardiac restricted deletion of Rad has been demonstrated to provide stable positive inotropy without pathological remodeling in healthy mice, and has proven salutary in a dilated cardiomyopathy model, yet the underlying downstream alterations to channel biophysics and calcium handling--especially critical calcium dependent kinases such as CaMKII-- have not been fully elucidated.

Objective: To evaluate how Rad deletion alters activity dependent regulation of L-type calcium current

Methods: Isolated ventricular myocytes with and without Rad deletion were patched in the whole cell configuration. Western blots were performed on isolated murine ventricles.

Results: Rad deletion results in elevated calcium dependent facilitation via activation of CaMKII. Beta-adrenergic agonist isoproterenol phenocopies Rad deletion effect on calcium dependent facilitation, suggesting a Rad dependent mechanism for PKA mediated increase in CDF.

Conclusions: These results suggest Rad has the capacity to alter local CaMKII activity without triggering CaMKII mediated pathological remodeling.

Angiotensin Biomarkers for Drug Monitoring and Primary Aldosteronism Screening in Resistant Hypertension

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Background: Medication non-adherence and undetected primary aldosteronism are major contributors to resistant hypertension, a clinical entity with high cardiovascular risk. We tested whether direct quantification of angiotensin peptides and aldosterone enables simultaneous drug monitoring and mechanism-based screening for PA under standard antihypertensive treatment.

Methods: In the Prospective Cohort: Resistant Hypertension (PRIO) study of 183 patients standardized to triple therapy with ACEi or ARB, we defined drug class-specific thresholds for PA detection based on adherence status using angiotensin (Ang)-based biomarkers. Concentrations of Ang peptides and aldosterone were quantified from serum samples of 67 patients with PA and 116 with resistant hypertension using a validated mass spectrometry-based platform. Receiver operating characteristic analyses defined cut-off values for the ratio of Ang II to Ang I (ACE-Q) and Ang II for ACEi and ARB exposure, respectively, as well as aldosterone to Ang II ratio (AA2-R) thresholds for PA, which were integrated into a medication-adjusted screening algorithm.

Results: ACE-Q values < 1.00 pM/pM detected ACEi use with 100% sensitivity and 99% specificity. Similarly, Ang II concentrations > 163.0 pM detected ARB use with good performance. Derived from adherent patients, drug class-specific AA2-R thresholds for PA differed substantially between ACEi- and ARB-treated patients (20.41 and 2.00 pM/pM). Following stratification by RAAS inhibitor status, adjusted AA2-R thresholds were applied for PA detection. Compared with a conventional single AA2-R threshold applied across the entire cohort, the medication-adjusted algorithm markedly improved diagnostic performance (sensitivity/specificity: 94.3%/91.9% vs. 67%/78%).

Conclusions: Angiotensin biomarkers enable mechanism-based drug monitoring and drug class-specific PA screening under standard therapy.

Keywords: medication adherence, aldosterone, angiotensin II, mass spectrometry, diagnostic algorithm

Alternative splicing generates a conserved, brain-enriched Aster-B isoform (Aster-B^{ICFS}) in the developing mouse brain

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Cholesterol is essential for central nervous system membrane integrity, synaptic function, and myelin formation. Because the blood–brain barrier largely prevents uptake of circulating cholesterol, the brain relies on local synthesis and on tightly regulated intracellular cholesterol transport. Aster/GRAMD1 proteins (Aster-A/B/C) are endoplasmic reticulum (ER)-anchored proteins that mediate nonvesicular cholesterol transport from the plasma membrane (PM) to the ER, yet whether neuronal Aster isoforms are developmentally regulated has not been examined.

Comparative analysis of RNA-seq reads across the Aster-B (*Gramd1b*) locus in mouse brain and brown adipose tissue revealed a previously unrecognized splice junction in the brain. Sanger sequencing of the isoform-flanking PCR product, followed by CRISP-ID deconvolution, identified a short 12-bp exon (Exon 15) that introduces four amino acids (ICFS) into the Aster-B transmembrane region, defining a new isoform, Aster-B^{ICFS}. The ICFS-containing transmembrane region is conserved across vertebrates.

Using isoform-specific primers and absolute quantification with plasmid standard curves, we found that Aster-B^{ICFS} is strongly enriched in the brain, with minimal expression in peripheral tissues. Across development, Aster-B^{ICFS} expression increased more than 100-fold from E15 to adult in both hippocampus and cortex, becoming comparable to the classical isoform and accounting for nearly 50% of total Aster-B transcripts in adult brain. Consistent with neuronal enrichment, Aster-B^{ICFS} was detected in N2a cells but not in astrocytes, despite higher total Aster-B expression in astrocytes. Genome-browser analysis revealed PTB-associated signals near the ICFS exon, suggesting PTBP1 and PTBP2 as candidate regulators of its inclusion.

Together, these data identify Aster-B^{ICFS} as a conserved, brain-enriched, and developmentally induced Aster-B isoform and establish N2a cells as a tractable model for determining whether and how this isoform regulates neuronal cholesterol transport and differentiation.

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Abstracts for Trainee Showcase Presentations

Title: “Combining Volume Electron Microscopy and Computational Fluid Dynamics Analysis to Understand How Arterial Thrombi Become Occlusive”

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Abstract Text: Occlusive thrombosis causes 1 in 4 deaths worldwide, yet the native mechanical environments experienced by platelets during growth of biologically formed arterial thrombi remain poorly defined. This research project developed a same specimen workflow integrating volume electron microscopy (EM) with computational fluid dynamics (CFD) to relate local near-surface flow deformation to platelet ultrastructure in a partially occlusive mouse common carotid artery. Ferric chloride injury was used to generate partial thrombi and laser speckle contrast imaging was used to observe occlusion. Fixed arteries with thrombi were imaged by vEM to reconstruct lumen and thrombus geometry and identify activation-associated platelet ultrastructural phenotypes via segmentation. A specimen derived fluid volume was extracted and simulated in ANSYS Fluent using three-dimensional, transient, laminar incompressible flow, a literature derived pulsatile mouse common carotid plug velocity inlet condition, and a Carreau non-Newtonian viscosity blood flow model. CFD fields were spatially co-registered with matched EM sections in ImageJ. Time-averaged velocity contours demonstrated split high velocity jets along the lateral thrombus margins and a lower velocity region above the thrombus surface. In a representative matched thrombus-flow interface, the direction-specific rate of deformation tensor component D_xx showed a positive Spearman correlation with highest activation-associated platelet ultrastructure (rho = 0.65), whereas strain rate magnitude alone showed little association (rho = -0.15). These preliminary findings suggest that direction-specific flow deformation may capture platelet-flow relationships that are not apparent from scalar strain-rate magnitude alone which is typically studied in platelet flow chamber mechanobiology experiments. This integrated volume EM-CFD framework enables direct spatial interrogation of how biologically generated thrombus geometry shapes local flow and platelet ultrastructure and provides a platform for mechanistic studies of arterial thrombus growth and occlusion.

Diagnosis and Treatment of Patent Ductus Arteriosus in Full-Term Infants: A Single-Center Experience

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Abstract

Background: Patent ductus arteriosus (PDA) is a congenital cardiac defect caused by persistence of the fetal ductus arteriosus after birth. While PDA is commonly associated with prematurity, it can also occur in full-term infants and may result in pulmonary overcirculation, heart failure, and impaired growth. Management strategies include conservative observation, pharmacologic therapy, catheter-based intervention, and surgical repair; however, limited data exist regarding treatment patterns and outcomes among full-term infants. This study aimed to characterize the presentation, management, and outcomes of full-term infants diagnosed with PDA at a single academic medical center.

Methods: Following Institutional Review Board (IRB) approval, a retrospective chart review was conducted of 56 full-term infants (36–42 weeks gestational age) diagnosed with PDA at the University of Kentucky between 2014 and 2024. Patient identification was facilitated through a Center for Clinical and Translational Science (CCTS)-supported electronic health record data extraction, and clinical data were subsequently reviewed in Epic. Demographic characteristics, clinical presentation, diagnostic findings, treatment strategies, and outcomes were collected and analyzed descriptively.

Results: Mean gestational age was 38.67 ± 1.41 weeks, and mean birth weight was 3330 ± 526 g. The cohort consisted of 25 males and 31 females. Eighteen patients (32.1%) were symptomatic at diagnosis, and all patients were diagnosed by echocardiography. Management was predominantly conservative, with watchful waiting utilized in 51 patients (91.1%) and catheter-based intervention in 4 patients (7.1%). Three catheter closures were performed for persistent PDA at 5, 6, and 8 years of age, while one patient underwent intervention following a failed PDA stent complicated by aortic dissection requiring aortic stenting and subsequent PDA ligation. No patients received pharmacologic or primary surgical treatment. Seven patients died between 1 and 22 days of life; deaths were attributed to severe comorbid conditions, including hypoxic-ischemic encephalopathy and multisystem organ failure, and were not directly related to PDA.

Conclusions: Despite the availability of multiple treatment options for PDA, most full-term infants in this study required minimal intervention. Conservative management through watchful waiting was the predominant treatment strategy, with invasive intervention required in only a small minority of patients. These findings suggest that PDA in full-term infants is frequently managed successfully without intervention and highlight the generally low need for invasive treatment in this population.

Isoflurane effects on larval *Drosophila* cardiac tissue

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Abstract

Isoflurane is a dose-dependent anesthetic clinically administered via inhalational and volatile methods for general anesthesia. It can affect ion channels (i.e. K2P channels). A clinically significant side effect is its ability to trigger malignant hyperthermia due to calcium dysregulation via ryanodine (RY) receptors in skeletal muscle. However, direct actions on the heart are not well understood as a patient's temperature rises quickly, increasing heart rate and sympathetic drive. The larval *Drosophila* heart is myogenic and not innervated by neurons; thus it can be maintained *in situ*. This study aims to determine the mechanistic action of isoflurane on larval *Drosophila* cardiac responses, the role of the RY receptor in cardiac tissue, and the effect on the membrane potential, since it may also result in hyperpolarization. Canton S (n=10) and ORK1>M6M7 (n=10) *Drosophila* early 3rd instar larvae were dissected via a ventral incision and pinned to expose the heart tube. Using a microscope, the number of heart beats per minute in each saline bath and in isoflurane solution were counted . Isoflurane was freshly added to saline, and the solution was vortexed. Exposure to 0.1% isoflurane produced an increase in heart rate, with a significant increase by one-tailed paired t-test ($p = 0.044$) but not by two-tailed analysis ($p = 0.088$). A 0.2% significantly reduced heart rate (two-tailed paired t-test, $p = 0.0015$). Further, 1.0% isoflurane stopped the heart completely (rank sum test, $p<0.05$). These findings demonstrate concentration-dependent effects of isoflurane on the larval *Drosophila* heart. This work helps provide a basic understanding of isoflurane's potential actions on cardiac tissue in other organisms. Future studies will measure resting membrane potential in controls and K2P-overexpressing hearts.

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Late Sleep Timing is Associated with Metabolic Risk in Postmenopausal Women

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Postmenopausal women are vulnerable to metabolic dysfunction. Compelling evidence suggests this is because they lack the protective effects of estrogens. We have shown that circulating estrogens regulate daily eating and activity rhythms in female mice and protect them from obesity and metabolic dysfunction. However, few studies have investigated whether postmenopausal women have disrupted eating and sleep-activity rhythms that could contribute to metabolic dysfunction. The purpose of this study was to investigate the relationship between sleep and eating rhythms and metabolic risk in postmenopausal women. We studied 7 days of sleep and food intake behaviors in overweight and obese postmenopausal women. Sleep timing was assessed by actigraphy and sleep logs. First and last calorie intake times were collected daily using text messaging. Abdominal visceral fat, collected from a total body DXA scan, and insulin resistance, calculated as Matsuda Index from an oral glucose tolerance test, were analyzed as markers of metabolic risk. In 122 postmenopausal women (age mean ± SD: 58.1 ± 3.6 years) we found that later sleep onset was associated with greater abdominal visceral fat, but not insulin resistance. Next, we used a random-forest model to quantify the relative contributions of sleep and meal timing variables to predict abdominal visceral fat. The top predictors of visceral fat were time of sleep onset, sleep duration, and time of last caloric intake. These data suggest that interventions that advance sleep onset may reduce metabolic risk in postmenopausal women.

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Lipoproteins are susceptible to proteolytic modifications that may alter their atherogenicity

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Introduction: Inflammation drives atherosclerotic cardiovascular disease, with recent clinical trials successfully targeting cytokines to reduce cardiovascular risk. However, immune cells that secrete cytokines *also* secrete proteases. These proteases accelerate plaque progression but are not clinical targets. For example, neutrophil elastase is a serine protease secreted by neutrophils and macrophages in atheromatous plaques. Inflammatory stimuli can also increase plasma elastase levels ten-fold. *In vitro*, elastase modifies the protein components of low-density lipoprotein (LDL) and high-density lipoprotein (HDL), and these elastase-modified lipoproteins are rapidly internalized by macrophages. These data point to a proatherogenic role of elastase through its ability to modify lipoproteins and amplify foam cell formation. It is unknown, however, how these modified lipoproteins are metabolized in plasma or how they affect plaque development *in vivo.*

Approach: Alpha-1-antitrypsin (AAT) is the endogenous inhibitor of neutrophil elastase. Here, we aim to determine the effects of elastolytic activity on lipoprotein metabolism and atherosclerosis using a two-pronged approach: (1) acute administration of elastase to lipoproteins in plasma and (2) use of an AAT-deficient (*Serpina1*$^{-/-}$) mouse as a model for a long-term inability to inhibit elastase.

Results: We first characterized the impact of neutrophil elastase on lipoproteins in whole plasma *ex vivo*. Western blotting revealed dose-dependent degradation of apolipoproteins in plasma from wild type and *Serpina1*$^{-/-}$ mice. *Serpina1*$^{-/-}$ plasma proteins were more susceptible to degradation than wild type, and ApoB was more susceptible than ApoA1. When elastase was injected *in vivo* into *Serpina1*$^{-/-}$ mice, ApoB was rapidly cleaved in plasma. Elastase also degraded ApoB of human LDL and caused a decrease in LDL size. Interestingly, elastase-modified LDL injected into wild type mice was cleared slower than unmodified LDL. To determine how inability to inhibit elastase affects cardiovascular risk, we then conducted an atherosclerosis study in *Serpina1*$^{-/-}$ mice. Surprisingly, *en face* analysis demonstrated that male *Serpina1*$^{-/-}$ mice exhibit ~40% less plaque lesion area in the aortic arch compared to wild type mice. Consistent with this finding, male *Serpina1*$^{-/-}$ mice had significantly lower plasma cholesterol and triglycerides. These mice also had reduced hepatic lipids, suggesting that AAT plays a broader role in lipid metabolism than previously appreciated. No difference in lipids or lesion area was observed in female mice.

Conclusions: Neutrophil elastase modifies lipoproteins *ex vivo* and *in vivo*, and LDL is more susceptible to cleavage than HDL. Elastase-modified LDL has impaired plasma clearance, which may increase its likelihood to accumulate in plaque. Male AAT-deficient (*Serpina1*$^{-/-}$) mice are resistant to atherosclerosis, hyperlipidemia, and hepatic steatosis. These findings suggest that AAT plays a novel role in hepatic lipoprotein metabolism, which we are now investigating.

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A high-fat, high-sugar diet drives regional plaque deposition, vascular remodeling, and brain lipid changes in a model of amyloid pathology

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Background Type 2 diabetes (T2D) is associated with 2-4-fold increased risk for the development of Alzheimer's disease (AD). Both T2D and AD are strongly associated with increased neuroinflammation and vascular disease; however, the specific drivers underlying their relationship remain unclear. This study seeks to characterize how an obesogenic diet impacts circulating metabolites and lipids within the brain's interstitial fluid (ISF) and how it impacts pathological changes associated with amyloid plaques and the neurovascular unit, to discover the drivers of the neuroinflammation seen with AD.
Experimental Approach Here, we compared APP/PS1 mice, a genetic model with amyloid beta (Aβ) overexpression and amyloid plaque formation, to wildtype (WT) control mice, on either a high-fat, high-sugar-diet (HFHSD) or lean-chow (LC) control diet beginning at 2 months. At 6 months, hippocampal ISF was collected using *in vivo* microdialysis and subjected to lipidomic, metabolomic, and lipid particle analysis. Post-mortem tissue was collected for bulk RNA-seq and immunohistochemistry.
Results APP/PS1 mice fed HFHSD had increased amyloid plaques within the perirhinal/piriform cortices (PRh+Pir). AQP4+ astrocytic endfeet in the PRh+Pir were significantly greater in percent area in APP/HFHSD mice than all other conditions. CD31+ endothelial cells were increased in APP/PS1-HFHSD mice compared to the APP/PS1-LC mice. GFAP+ reactive astrocytes increased with plaques but showed no diet-induced effects. APP mice, regardless of diet, had increased Iba1+ microglia in the PRh+Pir relative to WT-LC mice; these differences were not observed when compared to WT-HFHSD. Bulk RNA-seq revealed APP-associated transcriptional signatures of altered cholesterol metabolism and neuroinflammation, while HFHSD further shifted the APP transcriptome towards vascular remodeling. Metabolomic profiling revealed compartment- and diet-dependent disruption of amino acid metabolism, including altered arginine, tryptophan, valine, and serine. Lipidomic profiling further identified increased ISF TG(51:1) in APP/PS1 mice and an overall reduction in larger triglyceride species in APP/PS1 mice and high-fat-diet–fed WT mice, consistent with altered lipid storage and trafficking.
Conclusions At 6 months, HFHSD did not broadly worsen all aspects of AD pathology but instead interacts with amyloid pathology to promote regional plaque accumulation, neurovascular remodeling, and disruptions in extracellular fatty acid homeostasis. This suggests that vascular and metabolic dysfunction may represent early mechanisms linking obesity-related metabolic disease to accelerated Alzheimer's pathology.

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