25TH 2023

CARDIOVASCULAR RESEARCH DAY

UNIVERSITY OF KENTUCKY

October 20, 2023

Central Bank Center

Lexington, KY

Schedule of the Day

25TH 2023 CARDIOVASCULAR RESEARCH DAY UNIVERSITY OF KENTUCKY

October 20, 2023 | Central Bank Center Ballrooms

8:30 am Check-In and Breakfast

Session 1

9:00 am Welcome

Alan Daugherty, PhD, DSc
Director, Saha Cardiovascular Research Center
Chair, Physiology
Gill Foundation Chair of Preventive Medicine

9:05 am Rapid Fire Presentations

Jimmy Xue
Graduate Student | Xiangan Li Lab
University of Kentucky
"Scavenger Receptor BI-mediated Heme Uptake Is an Essential Heme Clearance Pathway During Severe Hemolysis"

Yanming Li, PhD
Instructor | Ying Shen Lab
Baylor College of Medicine
"Dynamic Phenotypic Transition of Smooth Muscle Cells During Human Ascending Thoracic Aortic Disease Progression: From Compensation in Aortic Aneurysm to Decompensation in Aortic Dissection"

Sarisha Lohano
Undergraduate | Jonathan Satin Lab
University of Kentucky
"Cardiomyocyte-Restricted Deletion of RAD Improves Cytosolic Calcium Levels and Abnormal Calcium Release Events"

Matt Thomas, PhD
Postdoctoral Fellow | Julie Pendergast Lab
University of Kentucky
"Sleep and Eating Rhythms are Associated with Metabolic Risk in Postmenopausal Women"

Schedule of the Day

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UNIVERSITY OF KENTUCKY

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9:40 am Early Career Faculty Presentations

Lindsay Czuba, PhD
Assistant Professor, College of Pharmacy
"Altered Bile Acid Homeostasis in Obesity Pathogenesis"

Cheavar Blair, PhD
Research Assistant Professor, College of Medicine
"The Polyamine Spermidine Enhances the Contractile Function of Single-Cell hiPSC-CMs Post Doxorubicin Treatment"

Hongbing Fan, PhD
Assistant Professor, College of Agriculture, Food and Environment
"Food-Derived Peptides Reduce Blood Pressure Via Regulating Angiotensin-Converting Enzymes"

10:25 am Break

Session 2

10:45 am Gill Award for Early Career Achievements

Rebecca Haeusler, PhD
Associate Professor, Pathology & Cell Biology
Naomi Berrie Diabetes Center
Columbia University
"How Hepatic Insulin Signaling Contributes to Cardiovascular Health"

Schedule of the Day

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October 20, 2023 | Central Bank Center Ballrooms

11:30 am Poster Pitches

11:40 am Poster Session

Odd numbered posters are presented for judges then available for viewing.

Session 3

12:40 pm Lunch

Career Development Speaker

Joseph Hill, MD, PhD

Professor of Medicine and Molecular Biology
James T. Willerson M.D. Distinguished Chair in Cardiovascular Diseases
Frank M. Ryburn, Jr. Chair in Heart Research
Director, Harry S. Moss Heart Center
University of Texas Southwestern Medical Center
Editor-in-Chief, *Circulation*
"Disseminating Biomedical Information in Evolving Times"

1:40 pm Break

Schedule of the Day

25TH 2023 CARDIOVASCULAR RESEARCH DAY
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Session 4

2:00 pm Trainee Presentations

Alexis Smith
Graduate Student | Sidney Whiteheart Lab
University of Kentucky
"The Role of Cysteine String Protein-α in Platelet Exocytosis"

Tyler Benson, PhD
Postdoctoral Fellow | Phillip Owens Lab
University of Cincinnati
"Vascular Smooth Muscle Cell–specific Deletion of Protein Kinase R-like Endoplasmic Reticulum Kinase Attenuates Microbiome-enhanced Abdominal Aortic Aneurysm"

2:30 pm Poster Pitches

2:40 pm Poster Session
Even numbered posters are presented for judges then available for viewing.

Session 5

3:40 pm Gill Award for Outstanding Contributions to Cardiovascular Research

Joseph Wu, MD, PhD
Director, Stanford Cardiovascular Institute
Simon H. Stertzer, MD, Professor of Medicine & Radiology
Stanford University
President, American Heart Association
"Stem Cells & Genomics: From Precision Medicine to Clinical Trials in Dish"

Schedule of the Day

25TH 2023 CARDIOVASCULAR RESEARCH DAY
UNIVERSITY OF KENTUCKY

October 20, 2023 | Central Bank Center Ballrooms

4:20 pm Alumni Presentation

Alison Bailey, MD

Physician Director
Cardiovascular Disease
HCA Healthcare, Chattanooga, TN
Editor-in-Chief, American College of Cardiology Extended Learning
"Social Determinants of Health: What Are They and Why You Should Care"

4:40 pm Special Presentation

Mary Walsh, MD

Medical Director
Cardiovascular Research Institute
St Vincent Heart Center, Indianapolis, IN
Past President, American College of Cardiology
"The Power of Cardiovascular Research Touches Patients Everyday: A Clinician's Perspective"

5:00 pm Awards Presentation

Saha Awards
Poster Presentation Award Winners

5:15 pm Networking Reception

Sponsors

A special thank you to the sponsors of the
2023 University of Kentucky Cardiovascular Research Day.

Gill Foundation
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Research & Education

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Institutional Support

Institutional support for the 2023 University of Kentucky Cardiovascular Research Day is provided by the following units

Gill Heart and Vascular Institute Outstanding Contributions to Cardiovascular Research Award
$25,000 Prize

Joseph Wu, MD, PhD

Director, Stanford Cardiovascular Institute
Simon H. Stertzer, MD, Professor of Medicine and Radiology
Stanford University School of Medicine
President, American Heart Association

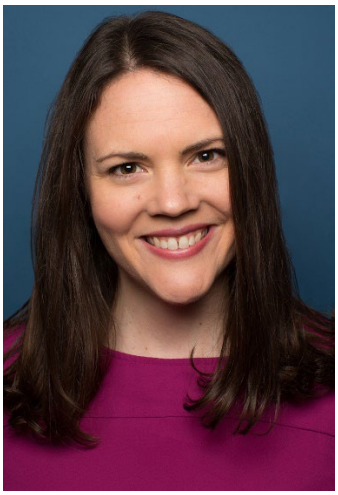
Joseph C. Wu, MD, PhD is Director of Stanford Cardiovascular Institute and Simon H. Stertzer, MD, Professor of Medicine and Radiology at Stanford University. Dr. Wu received his MD from Yale University and PhD (Molecular & Medical Pharmacology) at University of California, Los Angeles. He is board certified in cardiovascular medicine.

His lab works on cardiovascular genomics and induced pluripotent stem cells (iPSCs). The main goals are to (i) understand basic disease mechanisms, (ii) accelerate drug discovery via "clinical trial in a dish" concept, and (iii) implement precision medicine for patients. Dr. Wu has published >500 manuscripts with H-index of 125 on Google scholar. He is listed as top 0.1% of highly cited researchers by Web of Science for past 5 years (2018, 2019, 2020, 2021, 2022).

Dr. Wu has received several awards, including the NIH Director's New Innovator Award, NIH Roadmap Transformative Award, Presidential Early Career Award for Scientists and Engineers (PECASE) given out by President Obama at the White House, American Heart Association (AHA) Distinguished Scientist Award, AHA Merit Award, and Burroughs Wellcome Foundation Innovation in Regulatory Science Award. Dr. Wu serves on the FDA Cellular, Tissue, and Gene Therapies Advisory Committee. He is on the Board of the Keystone Symposia. He is the current President of the American Heart Association (July 2023 – June 2024).

Dr. Wu is an elected member of American Society for Clinical Investigation (ASCI), Association of University Cardiologists (AUC), American Institute for Medical and Biological Engineering (AIMBE), American Association for the Advancement of Science (AAAS), American Association of Physicians (AAP), Academia Sinica (Taiwan), National Academy of Inventors (NAI), and National Academy of Medicine (NAM).

Gill Heart and Vascular Institute Early Career Award
$10,000 Prize



Rebecca Haeusler, PhD

Associate Professor of Pathology and Cell Biology
Naomi Berrie Diabetes Center
Columbia University Medical Center
Gill Heart and Vascular Institute

Rebecca Haeusler is an Associate Professor of Pathology and Cell Biology (with tenure) at Columbia University in New York, NY. Dr. Haeusler is originally from Michigan, and she obtained her undergraduate degree in biology at MIT and her PhD in biological chemistry at the University of Michigan. A central focus of Dr. Haeusler's research is on the proatherogenic metabolic abnormalities that occur during insulin resistance, with a particular focus on bile acids and lipoproteins. She is funded by grants from the National Institutes of Health and the American Diabetes Association, and she has won awards for her research, including the David L. Williams award for research on lipids and lipoproteins, the Journal of Clinical Investigation lectureship award, and the ATVB Special Recognition Award in Vascular Biology. Dr. Haeusler serves on the boards of the Deuel Lipid Conference and the Kern Lipid Conference, is an associate editor of Diabetes and an academic editor of PLOS Biology. At Columbia, Dr. Haeusler is associate director of the Digestive and Liver Disease Center and co-director of the Integrated PhD program.

Career Development Presentation

Joseph A. Hill, MD, PhD

Professor of Medicine and Molecular Biology
James T. Willerson, MD Distinguished Chair in Cardiovascular Diseases
Frank M. Ryburn, Jr. Chair in Heart Research
Director, Harry S. Moss Heart Center
UT Southwestern Medical Center

Dr. Hill is a cardiologist-scientist whose research focuses on molecular mechanisms of remodeling in the disease-stressed myocardium. He graduated with an MD, PhD from Duke University. Next, he pursued postdoctoral scientific training at the Institut Pasteur in Paris, followed by clinical training in Internal Medicine and Cardiology at the Brigham and Women's Hospital, Harvard Medical School. Dr. Hill served on the faculty of the University of Iowa for 5 years before moving in 2002 to the University of Texas Southwestern Medical Center to assume the role of Chief of Cardiology and Director of the Harry S. Moss Heart Center.

Dr. Hill's research group strives to decipher mechanisms of structural, functional, metabolic, and electrical remodeling in heart disease with an eye toward therapeutic intervention. Dr. Hill serves on numerous committees, boards, and study sections, and he lectures widely. In addition, he serves on several editorial boards, including *Circulation Research: Senior Consulting Editor*, *American Journal of Physiology, Heart and Circulatory Physiology, and American Journal of Cardiology*. He is Editor-in-Chief of the textbook *Muscle: Fundamental Biology and Mechanisms of Disease*. He has received numerous recognitions and awards, including election to the Association of American Professors; he recently served as President of the Association of University Cardiologists and chair of the Academic Council of the American College of Cardiology. He received the 2018 Research Achievement Award from the International Society for Heart Research, the 2020 Lucian Award from McGill University, and delivered the William Harvey Lecture this year at the European Society of Cardiology. Presently, he serves as Editor-in-Chief of *Circulation*. Dr. Hill maintains an active clinical practice focusing on general cardiology, heart failure, and hypertension.

Invited Speakers

Distinguished Alumni Presentation

Alison Bailey, MD

Physician Director
Cardiovascular Disease
HCA Healthcare, Chattanooga, TN

Alison L. Bailey, MD, FACC, FASPC, is chief of cardiology for Centennial Heart at Parkridge Medical Center in Chattanooga, Tennessee and a Physician Director of Cardiovascular Disease for HCA Healthcare. Her clinical practice focuses on the prevention of cardiovascular disease, women's cardiovascular care and the diagnosis and treatment of heart failure.

Dr. Bailey is a native of eastern Kentucky and grew up in Manchester, KY. She completed her undergraduate degrees, medical school, Internal Medicine residency and Cardiovascular Diseases fellowship at the UK. She then served as faculty and associate program director for the fellowship at the Gill Heart Institute at UK until 2015. She moved to Chattanooga, TN to establish a new Cardiovascular Disease Fellowship program at the University of Tennessee at Chattanooga and served in that role until 2020 when she formed a new cardiovascular group focused on optimizing patient care.

She is passionate about medical education and has been involved with the design and delivery of cardiovascular education for over 10 years. She has served as cardiovascular disease fellowship program director, developed education curricula for GME and clinical practice improvement and bedside education. She remains dedicated to the education of the cardiovascular care team with her work at HCA's Graduate Medical Education group as well as multiple professional societies.

She currently serves as the editor-in-chief of the American College of Cardiology's Extended Learning (ACCEL) editorial board, is the immediate past governor of the ACC's Tennessee Chapter and a board member and Secretary for the American Society of Preventive Cardiology.

Invited Speakers

Special Presentation

Mary Norine Walsh, MD, MACC

Medical Director, Heart Failure and Cardiac Transplantation
St Vincent Heart Center, Indianapolis, IN
Past President, American College of Cardiology

Mary Norine Walsh, MD, MACC, earned both her undergraduate and medical degrees from the University of Minnesota. She completed her internship and residency at the University of Texas Southwestern, and her cardiology fellowship at Washington University School of Medicine in St. Louis, MO. She served as an assistant professor of medicine in the division of cardiology, as well as an assistant professor of radiology at the Hospital of the University of Pennsylvania from 1990 to 1992. Walsh joined what is now Ascension St. Vincent Medical Group in Indianapolis, IN in 1992. Her areas of expertise include nuclear cardiology, heart failure, and cardiac transplantation with a special interest in cardiovascular disease in women.

She is the medical director of the heart failure and cardiac transplantation programs at Ascension St. Vincent Heart Center, and the Ascension St Vincent Cardiovascular Research Institute. Walsh is program director of the St. Vincent Advanced Heart Failure and Transplantation Fellowship and leads the Ascension Health heart failure service line. She is a deputy editor of JACC Case Reports, associate editor for JACC: Heart Failure and a reviewer for multiple scientific journals. She is an author of more than 200 articles and book chapters.

Walsh has been active in the ACC both locally and nationally. She was previously elected as president of the Indiana Chapter, and has served on and chaired multiple committees and work groups including serving as the organization's national president in 2017-2018.

Her teaching activities include instruction of students, residents and fellows and she lectures frequently on heart failure, heart disease in women, and topics in patient-centered healthcare. She is actively involved in clinical research in heart failure and systems approaches for quality initiatives in the practice setting. She has received numerous awards and recognitions including the Wenger Award for Medical Leadership in 2014, the St Vincent Distinguished Physician Award in 2018, the Women's Day Red Dress Award in 2019 and has been elected by her peers for inclusion in Best Doctors in America annually since 2005.

Early Career Faculty Presentations

Assistant Professor Showcase

Lindsay Czuba, PhD
Assistant Professor
College of Pharmacy

Cheavar Blair, PhD
Assistant Research Professor
College of Medicine

Hongbing Fan, PhD
Assistant Professor
College of Agriculture, Food and Environment

Selected Abstracts

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

Jimmy Xue
Graduate Student
Xiangan Li Lab
College of Medicine
University of Kentucky

Yanming Li, PhD
Instructor
Ying Shen Lab
Department of Surgery
Baylor College of Medicine

Sarisha Lohano
Undergraduate
Jonathan Satin Lab
College of Medicine
University of Kentucky

Matt Thomas, PhD
Postdoctoral Fellow
Julie Pendergast Lab
College of Arts and Sciences
University of Kentucky

Alexis Smith
Graduate Student
Sidney Whiteheart Lab
College of Medicine
University of Kentucky

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

Poster Pitch Participants

Sam Karem
Medical Student
University of Kentucky

Mikala Zelows
Graduate Student
University of Kentucky

Garrett Elmore
Graduate Student
University of Kentucky

Samantha Xu
Medical Student
Baylor College of Medicine

Garrett Anspach
Medical Student
University of Kentucky

Vivek Pandey
Post-Doctoral Scholar
University of Kentucky

Taesik Gwag
Staff
University of Kentucky

Shayan Mohammadmoradi
Post-Doctoral Scholar
University of Kentucky

Qian Wang
Post-Doctoral Scholar
University of Kentucky

Julianne Sharpe
Undergraduate
University of Kentucky

Seth Brunner
Undergraduate
University of Cincinnati

Caris Wadding-Lee
Graduate Student
University of Cincinnati

Daniëlle Coenen
Post-Doctoral Scholar
University of Kentucky

Alex Pettey
Graduate Student
University of Kentucky

Meghan Turner
Graduate Student
University of Kentucky

Ezekiel Rozmus
Graduate Student
University of Kentucky

Alex Pickering
Undergraduate
Mississippi State University

Bowen Li
Post-Doctoral Scholar
University of Kentucky

Poster Participants

Abstract:

Left Ventricular Assist Device (LVAD) In The Management of Heart Failure, A Single Center Experience

Sam Karem: MS3 at University of Kentucky College of Medicine, Sibu Saha, MD, FACS: University of Kentucky Healthcare

Objective:

Heart failure has become more prevalent today, with the CDC estimating 6.2 million Americans diagnosed with heart failure, and costing around $40.6 billion in 2020 (Kruse, et.al 2021). Many heart failure patients are successfully managed medically, but with worsening disease the Left Ventricular Assist Device (LVAD) has become more clinically relevant in treating patients with heart failure, either as a bridge to transplant, or destination therapy. The aim of this study is to review our experience with LVAD therapy at the University of Kentucky Healthcare system.

Methods:

This study was conducted with IRB approval from the University of Kentucky. Patients were treated with LVAD from 1/1/2017 to 12/31/2021. There were 127 patients in the study with an age range of 18-83. Patients' operative diagnoses consisted of 40 (31.4%) patients with ischemic cardiomyopathy, 36 (28.3%) with non-ischemic cardiomyopathy, 26 (20.5%) patients with chronic systolic heart failure, and 26 (20.5%) patients with other diagnoses. Patients' pre-operative NYHA heart failure class show 76 (60%) in class IV failure, 24 (18.9%) patients class III, 6 (4.7%) in class III/IV failure, 2 (1.6%) in class II failure, and 19 (15%) patients did not have pre-operative NYHA status listed. Patient demographics reveal 111 (86.7%) of recipients are white, 16 (12.5%) are black, 105 (82%) male, and 23 (18%) female.

Results:

Results show average length of admission for LVAD implantation is 41 days. Quality of life is reported using NYHA classification at follow up after LVAD implant which shows 6 (4.7%) patients in Class I, 52 (40.9%) patients in Class II, 21 (16.5%) patients in class III, 3 (2.3%) patients in Class I-II, 8 (6.3%) patients in Class II-III, and remaining patients were deceased before discharge, or did not go below class IV heart failure. At the time of discharge, results show 37 (29.1%) patients discharged home, 43 (33.9%) patients discharged home with home health, 30 (23.6%) patients discharged to a rehab facility, and 17 (13.4%) patients deceased before discharge. Prior to 2021, there are 105 patients, and 65 (61.9%) patients were alive at 2 years of follow up; and 18 out of 23 (78%) patients who received LVAD after 2021 are still living.

For post-operative complications, 33 patients (25.9%) developed stroke/ischemia, 31 (24.4%) developed GI bleeding, 33 (25.9%) developed renal failure, 57 (44.9%) developed respiratory failure (post-op or acute failure), 45 (35.4%) developed driveline infection, 18 (14.2%) developed RV dysfunction/failure, and 26 (20.5%) developed an LVAD complication.

Conclusion:

Heart failure is becoming a more common disease with increasing number of admissions into the hospital. While most heart failure symptoms can be managed medically, LVAD has been implemented to try to successfully manage late-stage heart failure symptoms, prolong life, and bridge patients to transplant.

The Anticoagulant Systems Cooperate Synergistically on Phospholipid Vesicle and Endothelial Cell Membranes, But Not on Platelets

Martha M.S. Sim[1,2], Dlovan F. D Mahmood[1], and Jeremy P. Wood[1,2,3]

[1]Saha Cardiovascular Research Center, University of Kentucky
[2]Department of Molecular and Cellular Biochemistry, University of Kentucky
[3]Division of Cardiovascular Medicine Gill Heart and Vascular Institute, University of Kentucky

Background: Thrombin, the enzyme which converts fibrinogen into a fibrin clot, is produced by the prothrombinase complex, composed of factor Xa (FXa) and factor Va (FVa). Down-regulation of this process is critical, as excess thrombin can lead to life threatening thrombotic events. FXa and FVa are inhibited by the anticoagulants tissue

factor pathway inhibitor alpha (TFPIα) and activated protein C (APC), respectively, and their common cofactor protein S (PS). TFPIα, APC, and PS function synergistically on the surface of phospholipid vesicles (PLs). We have also recently reported a shear-dependent interaction between PS and von Willebrand factor (VWF), which partially blocks its anticoagulant function. Here, we investigated the function of the TFPIα/APC/PS anticoagulant system on physiologic membrane surfaces (platelets and endothelial cells, or ECs), and the effect of VWF on prothrombinase inhibition in these different membrane environments.

Methods: Thrombin generation was measured in plasma and platelet-rich plasma (PRP) by calibrated automated thrombography, purified prothrombinase activity was assayed using a chromogenic substrate, and FVa cleavage was monitored by immunoblotting. Experiments were performed with synthetic PLs, washed platelets, or cultured EA.hy926 cells, an EC line. Experiments involving VWF were performed in the presence or absence of shear.

Results: In plasma TG, as we have previously published, PS anticoagulant activity was greatly enhanced by addition of thrombomodulin (TM), to promote protein C (PC) activation. TM decreased thrombin generation in a PS-dependent manner. The effect of TM and PS was enhanced by the addition of TFPIα, particularly at low PS concentrations. Conversely, inhibitory antibodies against TFPI and PC had an additive effect on plasma TG which was mimicked by inhibiting PS alone. Similar results were obtained in plasma supplemented with ECs (which provide TM) or in PRP. However, in PRP, the antibodies reversed TG beyond what was seen with tissue factor-initiation alone.

In purified protein systems, performed using either PLs or ECs, physiologic TFPIα concentrations had no detectable effect on thrombin activation by prothrombinase, unless APC was present in the system. The greatest inhibitory activity was observed when TFPIα, APC, and PS were all present, and was similar on ECs and PLs. Similarly, inhibition of FXa by TFPIα was found to render FVa susceptible to proteolysis by APC on either PLs or ECs. However, prothrombinase assembled on activated platelets was protected from inhibition, even in the presence of TFPIα, APC, and PS, as measured either by thrombin activation or FVa cleavage.

Finally, we assessed the functional consequences of the PS/VWF interaction. In purified systems with PLs, sheared VWF dose-dependently reversed FXa inhibition by PS/TFPIα, measured using a chromogenic FXa substrate. Conversely, APC cofactor function, as measured by FVa cleavage, was unaffected on either PLs or activated platelets. Consistent with this, thrombin activation by prothrombinase was unaffected by sheared VWF on these surfaces. Interestingly, sheared VWF promoted prothrombinase function on ECs, increasing the rate of thrombin activation beyond that observed in the absence of the anticoagulants.

Conclusions: We propose a model of prothrombinase inhibition through combined targeting of both FXa and FVa, and that this mechanism enables down-regulation of thrombin activation outside of a platelet

clot. On PL or EC membranes in the plasma environment, APC, TFPIα, and PS cooperate synergistically to inhibit prothrombinase activity, preventing excessive clot propagation. Conversely, platelets protect prothrombinase from inhibition, supporting a procoagulant environment within the clot. The anticoagulant system appears to function similarly on PLs and on ECs, except that VWF has greater effect on ECs, suggesting that the protection mediated by platelets is due to a platelet component not present in ECs. We hypothesize that these regulatory mechanisms serve to localize procoagulant activity within the platelet plug and suppress it on ECs downstream of a clot.

Critical Role of Endothelial Injury in the Initiation of Aortic Dissection

Authors: Chen Zhang, MD[1,2]; Deborah C. Vela , PhD[2]; Laxman.Devkota, PhD[4]; Yanming Li, PhD[1,2]; Samantha Xu, MPH[1,2]; Lin Zhang, MS[1,2]; Abhijit Chakraborty, PhD[1,2]; Yang Li, PhD[1,2]; Kimberly R. Rebello, MD, MSc[1,2]; Hernan G. Vasquez, PhD[1,2]; Ketankumar B. Ghaghada, PhD[4]; Alan Daugherty, MD, PhD; Dianna M. Milewicz, MD, PhD; Scott A. LeMaire, MD[1,2,3]; Ying H. Shen, MD, PhD[1,2,3]

[1]Division of Cardiothoracic Surgery, Michael E. DeBakey Department of Surgery, Baylor College of Medicine, Houston, Texas; [2]The Texas Heart Institute, Houston, Texas; and [3]Cardiovascular Research Institute, Baylor College of Medicine, Houston, TX; and [4] Translational Imaging Group, Department of Radiology, Texas Children's Hospital/Baylor College of Medicine, Houston, TX.

Introduction: Endothelium forms a protective barrier and maintains vascular hemostasis. However, the role of endothelial injury in aortic aneurysms and dissections (AAD) remains poorly understood. The receptor-interacting protein kinase 3 (RIP3)-mediated necroptosis and gasdermin D (GSDMD)-meditated pyroptosis trigger necrotic cell death. In this study, we tested the hypothesis that endothelial cell (EC) death induced by necroptosis and pyroptosis contributes to AAD formation.

Methods: Endothelial integrity and gene expression were examined in ascending aortic tissues from ascending thoracic aortic aneurysm (ATAA) patients (n=9) and organ donor controls (n=8) by single-cell transcriptome analysis. The effects of EC death on AAD formation were determined in EC-specific Rip3 knockout mice (EC-Rip3-/-), EC-specific Gsdmd knockout mice (EC-Gsdmd-/-), and necroptosis/pyroptosis inhibitor necrosulfonamide treated mice in sporadic AAD models induced by angiotensin II (Ang II) infusion. Endothelial hyperpermeability was detected by Evans blue staining and nanoparticle-mediated contrast-enhanced CT (n-CECT), which is hypersensitive to early-stage endothelial injury.

Results: Single-cell transcriptome and immunostaining analyses revealed significant upregulation of pro-death genes (e.g., RIP3 and GSDMD), but downregulation of cell junction genes (e.g., TJP1 and GJA1) in ECs of ATAA patients compared with controls. In the mouse AAD model, endothelial injury and hyperpermeability were detected 2-5 days after Ang II infusion and were associated with intramural nanoparticle accumulation, elastic fiber fragmentation, and macrophage infiltration. Importantly, EC-Rip3-/- mice and EC-Gsdmd-/- mice showed preserved EC barrier function, reduced nanoparticle accumulation and elastic fiber fragmentation, and reduced AAD incidence. Blocking necroptosis/pyroptosis by necrosulfonamide treatment reduced the incidence and severity of AAD.

Conclusions: Endothelial injury and subsequent barrier dysfunction and infiltration are key features of AAD formation. Prevention of EC necrotic cell death can be a potential AAD therapeutic target.

Platelet-derived thrombospondin 1 promotes immune cell liver infiltration and exacerbates diet-induced steatohepatitis

Taesik Gwag, PhD [1, 2], Sangderk Lee, PhD [3], Zhenyu Li, PhD [4], Steven A. Weinman, MD [5,6], Ying Liang [7], Changcheng Zhou [8] and Shuxia Wang, PhD [1, 2 *]

[1] Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY 40536; [2] Lexington Veterans Affairs Medical Center, Lexington, KY 40502; [3] Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536; [4] Irma Lerma Rangel School of Pharmacy, Texas A&M University, College Station, TX, 77843; [5] Department of Internal Medicine, University of Kansas Medical Center, Kansas City, KS 66160; [6] Research Service, Kansas City VA Medical Center, Kansas City, MO 64128; [7] New York Blood Center, 310 East 72nd Street, New York, NY 10065; [8] Division of Biomedical Sciences, School of Medicine, University of California, Riverside, CA92521

Background and Aims: Recent studies have implicated platelets, particularly α-granules, in the development of steatohepatitis (NASH). However, the specific mechanisms involved have yet to be determined. Notably, thrombospondin 1 (TSP1) is a major component of platelet α-granules released during platelet activation. The role of platelet-derived TSP1 in NAFLD remains unknown and was investigated in this study. **Approach and Results**: Platelet-specific TSP1 knockout mice (*TSP1*$^{\Delta pf4}$) and their wild type littermates (*TSP1*$^{F/F}$) were utilized. NASH was induced by feeding the mice with a diet enriched in fat, sucrose, fructose, and cholesterol (AMLN diet). Additionally, a human liver NASH organoid model was employed. While TSP1 deletion in platelets did not affect diet-induced steatosis, platelet-specific TSP1 deficient mice exhibited attenuated NASH and liver fibrosis, accompanied by improvements in plasma glucose and lipid homeostasis. Moreover, intrahepatic platelet accumulation, activation and chemokine production were reduced in the platelet-specific TSP1 deficient mice, which correlated with decreased immune cell infiltration into the liver. Consequently, this led to diminished pro-inflammatory signaling in the liver and mitigated the progression of NAFLD. Moreover, *in vitro* data revealed that co-culturing TSP1-deficient platelets in a human liver NASH organoid model attenuated hepatic stellate cell

activation and NASH progression. Additionally, TSP1 deficient platelets also play a role in regulating brown fat endocrine function, specifically impacting Nrg4 production. A crosstalk between brown fat and liver may also influence NAFLD progression. **Conclusions**: Taken together, these data suggest that platelet α-granule-derived TSP1 is a significant contributor to the diet-induced NASH and fibrosis, and may serve as a new therapeutic target for this severe liver disease.

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

An-Hsuan Lin[1], Wen Su[1], Zhenheng Guo[2], and Ming C. Gong[1]

[1]Department of Physiology; [2]Department of Pharmacology and Nutritional Sciences, University of Kentucky

Objective - Men are more prone to hypertension than women, especially below the age of 50. Recently, it is appreciated that not only the level but also the normal circadian rhythm of blood pressure (BP) are essential. Disruption of BP circadian rhythm is emerging as an index for detrimental cardiovascular outcomes. Previous studies have found if change food intake pattern can alter BP rhythm in several animal species. However, it is mostly unknown whether, and if so, via what mechanism, male sex steroids regulate the circadian rhythm of BP. Additionally, whether the BP rhythm regulation by male sex steroids is associated with food intake rhythm still need to be elucidate. The current study investigates the role of male steroids on BP/food intake circadian rhythm and clock gene per2 oscillations.

Approach and Results - We monitored BP, heart rate (HR), and locomotor activity in 36-weeks-old male mice at baseline, and 1-, 2-, 3- and 4-weeks after orchiectomy by radiotelemetry. In addition, we also measured food intake at baseline and 2 weeks after orchiectomy in BioDAQ cages. We found that the amplitude and robustness of circadian rhythms in BP, food intake HR, and locomotor activity were significantly reduced 2 weeks after the orchiectomy. A reduction in the active dark-phase mostly accounted for the decrease in the BP and locomotor activity circadian rhythms. In contrast, a reduction in the resting light-phase accounted for the decrease in HR circadian rhythm. The 24-hour average BP and locomotor activity were lowered from 111.5 mmHg to 105.2 mmHg and from 4.5 counts/min to 3.1 counts/min by orchiectomy ($p<0.05$, $n=8$). In contrast, the 24-hour average HR was not significantly altered by orchiectomy (533.0 beats/min vs. 546.2 beats/min). To investigate the potential role of clock gene in the orchiectomy-induced changes in the circadian rhythms, we used mPer2Luc mice, in which clock gene Per2 is fused with a luciferase reporter thus allow real-time monitoring of the clock gene Per2 oscillations. We investigated the Per2 oscillation ex vivo by LumiCycle and clock genes mRNA expression by real-time qPCR (RTqPCR) in various tissues. The preliminary results of the ex vivo monitoring found that the peripheral oscillators in aorta, thymus and white adipose tissue were phase advanced to various extents by orchiectomy. Our preliminary analysis of RTqPCR showed orchiectomy altered Bmal1 and Per2 mRNA levels in aorta, WAT and thymus compared to sham surgery group.

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

Enhancement of High Density Lipoprotein-Associated Elastase Inhibitor Activity Prevents Atherosclerosis Progression

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Background: Inflammatory cells within atherosclerotic lesions secrete various proteolytic enzymes that contribute to lesion progression and destabilization increasing risk of an acute cardiovascular event. The relative contributions of specific proteases to atherogenesis is not well understood. Elastase is a serine protease produced by macrophages and neutrophils in plaque and may contribute to development of unstable plaque. We have previously demonstrated enrichment of protease inhibitor proteins on plasma high density lipoprotein (HDL), including alpha-1-antitrypsin, an inhibitor of elastase. These data support a potential role for HDL as an endogenous modulator of protease activity. In this study, we test the hypothesis that enrichment of HDL-associated elastase inhibitor activity is protective against atherosclerosis lesion progression.

Methods and Results: We designed an HDL-targeting protease inhibitor (HTPI) to bind to HDL and confer elastase inhibitor activity. HTPI is a small (1.6 kDa) peptide with an elastase inhibitor domain, a soluble linker, and an HDL-targeting domain. When incubated with Human plasma *ex vivo*, HTPI predominantly bound to HDL. Venous administration to mice resulted in binding to plasma HDL and increased elastase inhibitor activity on isolated HDL from mice that received HTPI. Accumulation of HTPI within plaque was observed after administration to *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice. To examine the impact of HTPI treatment on atherosclerosis, *Ldlr*$^{-/-}$ mice were fed Western diet for 12 weeks to establish atherosclerosis (WD-Saline) followed by two additional weeks continued on diet while receiving either saline or HTPI (2.5 mg/kg body weight) three times per week. Lesion area quantification by en face analysis revealed that HTPI prevented further lipid deposition in plaque. Histology and immunofluorescence staining of aortic root sections were used to examine the impact of HTPI on lesion morphology and inflammatory features.

Conclusions: These data support the hypothesis that HDL-associated anti-elastase activity could contribute to the athero-protective functions of HDL and support the potential utility of enrichment of anti-protease activity on HDL for stabilization of high-risk atherosclerotic lesions.

Title: History of Vascular Surgery

Authors: Nick Demas, MSII; Sibu Saha, MD, MBA, FACS

Background:
Vascular surgery continues to grow due to advances in technique and technology. This exhibit will depict the role of pioneers, medications, and devices that have made a significant difference in the advancement of this specialty from antiquity to today.

Synopsis:
Most vascular surgery cases are due to trauma, occlusive disease, and aneurysmal disease. Vascular surgery began with attempts to control bleeding in cases of trauma, but the field has tremendously developed and improved since then. The management of occlusive and aneurysmal diseases of arteries continues to evolve with the advancement of techniques and devices thanks to the many contributions of pioneers throughout history. Such prominent individuals include Sushruta and his work in ancient India as the father of surgery; Rudolph Matas and the first surgical aneurysm repair; Alexis Carrel and his work on vascular suturing; Charles Dotter and his invention of numerous catheters and the first angioplasty; Charles Dubost and the first successful abdominal aortic aneurysm repair using a homograft; and Juan Parodi and the creation of endovascular repair techniques. Charles Dotter and his groundbreaking work revolutionized the field in 1964 with the first successful use of a dilating catheter for a stenosed femoral artery. Around the same time, Thomas Fogarty developed balloon embolectomy catheters after seeing how unsuccessful embolectomies were while working as an operating room technician. Their contributions would open the door for Juan Parodi and his endovascular repair techniques. Parodi worked for years to refine his endovascular graft that would be used in 1990 for the first successful endovascular repair of an abdominal aortic aneurysm. It is essential not to forget the invention of the antiplatelet and anticoagulant medications, like heparin and warfarin, that allow vascular surgeries to occur. Long before these medications were available, surgeons relied on various methods to control bleeding, like ashes, astringents from tree bark, and cauterization with burning metal or oil. It was not until 1940 that Joseph Murray described how heparin was important for preventing thrombosis during vascular surgery. Another crucial aspect is the advancement of surgical devices. Early vascular surgeries utilized grafts from other species, such as dogs, pigs, and cows, or even metal prostheses that thrombosed or failed for other reasons. It was not until the early 1900s that venous autografts were used successfully for arterial reconstruction with reverse vein grafts. Decades later during the 1980s and 1990s, Karl Hall reported his success using in situ bypasses with saphenous vein grafts. In the 1950s, the use of allografts and synthetic grafts to repair aneurysmal disease made a significant difference in treatment outcomes, up until recently with the creation of endovascular grafts. Many of these older techniques are now obsolete, thanks to technological advancements that made endovascular reconstruction possible. Many pioneers and their developments of new techniques, medications, and devices allowed the field of vascular surgery to flourish and impact countless patient lives throughout history. Currently, nearly 90% of cases utilize endovascular techniques with good outcomes and short hospital stays.

Unraveling the Impact of VAMP8 Deficiency on Aortopathies: A Fresh Perspective on Platelet Involvement

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Introduction and Objective: Platelet activation and its secreted mediators promote thrombus formation and the accumulation of inflammatory cells, which may play an important role in the development of aortopathies by destroying the structural integrity and stability of the vessel wall. This study first explores the role of angiotensin II (AngII) in platelet activation and pivots on the soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) protein, the vesicle associated membrane protein 8 (VAMP8) – the primary vesicle membrane (v-SNARE) and a major facilitator of platelet secretion.

Approach and Results: Washed platelets from saline or AngII-infused mice (28 days) exposed to thrombin (0.05 U/mL) showed elevated P-selectin, suggesting AngII-driven platelet activation. Hematological studies showed increased mean platelet volume (MPV) and platelet distribution width (PDW) in the AngII group. Washed platelets from wildtype (WT) mice, treated with varying AngII doses, also exhibited elevated P-selectin and Jon/A levels. Additionally, abdominal aortas from 28-day AngII-infused mice, revealed platelets abundance at elastin break sites and false lumen formation compared to saline-infused counterparts.

Given the profound impairment in platelet cargo secretion due to VAMP8 deficiency, we investigated its role in aortopathies. Using RNA-seq data from washed platelets of VAMP8$^{-/-}$ mice vs. WT, we analyzed the molecular signature of VAMP8 deficiency. Gene ontology analysis revealed that the major differences in enriched genes between the two groups involved triglyceride biosynthetic process and platelet calcium homeostasis. Further, ApoE$^{-/-}$ mice crossed with VAMP8$^{-/-}$ mice exhibited a substantial reduction in atherosclerotic lesion development when compared to the control group while plasma cholesterol remained consistent across all groups. Additionally, proprotein convertase subtilisin/kexin type 9 (PCSK9)-induced hypercholesterolemic VAMP8$^{-/-}$ or WT male mice fed a Western diet were infused with AngII (1,000 ng/kg/min) for 4 weeks and aortic *ex vivo* and *en face* analysis indicated that the VAMP8 deficiency profoundly attenuated AngII-induced aortic aneurysms and atherosclerosis compared to control group.

Conclusion: Our findings reveal that VAMP8 deficiency markedly mitigates aortic aneurysm and atherosclerosis, thereby proposing a novel perspective to explore the influence of platelet cargo secretion on the inception of vascular complications.

Phenotyping daily rhythms of eating behavior during high-fat feeding for quantitative trait loci analysis in male mice

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Aberrant eating behavior rhythms, such as nighttime eating, are associated with obesity in humans. Obesity is also affected by the timing of eating in male mice. When male obesity-prone strains of mice were fed high-fat diet (HFD), their daily rhythm of eating behavior became disrupted. However, eating behavior rhythms in strains of male mice resistant to diet-induced obesity (DIO) were not disrupted by HFD feeding. The long-term goal of this study is to use quantitative trait loci (QTL) analysis to discover genes that regulate disruption of eating behavior rhythms in response to HFD feeding. QTL mapping identifies regions of chromosomes that correlate with a trait. QTL mapping requires ~200 mice that have a variety of genotypes and are phenotyped for the trait of interest. Therefore, we studied male F2 male mice that were generated from intercrossing F1 crosses of obesity-prone 129X1/SvJ and obesity-resistant SWR/J mouse strains. At 8 weeks old, male F2 mice were fed HFD for 6 weeks. We measured body weight, food intake, adiposity, and fasting blood glucose, as well as eating behavior rhythms with infrared video cameras. Thus far we have phenotyped eating behavior rhythms and metabolism in 38 F2 mice. HFD feeding had variable effects on the amplitude, or strength, of the eating behavior rhythms in F2 male mice. We found that adiposity was negatively correlated with the amplitudes of eating behavior rhythms, such that mice with greater adiposities had weaker eating behavior rhythms. Additionally, body weight was positively correlated with fasting blood glucose. Our data show that these F2 generation male mice are an appropriate model for QTL analysis based on the variation in the amplitudes of their eating behavior rhythms.

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LIMA1 knockdown reduces cholesterol levels in plasma, liver, and bile but does not inhibit hepatic NPC1L1 function

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LIM domain and actin binding 1 (LIMA1), generally considered a prognostic marker of malignancy in various types of cancer, was recently identified as an important regulator of cholesterol metabolism in humans. Heterozygous carriers of a rare frameshift variant in the LIMA1 gene have low LDL-cholesterol and decreased cholesterol absorption due to impaired NPC1L1 uptake from the surface of enterocytes. In humans but not mice, the liver expresses NPC1L1 which reclaims cholesterol from the bile. In the current study, our lab tested the hypothesis that hepatic LIMA1 knockdown would inhibit NPC1L1 in the liver resulting in increased biliary cholesterol. Male Ldlr-/- and Ldlr-/- with hepatic expression of a human NPC1L1 transgene (L1Tg) were treated for 6 weeks with either control antisense oligonucleotide (ASO) or an ASO targeting LIMA1. After 2 weeks of ASO treatment, the mice were fed a high fat (42% Kcal)/high cholesterol (0.2% wt/wt) diet for 4 weeks. LIMA1 ASO treatment decreased hepatic and intestinal Lima1 mRNA by >95% and 35%, respectively. Contrary to our hypothesis, knockdown of Lima1 did not increase biliary cholesterol in the Ldlr-/- L1Tg mice. However, LIMA1 ASO treatment did significantly decrease biliary, liver, and plasma cholesterol levels in Ldlr-/- mice. The reduction in plasma cholesterol was due to significant decreases in cholesterol associated with VLDL and LDL. Despite a >60% reduction in biliary cholesterol, mice with Lima1 knockdown had unchanged fecal cholesterol excretion, which could reflect decreased cholesterol absorption. In conclusion, partial knockdown of intestinal Lima1 may be reducing cholesterol absorption consequently decreasing cholesterol levels in liver, plasma, and bile. Further studies using GalNAc LIMA1 ASO or hepatocyte-specific Lima1 knockout mice are needed to determine whether targeting LIMA1 in liver, intestine or both provides optimal plasma cholesterol lowering.

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

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Abstract

Objective

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

Methods

Exenatide, a short-acting GLP-1 RA, was intraperitoneally injected at the onset of the light (ZT0) or dark (ZT12) phase in male diabetic *db/db* mice. BP and food intake were simultaneously monitored using radio-telemetry and the BioDAQ, respectively. Norepinephrine content was determined by HPLC in 6-hour urine samples collected over 24 hours under basal conditions, after ZT0 and ZT12 exenatide administration.

Results

db/db mice exhibited nondipping BP and were hyperphagic compared to controls. ZT0 exenatide administration decreased light-phase BP, restoring dipping BP. In contrast, ZT12 exenatide administration decreased dark-phase BP, worsening BP circadian rhythm to reversed dipping. The alterations in BP induced by exenatide were accompanied by corresponding changes in food intake. When fasting was initiated while exenatide was administered at ZT0, the BP dipping-restorative effects of exenatide were abolished. Furthermore, the urinary norepinephrine content, a marker for sympathetic activity, was significantly reduced 6 hours following exenatide administration at both ZT0 and ZT12.

Conclusion

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

Serotonin 3 receptors contribute to angiotensin II-induced aortopathies: potential role of periaortic fat-derived serotonin

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Background: Serotonin 3 receptors (5HTR3) are ligand-gated ion channels implicated in inflammation. Aortopathies induced by angiotensin II (AngII) exhibit macrophage-induced inflammation that occurs in ascending, thoracic and abdominal aortas of male hypercholesterolemic mice. Periaortic fat, a potential source of serotonin (5HT), the endogenous 5HTR3 ligand, has been implicated in aortopathy development. We defined effects of 5HTR3 antagonism on AngII-induced aortopathies, with a focus on periaortic fat as a source of 5HT to act at macrophage 5HTR3.

Methods: RNA seq of aortas from mice with varying sex chromosome complement was used to define differential regional aortic 5HTR3 expression. We administered tropisetron (Tropi), an 5HTR3 antagonist, to male AngII-infused low density lipoprotein receptor (Ldlr) deficient mice and quantified aortopathies in the distal thoracic (DTA) and abdominal aorta (AA). We examined effects of AngII on periaortic fat 5HT levels and evoked release of [3H]5HT from periaortic slices. We quantified effects of an 5HTR3 agonist on markers of inflammation and calcium influx in J774 macrophages.

Results: 5HTR3 expression differed markedly between thoracic and abdominal aortas of XX, but not XY mice. Tropi dose-dependently abrogated aortic lumen diameters, aortopathy incidence, maximal diameters and aortic weights of AngII-infused male mice. As a potential source of 5HT, there was positive 5HT immunostaining in thoracic brown and abdominal white periaortic fat. AngII infusion reduced 5HT levels in brown, but not white fat and AngII facilitated the evoked release [3]5HT release from thoracic, but not abdominal periaortic fat. J774 macrophages responded to 5HT or m-chlorophenylbiguanide (m-CPBG; 5HTR3 agonist) to increase iNOS expression and extracellular Ca^{2+} influx, which was reduced by Tropi.

Conclusions: These findings indicate a novel role for 5HTR3 in aortopathy development, with a potential role for periaortic fat as a source of 5HT, and macrophage 5HTR3 as a cell type critical to aortopathy development.

Carnitine Palmitoyltransferase 1a Modulates Hepatic Lipid and Lipoprotein Metabolism

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Background: Nonalcoholic fatty liver disease (NAFLD) affects almost 1 billion people worldwide and is associated with cardiometabolic risk factors. Genome- and epigenome-wide association studies have associated variants and methylation status of carnitine palmitoyltransferase 1a (CPT1a) to perturbations in very low-density lipoprotein (VLDL) cholesterol and triglyceride levels. The primary goal of this project is to determine the mechanism by which CPT1a alters hepatic and lipoprotein metabolism.

Methods: Eight-week-old *Cpt1a* floxed mice expressing the human apoB100 transgene ($Cpt1a^{fl/fl}/B100^{Tg}$) were administered control adenoassociated virus (AAV) or AAV encoding Cre-recombinase under control of a liver-specific promoter (TBG-Cre). Control and LKO mice were placed on low-fat control or western-type diet (WTD; 42% kcal fat, 0.2% cholesterol) for 16 weeks. Body weights were recorded weekly and body composition by MRI was performed at the study midpoint and end. Livers were collected and used for histological and lipid analysis, while gene and protein expression were measured by single-cell RNA sequencing and immunoblotting, respectively. FPLC and nuclear magnetic resonance (NMR) determined the lipoprotein composition in plasma.

Results: Mice with liver-specific deletion of *Cpt1a* displayed lower circulating apoB levels consistent with reduced triglyceride-rich lipoproteins and LDL particle number. Despite a reduction in steady-state plasma lipids, VLDL-triglyceride secretion was enhanced in LKO mice. WTD-feeding elevated hepatic triglycerides in LKO mice across both sexes, while cholesterol (free and esterified) increased by ~2.5-fold specifically in females. Consistent with greater accumulation of free cholesterol in female LKO mice, single-cell RNA sequencing revealed an M1 proinflammatory phenotype associated with increased expression of *Mmp12* and *Cxcl13*, while M2 antiinflammatory macrophage markers (*Slit3*, *Ccnd2*) were significantly decreased.

Conclusions: Despite accelerated VLDL secretion, liver-specific deletion of CPT1a reduces plasma LDL-cholesterol and triglycerides. Increases in hepatic free cholesterol levels were observed only in female LKO mice, which associates with a pro-inflammatory gene signature in macrophages that may contribute to the exacerbation of liver injury in these mice.

The Role of Lipids in Obesity-Mediated Protection in Sepsis

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Introduction and Objectives: Obesity is associated with improved survival in sepsis, a phenomenon termed the "obesity paradox." During sepsis, the body turns on several metabolic adaptation processes due to an increased energy demand, resulting in a high catabolic state and leading to the breakdown of energy sources. Adipose tissues encompass the body's largest endogenous energy supply providing stored triglycerides (TG) as an energy source. Therefore, we hypothesize that the availability of increased energy sources in the form of stored lipids contributes to obesity-mediated tolerance in sepsis. As a first step in testing our hypothesis, we analyzed the effect of obesity on changes in plasma lipids in mice during sepsis.

Approach: Male C57Bl/6 mice were procured from The Jackson's Lab Diet-induced Obesity colony at 20 weeks of age. The animals were fed a low-fat diet (n=10; LFD, 10% kcal from fat) or high-fat diet (n=11; HFD, 60% kcal from fat) continuously from 6 weeks of age and maintained on the diet until 26-27 weeks of age (diet duration 20-21 weeks) when sepsis was induced by cecal slurry (CS, 300 ul *i.p*) injection. Survival was monitored for 14 days, and blood was withdrawn by tail-snip at baseline, 24, 72, and 120 h after CS injection. For short-term experiments, mice were euthanized 24 h after CS injection. Lipid levels were analyzed by enzymatic kits (Wako Chemicals, VA). Plasma total cholesterol, free fatty acid, and triglyceride concentrations were measured using enzymatic kits (Wako Chemicals, Richmond VA.).

Results: HFD-fed mice showed significantly increased sepsis survival compared to LFD-fed mice after CS-induced septic challenge ($p=0.0043$; 36.4% HFD vs. 0% LFD), confirming our previous result. Plasma total cholesterol (TC) levels decreased steadily with time following the sepsis challenge in both groups of mice; however, the levels in HFD-fed mice were maintained significantly higher ($p<0.0001$) throughout the study compared to LFD-fed mice. Interestingly, increased cholesterol levels in HFD-fed mice were contributed by non-HDL lipoproteins. Baseline plasma triglycerides (TG) were significantly lower in HFD-fed mice compared to LFD-fed mice; however, TG levels declined sharply in LFD-fed mice following sepsis which did not occur in the HFD-fed mice. FFAs inversely increased following CS injection in the LFD group but remained constant in the HFD group.

Conclusions: The development of sepsis induces a decrease in plasma lipid levels in mice. However, the obese, HFD-fed mice maintained significantly higher levels of plasma lipids than LFD-fed mice, which may contribute to improved sepsis survival in obesity by providing substrates for energy production.

Impact of Maternal Obesity on Fetal Imprinting of Cardiovascular Risk: Unveiling Sex Differences in Fetal Programming

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Background: The “the fetal origins” hypothesis proposes that adverse environmental exposures leads to irreversible adaptations in organ structure and/or function and predispose the individual to the later onset of chronic disease in adulthood. Although the incidence of obesity continues to raise worldwide, the impact of maternal obesity (and related nutrition) on fetal imprinting of later cardiovascular risk remains poorly defined. Here we tested the hypothesis that maternal prenatal high fat diet (pHFD) alters normal cardiovascular development and function, and thereby predisposes offspring to cardiovascular disease in adulthood.

Methods: We assessed the cardiac functions of male and female *C57BL/6* mice that were prenatally exposed to pHFD or control diet (pCD). Cardiac function at basal level and after stress (isoproteronol, 0.25mg/kg body weight, ip) was assessed with echocardiogram at age of 8 weeks. Mice were then received either a single dose of LPS (1mg/kg, ip) or saline. Mice were sacrificed at 24h after the treatment. Plasma, cardiac, and hepatic samples were collected.

Results: Fasting lipid profiles showed no difference among male and female offspring. Prenatal high fat diet feeding resulted I lower body weight of male offspring but not females. At a baseline level, female offspring from pHFD dams displayed reduced aortic flow maximum velocity (AO Vmax, 119.5±33.0 vs. 141.9±24.1 cm/s, respectively, $p<0.05$), lower stroke volume (SV, 39.8±10.1 vs. 46.0±7.6 uL, respectively, $p<0.05$), and slightly reduced cardiac output (CO, 18.1±6.4 vs. 21.5±4.5 mL/min, respectively, $p=0.0536$) compared to pCD offspring. However, there were no significant function difference between pHFD and pCD exposure in male offspring at basal level. In contrary, male offspring from prenatal HFD had more significant deficit of stress response with lower % change after isoproterenol in AO Vmax (18.6±19.1 vs. 52.8±42.3 %, $p<0.01$), SV (23.5±15.5 vs. 53.4±52.5 %, $p<0.05$), CO (35.4±22.3 vs. 74.3±58.4 %, $p<0.05$), and fractional shortening (51.1±35.5 vs. 77.5±34.2 %, $p<0.05$).

Conclusions. Our findings demonstrate that prenatal exposure of high-fat diet could have long term effects on cardiac function in adult offspring and it is sex dependent. Prenatal HFD has more significant effect on contractile reserve in male mice than that in female mice.

Elevated free cholesterol levels due to impaired reverse cholesterol transport are a risk factor for polymicrobial sepsis in mice

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Abstract

Lipid metabolic abnormalities have been observed in septic patients. However, the extent and mechanism by which they contribute to sepsis remain largely unknown. Reverse cholesterol transport (RCT) plays a crucial role in regulating cholesterol metabolism in the bloodstream. During this process, high-density lipoprotein (HDL) collects cholesterol from peripheral tissues and transports it to its receptor, scavenger receptor BI (SR-BI) in the liver, where it is secreted via bile.

In this study, we used AlbCreSR-BI$^{fl/fl}$ mice as a model of impaired RCT to investigate the impact of RCT on sepsis. We found that AlbCreSR-BI$^{fl/fl}$ mice were significantly more susceptible to cecal ligation and puncture (CLP)-induced polymicrobial sepsis, evidenced by a 14.3% survival rate compared to an 80% survival rate in SR-BI$^{fl/fl}$ littermates. Additionally, CLP-AlbCreSR-BI$^{fl/fl}$ mice had a 5-fold increase in free cholesterol levels relative to CLP-SR-BI$^{fl/fl}$ littermates. Notably, administering the cholesterol-lowering drug, probucol, normalized the free cholesterol levels and improved survival in CLP-AlbCreSR-BI$^{fl/fl}$ mice.

Our results highlight that elevated free cholesterol levels, resulting from impaired RCT, pose a significant risk factor for sepsis. This suggests that selectively targeting dysregulated cholesterol metabolism could be a promising therapeutic strategy for sepsis.

Characterization of Nrac, a nutritionally-regulated adipose and cardiac-enriched microprotein

Taylor Coughlin and Catherine A. Makarewich

Objective: Nrac (nutritionally-regulated adipose and cardiac-enriched gene) was previously identified as a microprotein encoded by the long noncoding RNA A530016L24Rik (human C14orf180) with 2 conserved transmembrane domains. Nrac is highly expressed in the heart as well as in brown and white adipose tissue and its expression is decreased in response to both fasting and obesity. To date, the biological function of Nrac is unknown, however a previous study indicated it may localize to the plasma membrane in white adipocytes. The goal of this study was to examine the molecular function of Nrac in adipocytes and the heart.

Methods and Results: Using a brown adipocyte cell culture system, we differentiated pre-adipocytes to mature adipocytes and analyzed Nrac expression levels by qPCR. Nrac expression was undetectable in pre-adipocytes but markedly increased during differentiation and was robustly expressed in mature adipocytes. To determine the subcellular localization of Nrac, we transfected brown pre-adipocytes with a HA-V5-eptipe tagged Nrac fusion protein (HA-V5-Nrac), differentiated these cells to mature brown adipocytes, and performed immunostaining. These experiments showed that Nrac localizes specifically to the endoplasmic reticulum, and that overexpression of Nrac led to a striking increase in intracellular lipid droplet size as determined by BODIPY staining. To analyze Nrac in vivo, we generated a novel Nrac knockout (KO) mouse line using CRISPR/Cas9. Histological analysis of brown adipose tissue (BAT) from these mice revealed that intracellular lipid droplet size was markedly decreased in Nrac KO tissue compared to BAT from WT mice. RNA-sequencing analysis of heart tissue from WT and Nrac KO mice revealed a striking downregulation of genes related to adipogenesis and fatty acid metabolism in Nrac KO hearts and an upregulation in endoplasmic reticulum stress related genes.

Conclusions: Our data indicate that Nrac is an endoplasmic reticulum membrane-localized microprotein that is highly expressed in the heart and adipose tissue. Our studies indicate that Nrac is involved in adipogenesis and lipid droplet formation. Future studies will aim to define the molecular mechanisms that contribute to Nrac function in heart and adipose tissue.

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

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

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

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

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

THE IMPACT OF *Dennd5b* ON DIETARY LIPID ABSORPTION AND METABOLISM IN MALE AND FEMALE MICE

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Background: The absorption of dietary lipids by enterocytes is essential for maintenance of systemic lipid homeostasis. Our laboratory previously demonstrated a role for the gene *Dennd5b* in intestinal lipid absorption. Female *Dennd5b*$^{-/-}$ mice have reduced appearance of dietary triglycerides (TG) in plasma and are less susceptible to diet-induced hypercholesterolemia, atherosclerosis, and obesity. In addition, we found that a common human *DENND5B* gene variant is correlated with body mass index in females, but not males. While these previous findings have demonstrated an important role for *Dennd5b* in lipid homeostasis in females, the impact of biological sex on dietary lipid absorption and peripheral lipid metabolism is not fully understood. Furthermore, the mechanism by which *DENND5B*'s role in TG absorption may mediate these metabolic phenotypes is not known.

Objective: To determine if the *Dennd5b*$^{-/-}$ mouse model recapitulates the sex disparity in *DENND5B* effect on metabolic phenotype that was observed in humans and to determine the fate of unsecreted TG in intestinal tissue.

Methods and Results: In this study, using a non-absorbable dietary fatty acid tracer, we quantitatively assessed the impact of *Dennd5b*-deficiency on lipid absorption in both male and female mice. Interestingly, there was a relatively modest reduction in lipid absorption efficiency in *Dennd5b*$^{-/-}$ mice in both sexes (males -13.8%, $p<0.001$; females -3.67%, $p<0.01$), despite a near complete absence of plasma TG after oil gavage in both sexes. This observation could be due to utilization of TG within enterocytes, altered systemic lipase activity, or both. Metabolic cage studies on mice fed high-fat diet showed the respiratory exchange ratio (RER) in both wildtype and *Dennd5b*$^{-/-}$ mice shifts toward utilization of fatty acids as an energy source. This observation and the fact that most ingested fatty acids do not leave the enterocyte in *Dennd5b*$^{-/-}$ suggests increased fatty acid metabolism by enterocytes. We hypothesized enterocytes are disposing of unsecreted TG by beta oxidation. In electron microscopy studies, we observed frequent large electron-dense structures that resemble autophagosomes. Additionally, western blotting of intestinal lysates for Lc3, revealed a significant increase in the ratio of Lc3-II to Lc3-I in *Dennd5b*$^{-/-}$ mice on Western diet, an indication of increased autophagy. We also observed increased protein levels of the fatty acid sensing transcription factor, Hnf4g, and mRNA abundance of one of its target genes involved in beta oxidation, *Cpt1a*, in *Dennd5b*$^{-/-}$ mice on Western diet. These findings are consistent with increased fatty acid oxidation. We also observed altered mitochondrial morphology by electron microscopy in *Dennd5b*$^{-/-}$ enterocytes after oil gavage. To understand the impact of impaired lipid absorption on gut microbiome in high-fat diet (45% kcal fat) fed mice, we performed microbiome analysis on fecal samples collected from both male and female wildtype and *Dennd5b*$^{-/-}$ mice. We found that *Dennd5b*-deficiency results in striking changes in specific gut microbes characterized by the appearance of several microbe species not detected in wildtype mice.

Conclusions: This study suggests that the sexually dimorphic impact of *Dennd5b* may not be as prominent in mice as it is in humans. In mice, both sexes display a metabolic impact resulting from *Dennd5b*-deficiency. Our data support the hypothesis that unsecreted TG in the *Dennd5b*$^{-/-}$ enterocytes are degraded by autophagy, liberating free fatty acids which are utilized in mitochondrial oxidation. Overall, our findings demonstrate that *Dennd5b* plays a critical role in secretion of dietary TG by enterocytes that can impact systemic metabolic health by regulating lipid metabolism in the intestinal tissue.

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Vascular Smooth Muscle Cell–specific Deletion of Protein Kinase R-like Endoplasmic Reticulum Kinase Attenuates Microbiome-enhanced Abdominal Aortic Aneurysm

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BACKGROUND: We have previously linked the microbiome-derived metabolite trimethylamine N-oxide (TMAO) to abdominal aortic aneurysm (AAA) in both associative human and mechanistic mouse studies. TMAO is reported to bind protein kinase R-like endoplasmic reticulum kinase (PERK), resulting in selective activation of the unfolded protein response (UPR). Concordantly, our previous RNA sequencing results indicate that TMAO augments PERK-mediated UPR pathways in the aneurysm wall. Yet, the afflicted cell types and underlying mechanisms of how TMAO–mediated PERK activation drives AAA pathogenesis remain unknown.

METHODS & RESULTS: As global deletion of PERK is embryonically lethal, *Ldlr*$^{-/-}$ mice with conditional global *Perk* deletion were generated by crossing hemizygous male CAGCre-ER (B6.Cg-Tg(CAG-Cre/Esr1*)5Amc/J, Jackson Labs) to female *Perk* floxed mice (*Eif2ak3*$^{tm1.2Drc}$*/J*, Jackson Labs). Subsequently, both male and female CAGCre-ER positive (CAG^{CRE+}) and CARCre-ER negative (CAG^{CRE-}) mice were fed tamoxifen diet for 14 days. Surprisingly, CAG^{CRE+} died (14/24) during tamoxifen feeding compared with no deaths (0/14) in CAG^{CRE-} mice. A significant reduction in blood glucose after 10 days of tamoxifen diet accompanied by a pronounced reduction in pancreas size was observed in CAG^{CRE+} compared to CAG^{CRE-} mice. Next, given that global PERK deletion is lethal at any age and the pronounced role of VSMCs in AAA progression, *Ldlr*$^{-/-}$ mice with VSMC-specific deletion of *Perk* were produced by breeding hemizygous male mice expressing Cre under the control of the Transgelin promoter (*B6.Cg-TG(Tagln-cre)1Her/J*, Jackson Labs) to female *Perk* floxed mice (*Eif2ak3*$^{tm1.2Drc}$*/J*, Jackson Labs). Specific deletion of PERK in VSMCs was validated by western blot. Male mice were subjected to angiotensin II (AngII) infusion via osmotic mini pump (1,000 ng/kg/min; 28 days), while female mice underwent laparotomy and application of topical elastase (10 mg/mL porcine pancreatic elastase 5 minutes) to induce AAA. To augment circulating TMAO levels, mice were fed a high choline diet (0.2% total cholesterol + 1.2% choline supplementation) for one week prior to and throughout the study. Male mice with VSMC specific PERK knockout demonstrated significantly reduced AngII-induced aortic dilation (Cre+: 0.90 ± 0.048 mm; Cre-: 1.57 ± 0.156 mm; P = 0.001) and rupture induced death

relative to control. Similarly, female mice with VSMC specific PERK knockout displayed significantly reduced aortic diameter (Cre+: 1.17 ± 0.069 mm; Cre-: 2.65 ± 0.432 mm; P = 0.002) and rupture induced mortality as compared to control.

CONCLUSIONS: These results indicate that while global deletion of *Perk* is lethal, VSMC specific knockout of PERK blunts AAA formation in two independent murine models. Further studies are needed to elucidate the mechanism of PERK mediated ER stress in AAA, which may reveal novel therapeutic targets for AAA treatment.

Scavenger Receptor BI-mediated Heme Uptake Is An Essential Heme Clearance Pathway During Severe Hemolysis

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Background:

Severe hemolysis plays a harmful role in both sepsis and Sickle Cell Disease (SCD). In 2021, SCD accounted for 367,000 cases of mortality burden, while sepsis affected 11 million worldwide. An incomplete understanding of heme clearance during severe hemolysis constitutes a significant barrier to better treatments. When red blood cells rupture, hemoglobin (Hb) releases a substantial amount of heme, initiating a cascade of complications in pulmonary and systemic vasculopathy. Initial protection is provided by Haptoglobin (Hp) and hemopexin (Hx). Hp sequester Hb, limiting heme liberation, while Hx sequester heme. However, with disease progression, Hp and Hx are depleted, and plasma heme rises to a dangerous level. Given the limited protections offered by Hp and Hx, there is an urgent imperative to explore a novel heme clearance pathway for advancing therapies.

Methods & Results:

Firstly, we used the crystal structure of a related protein (LIMP-2) as a template within SWISS-MODEL to create an initial SRBI structural model. Simulation test revealed that heme binds SRBI in the hydrophobic pocket. Surface plasmon resonance (SPR) platform showed an equilibrium dissociation constant (Kd) of 18uM, which is pathologically meaningful. Following that, we used CHO, HEK293 cell lines stably expressing vector or SR-BI, primary hepatocytes from hepatic SRBI KO, and wild-type mice to examine cellular uptake of heme analog, ZnMP, via flow cytometry and confocal fluorescence microscopy. Cellular uptake of ZnMP exhibited dose dependence and was inhibited by competitive inhibition and BLT-1, an SRBI irreversible inhibitor. Heme binding proteins (HDL, Hx) inhibited cellular uptake, suggesting SRBI mediates free heme uptake. Secondly, we injected heme to liver SRBI KO and wild-type mice. Compared to the wild-type, liver SRBI KO mice showed a significant delay in plasma heme clearance, while lung and kidney accumulated more heme. Immunoblot showed that heme upregulates HO-1 expression more in cells expressing SRBI than in the vector control. Lastly, survival analysis showed mice without hepatic SRBI are more susceptible to PHZ and hemin-induced acute hemolysis, evidenced by lower RBC, Hb, higher spleen weights, plasma free heme, and mortality rates compared to wildtype littermates (100% survival in wildtype n=15 vs. 60% in liver SRBI KO mice n=20, both genders).

Conclusions:

Here, we demonstrated that SRBI mediates the uptake and clearance of free heme and protects against hemolysis-induced lethality. Interventions targeting SR-BI-mediated heme clearance may represent a new therapeutic category in the battle against hemolytic complications such as sickle cell disease crisis and sepsis.

Protease-activated Receptor 2 Attenuates Aneurysm Progression and Rupture in the Topical Elastase Murine Model

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Background: Accumulating evidence suggests protease-activated receptor 2 (PAR2) signaling promotes the release of proinflammatory cytokines which contribute to the pathogenesis of several cardiovascular diseases. Our laboratory has previously shown that PAR2 deficiency in low-density lipoprotein receptor deficient (*Ldlr*$^{-/-}$) mice attenuates the incidence of abdominal aortic aneurysm (AAA) in the angiotensin-II (AngII) mouse model. Our lab has also shown that pharmacologic inhibition of PAR2 with a cell-penetrating pepducin inhibits progression of AAA within the AngII model. The purpose of this study was to determine whether these effects of PAR2 deletion persist in the topical elastase model of aneurysm development.

Methods and Results: We examined either *Par2*$^{+/+}$ (n = 12 male, n = 12 female) or *Par2*$^{-/-}$ (n = 12 male, n = 11 female) in the topical elastase mouse model of aneurysm. Briefly, mice were opened via laparotomy and received topical administration of porcine pancreatic elastase (PPE, 5µl of 10mg/mL for 5 minutes) to the infrarenal aorta. After 28 days, mice were euthanized and a portion of their aortas at the site of largest expansion was measured (diameter). Abdominal aortas were then embedded, and histology examined. *Par2* deficiency attenuated the incidence of rupture-induced death in both male and female cohorts compared to *Par2* proficient mice ($P < 0.05$). Importantly, *Par2* deficiency attenuated infrarenal aortic diameter when compared to *Par2* proficient mice in both male and female cohorts ($P < 0.01$). Finally, male and female *Par2*$^{+/+}$ mice had less type I collagen deposition ($P < 0.01$ for males, $P < 0.05$ for females) and more type III collagen deposition ($P < 0.05$ for each cohort) than the *Par2*$^{-/-}$ groups.

Conclusion: These results suggest that PAR2 deletion attenuates aneurysm formation in a second mouse model (topical elastase), further advancing the notion that PAR2 plays an important role in the pathogenesis of abdominal aortic aneurysms.

Acculturation and Length of Stay in the U.S. Impact Cardiovascular Disease Risk in U.S. Latinos/as

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Abstract

Background: The high prevalence of multiple cardiovascular disease (CVD) risk factors in the U.S. Latino/a population has contributed to increased stroke, ischemic heart disease, and heart failure mortality rates in recent decades. While the results of some studies indicate that among Latino/a immigrants, acculturation and length of stay in the U.S. influence CVD risk, this has not been well studied in those residing in non-traditional U.S. immigrant settlement communities where culturally responsive health promotion resources are limited.

Objective: To examine associations among acculturation, length of stay, and CVD risk among Latino/a immigrants residing in a non-traditional U.S. settlement community.

Methods: This was a secondary analysis of baseline data from the *Corazón de la Familia* (Heart of the Family) study. Participants were 239 Latino/a immigrants, age 18 years of age and older with multiple CVD risk factors. Acculturation was assessed using Marin's Acculturation Scale; scores range from 12-60 points, with a higher score indicating a higher acculturation to the United States culture. Length of stay in the U.S. was self-reported in years. Framingham Risk Score (FRS) was used to evaluate CVD risk. Multiple linear regression was used to determine whether acculturation and length of stay predicted CVD risk, controlling for marital status, employment, financial comfort, and educational attainment.

Results: The sample was predominantly female (86.2%) and married or cohabitating with a partner (73.2%). The majority had completed a high school education or less (66.1%), were employed full or part-time (86.2%), and reported that they were financially comfortable (80.3%). The mean acculturation score was 21.9 ± 6.3, and the length of stay in the U.S. was 14.6 ± 7.7 years. The mean CVD risk score was 3.7 ± 4.5. Regression analyses showed that acculturation and length of stay were both significant predictors of CVD risk ($F [6, 232] = 4.73$, $p < .001$; $R^2 = .11$) with higher acculturation ($\beta = -.15$, $p = .03$) and shorter length of stay ($\beta = .35$, $p < .001$) associated with lower risk.

Conclusions: The outcomes of this study support findings from research with other immigrant populations that indicate that CVD risk is negatively associated with degree of acculturation and positively associated with length of time in the U.S. Future research is needed to identify factors underlying these associations and to develop and test interventions to reduce risk in this population.

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

Objective: Recent lineage tracing studies have shown vascular smooth muscle cells (VSMCs) develop macrophage-like characteristics (MLCs) in late-stage atherosclerosis resulting in accelerated inflammation. Our objective is to determine if the inflammatory receptor, protease activated receptor 2 (PAR2), accelerates VSMC-MLC in a mouse model of atherosclerosis.

Methods and Results: Our lab has demonstrated PAR2$^{-/-}$ mice have decreased atherosclerotic lesion area versus proficient controls via downregulation of VSMC migration and cytokine release. To examine the role of PAR2 in the VSMC-MLC dedifferentiation, *Par2*$^{+/+}$ and *Par2*$^{-/-}$ VSMCs were treated with cholesterol for 72 hours. While *Par2*$^{+/+}$ VSMCs demonstrated a loss of VSMCs markers (α-actin and myosin heavy chain) and upregulated macrophage markers (CD68 and Mac-2), *Par2*$^{-/-}$ VSMCs remained stable. In a hybrid mouse diversity panel conducted on 101 strains of mice with induced atherosclerosis, PAR2 was significantly correlated with an MLC super-gene Krüppel-like factor 4 (KLF4), which we determined to have lower genetic expression in PAR2 deficient VSMCs. Previous studies have demonstrated PAR2 mRNA is bound and stabilized by the RNA binding protein human antigen R (HuR). Our studies confirm these findings where *Par2*$^{-/-}$ VSMCs have less HuR mRNA expression and activity, suggesting a role of HuR in PAR2 mRNA stability. *Par2*^{flox} as well as *HuR*^{flox} mice bred with a TagIn Cre model have been used to establish two VSMC-knockout mouse lines in our lab to investigate the effects of VSMC-specific PAR2 and VSMC-specific HuR in atherosclerosis. We have found when knocking out PAR2 in VSMCs, we see observe no significant differences in atherosclerosis between Cre+ and their Cre- littermates. Interestingly, we see an attenuation in atherosclerosis in the HuR VSMC-knockout mice versus their Cre- littermates, suggesting a more prominent role of HuR in atherosclerosis than previously hypothesized in our lab.

Conclusions: These results suggest that VSMC HuR may play a prominent role in the development of atherosclerosis and may also assist in the mechanisms that lead to VSMC dedifferentiation. Future studies will continue to study VSMC dedifferentiation using various *in vitro* experiments as well as mouse models bred to *Par2*^{flox} and *HuR*^{flox} mice.

Protein S, TFPI, and Protein C in Hemophilia A: A Study of Their Function and Interplay

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Background: Hemophilia A, an inherited bleeding disorder caused by coagulation factor VIII deficiency, is treated with protein replacement therapy but carries the risk of inhibitory antibody development. Inhibitors of the anticoagulant Tissue Factor Pathway Inhibitor (TFPI) are in development as bypassing agents, which allow coagulation in the absence of factor VIII. However, TFPI works synergistically with other anticoagulants, including Activated Protein C (APC) and Protein S (PS), so it is unclear to what extent inhibiting TFPI increases the risk of thrombosis. We hypothesize that PS, PC, and TFPI cooperatively inhibit thrombin generation, and inhibiting TFPI will reduce PS/APC function, resulting in increased thrombin generation and formation of denser fibrin clots.

Method: Plasma thrombin generation was measured in healthy volunteers and patients with hemophilia A, in the presence or absence of thrombomodulin (which promotes protein C activation) to assess the APC/PS pathway.

Results: We have previously developed a thrombin generation protocol capable of measuring the activity of the APC/PS system via thrombomodulin supplementation. An anti-TFPI antibody was added to this system to mimic pharmacologic TFPI inhibition. Initial experiments determined that the inhibitory effect saturated with 10nM antibody. Further experiments were performed with the following conditions: i) tissue factor alone; ii) tissue factor with anti-TFPI; and iii) tissue factor with anti-TFPI and thrombomodulin.

In the control group, anti-TFPI decreased the lag time (the delay before thrombin is detected), relative to tissue factor alone, from 3.99±1.23min to 2.98±0.88min, consistent with the function of TFPI in blocking the initiation of coagulation by tissue factor. This was unchanged by the addition of thrombomodulin (2.64±1.05min), consistent with the need to generate thrombin before protein C activation. Anti-TFPI also increased the total amount of thrombin produced (endogenous thrombin potential; ETP), peak thrombin, and maximal velocity, and all three parameters were reduced when thrombomodulin was added. The findings in the control group are consistent with previous reports.

Consistent with the control group, anti-TFPI increased ETP, peak thrombin, and velocity in plasma from Hemophilia A patients (339.71±183.55nM*min, 18.01±6.48nM, and 2.68±1.67nM/min, respectively) compared to tissue factor alone (183.08±103.62nM*min, 9.77±5.49nM, and 1.03±0.97nM/min, respectively). This was followed by a significant reduction in all three values when thrombomodulin was added (14.19±15.99nM*min ETP, 1.54±0.35nM peak, and 0.26±0.09nM/min velocity). However, the effect of thrombomodulin on the lag time was dramatically different in patients. Anti-TFPI decreased the lag time as expected (from 4.86±1.84min to 2.77±0.39min), but the addition of thrombomodulin prolonged the lag to 5.69±1.29min. This was unexpected, as thrombomodulin requires thrombin to promote APC activation. It is possible that even less thrombin is necessary to activate protein C than previously thought, at least in the context of Hemophilia A.

Conclusions: Low baseline thrombin generation, even in the presence of TFPI inhibition, renders hemophilia A plasma more sensitive to the APC/PS system, which prolongs the lag time in patients but not healthy controls. As the lag time correlates with initial clot formation, understanding this effect will provide crucial insights into hemophilia A-associated bleeding, and help to tailor management strategies that minimize thrombotic complications.

The progression of EcoHIV infection in mouse

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Aside from their classical role in hemostasis, circulating platelets are considered as first-line vascular guardians that can influence both the innate and adaptive immune systems. Many systemic viremias, *e.g.,* HIV-1, present with mild thrombocytopenia and activated platelets. However, the literature is contradictory, with some reports claiming that platelets are essential for the immune responses, while others imply that platelets are viral reservoirs promoting infection. Thus, there is a need for mouse models that recapitulate the HIV-1 life cycle to identify the key mechanisms of HIV-1 interactions with platelets. In 2005, Potash *et al.,* developed a mouse infective HIV-1 model, *aka.* EcoHIV, which contains the HIV-1 genome with the gp120 gene being replaced by the murine leukemia virus' (MLV) gp80 gene. This change enables EcoHIV to infect rodents but not humans. In this project, we studied the effects of EcoHIV infection on platelets. Our results indicate that EcoHIV uses the spleen as a viral reservoir for replication for up to one week before going into a latent phase that persists out to at least 42 days. Interestingly, EcoHIV differed from HIV-1 during both acute and chronic stages of infection. EcoHIV virions were rapidly cleared from the blood circulation, by 48 hr post injection, which is not the case with HIV-1, which is only cleared from circulation (on the protein level) after treatment with combined antiretroviral therapy (cART). Acutely, EcoHIV-infected mice also did not experience thrombocytopenia nor increased platelet-leukocyte aggregate formation. Furthermore, sucrose purified EcoHIV is not endocytosed by platelets nor did it activate them *in vivo* or *ex vivo*. However, Polyethylene Glycol (PEG)-purified EcoHIV activate platelets nonspecifically as the PEG also precipitates other contaminants, which can activate platelets. One explanation for this lack of effect is that rodent platelets lack the MLV's receptor protein, mCAT-1, suggesting that platelets may not be general "viral interceptors" but may need specific viral receptors to be functional in viremias.

Obesity is Associated with Sleep Disruption in Postmenopausal Women

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Sleep is vital to good health. Epidemiological studies have shown that poor sleep quality is associated with increased metabolic risks, including weight gain and obesity. In women, the loss of estrogens after menopause is associated with poor sleep quality and increased abdominal fat. While complaints about poor sleep during menopause are well-documented, few studies have investigated the relationship between sleep and metabolic risks in postmenopausal women. The goal of this study was to study the relationship between sleep and metabolic risk in postmenopausal women. Twenty-eight overweight, postmenopausal women participated in the study. We used actigraphy and sleep logs to measure sleep timing and sleep quality for 7 days. Body fat % (total body DXA scans), BMI, abdominal circumference, and HbA1c were collected as markers of adiposity and metabolic risk. We found that later onset of sleep and shorter sleep duration were associated with greater BMI, waist circumference, and body fat %. Furthermore, we found that shorter sleep duration was associated with higher HbA1c. Interestingly, markers of sleep quality, including total sleep fragmentation index and sleep efficiency, were not associated with metabolic risk factors in postmenopausal women. Together, these findings suggest that interventions that modify behavior toward earlier sleep times could be effective in reducing metabolic risks in postmenopausal women.

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Contribution of platelet endo- and exocytosis to the initiation and progression of aortic aneurysms

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Background: Aortic aneurysms present as a widening and often catastrophic rupture of the aorta. While precipitated by an atherothrombotic and inflammatory environment, the underlying pathological mechanisms are unclear and adequate treatment strategies are lacking. Although antithrombotic drugs have been implied as a therapy, few studies have defined how platelets contribute to aortic aneurysms.
Aim: To investigate the role of platelet endo- and exocytosis in aortic aneurysm initiation and progression.
Methods: Aneurysm formation was induced in mice defective in platelet α-granule biogenesis (Nbeal2$^{-/-}$ and Serglycin$^{-/-}$), endocytosis (Arf6$^{-/-}$ and VAMP2/3$^{\Delta}$), and exocytosis (Munc13-4^{Jinx}) using: **1**) *i.p.* injection of an adeno-associated viral (AAV) vector expressing a gain-of-function mutation (D377Y) of mouse proprotein convertase subtilisin/kexin type 9 (PCSK9); **2)** western diet; and **3**) continuous, subcutaneous infusion of angiotensin II. *In vivo* ultrasound measurements were performed before and during infusion to assess aneurysm formation in both thoracic and abdominal aortic regions. At 4 and 12 weeks, or after rupture, aortas were harvested and analyzed *ex vivo*.
Results: During the study, 30% (3/10, p=0.0014) of the Nbeal2$^{-/-}$ and 21.4% (3/14, p=0.0145) of the Munc13-4^{Jinx} mice suffered thoracic and/or abdominal aortic rupture, compared to 0% (0/26) of wildtype mice. In the survivors, the inner diameter of neither the thoracic nor the suprarenal abdominal aorta of the α-granule-deficient Nbeal2$^{-/-}$ mice changed over time. However, *ex vivo*, Serglycin$^{-/-}$ mice showed a clear, more distributed, widening of the thoracic and descending aorta instead of local aneurysm formation with an increased maximum outer aortic diameter of the ascending/arch region compared to wildtype. Interestingly, Munc13-4^{Jinx} mice had an all-or-nothing phenotype; presenting with early-stage, massive aneurysm formation (females) or rupture (males), or showing a more distributed and atherosclerotic phenotype similar to Serglycin$^{-/-}$ mice. Animals with endocytosis-deficient platelets survived the study and at early stages, the differential increase in the average inner diameter of both the thoracic and suprarenal abdominal aorta was higher in female Arf6$^{-/-}$ mice compared to wildtype. Similarly, VAMP2/3$^{\Delta}$ mice had the largest inner thoracic diameter increase after 28 days of infusion. Arf6$^{-/-}$ and VAMP2/3$^{\Delta}$ mice showed a smaller maximum outer diameter of the descending aorta than wildtype mice.
Conclusions: Our data show that platelet endo- and exocytosis exert significant but perhaps contrasting effects on aortic aneurysm initiation and progression. By comparing the phenotypes of these strains, more detailed mechanistic insights into the role of platelets in aortic aneurysms seems possible.

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The role of platelet mitochondrial bioenergetics and glycogen mobilization in thrombosis and hemostasis

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Platelets are one of the most metabolically active cells in the bloodstream. They are known to show metabolic flexibility, switching between glycolysis and OxPhos depending on oxygen tension and the availability of substrates. Platelets also have metabolizable glycogen granules that contribute to the energy pool upon activation. The goal of this study is to investigate platelet energy metabolism under normal physiological conditions to better understand the relative roles of the major ATP-generating processes (glycolysis, OxPhos, and glycogenolysis). To address the role of glycogen, we used 2 glycogen phosphorylase inhibitors and tested their effects on standard platelet functions e.g., secretion, aggregation, clot contraction, and thrombus formation under shear. We also measured the glycogen levels in resting and activated platelets and in the presence of inhibitors. Consistent with previous studies, glycogen was utilized when platelets were activated with a 25% decrease upon thrombin activation. Both inhibitors blocked the use of glycogen and led to a 3-fold increase in glycogen compared to controls. This data suggests the active turnover of glycogen in resting platelets. Our inhibitor studies strongly indicated that glycogen is dispensable for low-energy processes like aggregation but contributes to secretion, clot contraction, and thrombus formation under shear. The inhibition was rescued by either increasing external glucose or bypassing glycolysis with added pyruvate. This study shows that the glycogen granules are metabolically active and contribute to the energy pool upon activation; this pool is important for high-energy platelet functions like clot contraction. Deletion of platelet glucose transporters (GLUT1 and GLUT3) revealed an essential role in platelet function and hemostasis and related glucose metabolism to hemostasis in vivo. Glucose can power platelets via glycolysis alone or in combination with OxPhos (in the mitochondria). We sought to probe the importance of platelet mitochondrial bioenergetics in hemostasis and thrombosis. Previously used mitochondrial inhibitors (antimycin, oligomycin) are toxic and cannot be used for in vivo studies, thus we developed two novel mouse models with altered mitochondrial function using a platelet-specific deletion of TFAM and QPC. TFAM (Transcription Factor A Mitochondrial), is essential for the maintenance, transcription, and translation of mitochondrial DNA. Its deletion is expected to disrupt platelet mitochondrial DNA, which encodes 13 subunits of OxPhos. QPC is a subunit of ubiquinol-cytochrome c reductase complex III. Its deletion is expected to disrupt Complex III, which is part of the platelet mitochondrial respiratory chain. We confirmed TFAM KO using Western blot analysis. Using Seahorse Analyzer, we confirmed that deleting TFAM disrupted platelet mitochondrial

bioenergetics in both resting and thrombin-stimulated platelets as seen as a 40% decrease in Oxygen Consumption Rate (OCR). We also observed a significant decrease in ATP production indicating the contribution of OxPhos to ATP generation. Aggregation was not affected
but in vitro clot contraction and thrombus formation under shear were defective (58%increase in lag time, 30% increase in AUC, and 60% decrease in surface area coverage in KO). Both the KO animals showed an increase in tail-bleeding time in the KO, an increase in occlusion time in the FeCl3 carotid injury model, and delayed hemostasis in a jugular puncture injury model with significantly higher rebleeding indicating that mitochondrial bioenergetics is important for clot stability. Using two novel mouse models with dysfunctional mitochondrial bioenergetics, we show that OxPhos is dispensable for low-energy demanding platelet functions such as aggregation but is important for secretion, clot contraction, hemostasis, and thrombosis. Platelets show considerable plasticity in energy metabolism using both glycolysis and oxidative phosphorylation. Using mouse models and inhibitors, we show the relative importance of these energy-producing processes (table) and the fuels and metabolic pathway choices made by platelets at different functional stages. Improving our understanding of platelet metabolism and the relative contributions of each pathway in the future could lead to a better understanding of how metabolic disorders can affect thrombotic risk in diabetic and obese patients.

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

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

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Background: Abdominal aortic aneurysm (AAA) is a dilatation of the infrarenal aorta and is defined as a >50% increase in diameter from baseline. Platelets are rapidly recruited to the forming intraluminal thrombus in AAA, stabilizing the aortic wall, and preventing rupture. Adhesion and activation of platelets releases a mix of inflammatory mediators such as transforming growth factor β (TGFβ), which mediates cellular proliferation, inflammatory signaling, and fibrosis. Importantly, while TGFβ neutralization results in increased rupture and disease progression in mouse models of AAA, the source of protective TGFβ signaling remains unknown. We hypothesized that platelet-derived TGFβ1 is the source of protective signaling and will attenuate aortic growth in murine AAA models.

Methods and Results: *Tgfβ1* floxed mice were obtained from Jackson Labs and bred with the platelet-specific Cre line platelet factor 4 (*Pf4*). Male *Tgfβ1-Pf4*$^{Cre-}$ (n = 7) and *Tgfβ1-Pf4*$^{Cre+}$ (n = 8) were subjected to laparotomy and topical elastase application (5µl 10 mg/mL porcine pancreatic elastase for 5 minutes). Ex vivo aortic diameter at day 28 showed *Tgfβ1-Pf4*$^{Cre+}$ mice had augmented abdominal aortic diameters compared to *Pf4*$^{Cre-}$ mice (*β1-Pf4*$^{Cre-}$: 1.70 ± 0.12 mm; *β1-Pf4*$^{Cre+}$: 2.37 ± 0.25 mm; P<0.01). Additionally, 25% (2/8) of Cre+ males died of an infrarenal aortic rupture with no deaths occurring in the control group. Similar results were found in female mice with platelet specific TGFβ1 depletion in the elastase model (*β1-Pf4*$^{Cre-}$: 1.38 ± 0.08 mm; *β1-Pf4*$^{Cre+}$: 2.21 ± 0.38 mm; P = 0.03).

Conclusion: Our results further support the view that TGFβ signaling plays a protective role in AAA. Furthermore, we identify TGFβ1 as a key isoform and platelets as an important source of TGFβ1 in aneurysm mitigation. Future studies will investigate potential cellular targets and underlying mechanisms of this protective phenotype.

The estrous cycle coordinates the circadian rhythm of eating behavior in mice

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The loss of circulating estrogens after menopause exacerbates the risk of cardiovascular disease in women. Estrogens impact cardiovascular function in many ways, ranging from direct effects on vasculature to regulation of insulin sensitivity. We previously found that exogenous estradiol treatment of ovariectomized mice regulates insulin sensitivity and metabolic risk by controlling the daily rhythm of eating behavior, or meal timing. Thus, another mechanism by which estrogens could regulate cardiovascular disease risk in females may be via meal timing. The goal of this study was to determine whether eating behavior rhythms are regulated by cycling ovarian hormones. We first studied diurnal eating behavior rhythms in female C57BL/6J mice fed low-fat diet in the light-dark cycle (12L:12D). The mice had regular 4- or 5-day estrous cycles as determined by vaginal cytology. Wheel revolutions also fluctuated with 4- or 5-day cycles and were greatest on the night of proestrus into estrus. We found that the amplitude, or robustness, of the eating behavior rhythm peaked every 4 or 5 days during proestrus or estrus. After removal of cycling hormones with ovariectomy, the amplitude of the eating behavior rhythm fluctuated at irregular intervals. Next, we studied the circadian eating behavior rhythm in constant darkness and found that the eating amplitude peaked every 3 to 5 days coincident with the greatest wheel activity, and thus ovulation. Together, these data show that fluctuations of ovarian hormones across the estrous cycle temporally organize the robustness of circadian eating rhythms so that it coincides with ovulation. While estrogen regulation of meal timing may have evolved to optimize reproductive success, another effect may be to reduce metabolic risk.

Effect of Prenatal Opioid Exposure on Cardiovascular Disease (CVD) Risk In Adult Offspring

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Adversity and dysfunctional households during childhood have been shown to increase the overall CVD risk in the long term. A common contributor to dysfunctional households is parental drug abuse, in which transplacental effects act as a stressor to the developing offspring. Chronic opioid exposure and opioid use disorder (OUD) are associated with a 2-fold increased risk of CVD and a 16% increase in coronary artery disease. OUD among pregnant women is an understudied area related to the opioid epidemic, causing a 5-fold increase in neonatal opioid withdrawal syndrome (NOWS) incidence. In former studies, we subjected female rats to two models of maternal opioid exposure: 1) morphine sulfate during gestation or 2) fentanyl citrate during preconception and gestation. At birth, female and male opioid-exposed offspring showed normal birth weight compared with vehicle-exposed offspring. Additionally, opioid-exposed offspring displayed NOWS-like somatic withdrawal signs after parturition and are significantly smaller from weeks 1-3 of life and catch up by week 4. As adults, in utero opioid-exposed offspring displayed increased mean arterial pressure, sympathetic activation, and CVD risk factors. Prenatal exposure to opioids has been shown to dysregulate the endogenous opioid peptides (EOPs) and alter behavioral outcomes later in life, but the effects on blood pressure regulation remain understudied. Therefore, this study aimed to investigate the expression of proenkephalin (PENK) in opioid-exposed adult offspring at two levels: a) centrally, in brain regions known to control the sympathetic outflow (Paraventricular nucleus (PVN) and rostral ventrolateral medulla (RVLM)), and b) peripherally, in the heart, kidneys, and liver. Protein was extracted from tissues for western blot analysis using the primary antibody for PENK (1:1000, invitrogen). PENK protein expression in RVLM and PVN showed a 35% and 30% reduction, respectively, in opioid-exposed offspring. In the heart, PENK protein expression was not statistically different in opioid-exposed offspring compared with vehicle-exposed offspring. However, in the kidneys and liver, PENK protein expression showed a 66% and 50% increase in male opioid-exposed offspring but not male opioid-exposed offspring compared to vehicle-exposed offspring. Similar to what is shown clinically in disease states of kidney damage and liver steatosis. As EOPs exert inhibitory effects on adrenergic signaling, our data indicate that prenatal exposure to opioids may induce a permanent downregulation of EOPs in the central nervous system that may contribute to increasing the sympathetic tone and desensitize the acute pressor responses to opioid peptides, suggesting that EOPs may be critical for blood pressure regulation. However, peripheral expression of PENK seems to reflect pathophysiological alterations in different tissue since elevated PENK level is clinically used to assess the severity of tissue damage.

Postdoctoral Fellow

Time Restricted Feeding in Mice Exposed to Light at Night Normalizes Day/Night Rhythms in Cardiovascular Function

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Introduction: Light at night disrupts circadian rhythms in cardiovascular and metabolic function and is associated with adverse health outcomes. Mice housed in dim light at night (dLAN) have disrupted day/night feeding and activity rhythms. Specifically, their rhythmic feeding behavior decreases, and they eat more calories during the light (inactive) cycle. We tested the hypothesis that time-restricted feeding to the dim light (active) cycle would normalize ~24-hour rhythms in cardiovascular function.

Methods: Male and female mice were implanted with telemetry devices to continuously record the electrocardiogram (ECG), blood pressure (BP), core body temperature (Tb), and activity. Mice were housed in thermoneutrality in 12 h light: 12 h dark cycles (LD, 200 lux: 0 lux) with ad libitum access to food (LD-ALF). Mice were then subjected to 12 h light: 12 h dim light cycles (dLAN-ALF; 200 lux: 5 lux) for two weeks. After 2 weeks, dLAN mice were restricted to dim-light cycle restricted feeding (dLAN-RF).

Results: dLAN mice ate more food during the light cycle, and they had a 25-40% reduction in the amplitude of the day/night rhythms in HR, BP, Tb, and activity. dLAN decreased the day/night differences between BP, HR, and Tb. dLAN also shifted the phase (peak time) for HR and Tb but not BP and activity. Time-restricted feeding to the dLAN cycle increased day/night rhythms in HR, BP, and Tb but not activity.

Conclusions: dLAN disrupted day/night rhythms in feeding behavior, HR, BP, Tb, and activity. Time-restricted feeding to the dLAN cycle normalized the amplitude in the day/night rhythms for HR, BP, and Tb but not activity. The data suggest that time-restricted feeding in mice exposed to dLAN mitigated the disruption of day/night rhythms in cardiovascular function but not activity cycles.

RAD Reduction Improves Heart Failure with Reduced Ejection Fraction

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Background: Heart failure with reduced ejection fraction (HFrEF) entails a systolic dysfunction of the heart. Treatment of HFrEF involves medications like Beta blockers and ACE inhibitors targeted towards reducing the pathologic remodeling that occurs with systolic failure. Ras associated with Diabetes (RAD) represents a promising target for heart failure therapy as previous studies have shown its depletion has increased systolic function without pathologic remodeling. A key response of the heart to chronic pressure overload is myocardial hypertrophy.

Hypothesis: Rad-depletion is cardioprotective for HFrEF

Methods: The present study used thoracic aortic constriction (TAC) as a model for pressure-overload in both Wild type (WT) and tamoxifen induced cardiomyocyte restricted RAD Knockout (cRadKO) mice. Congestive heart failure was determined by elevated lung weight. Cardiomyocyte hypertrophy was evaluated by cross-sectional area (CSA) in short axis histology slides. Male and female mice were studied.

Results: cRadKO was associated with improved ejection fraction following TAC. Survival and heart failure incidence was sexually dimorphic. In males, TAC promoted death and heart failure that was significantly attenuated by cRadKO. Females survived TAC and did not show eleveated lung weight. To assess myocardial hypertrophy, cross sectional area (CSA) was measured using a neural network novel model similar to previous work in skeletal muscle to allow analysis of a greater number of fibers than could feasibly be accomplished by hand measurement. This model demonstrates a high fidelity to hand measured cardiomyocyte CSA. CSA measurements showed hypertrophy in response to TAC, but no significant difference between cRadKO and WT, Work is in progress on male mice. The present study affirms Rad as a possible therapeutic target for treatment of heart failure, and presents an improved, high throughput workflow to measure myocardial hypertrophy.

Cardiomyocyte-Restricted Deletion of RAD Improves Heart Failure in a Mouse Model of Dilated Cardiomyopathy

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Background: Heart failure (HF) is the second leading cause of hospitalization and is projected to increase in prevalence by 46% by 2060. Despite advancements, the one-year mortality rate is 35%. Dilated cardiomyopathy (DCM) is the most common cardiomyopathy resulting in systolic heart failure with reduced ejection fraction (HFrEF). Current therapies fail to address a principal issue: loss of contractile force. Targeting proteins involved in excitation-contraction, such as the L-type calcium channel (LTCC) may offer a means to improve quality of life and attenuate adverse cardiac remodeling. RAD (Ras associated with diabetes) resides in the LTCC and loss of Rad modulates LTCC current ($I_{Ca,L}$). Modulated $I_{Ca,L}$ exhibits increased open probability, a more negatively shifted activation midpoint, and increased rate of current decay. Previously, cardiomyocyte-restricted deletion of RAD (cRADKO) has been shown to stably improve cardiac function in healthy mice.

Hypothesis: RAD ablation—after DCM onset—will rescue cardiac function via modulation of $I_{Ca,L}$ to promote positive inotropy.

Methods: The muscle lim protein knockout mouse (MLPKO) model of DCM was used to test if cRADKO induced in 10-week old mice improved function and attenuated pathology. Mice were evaluated $\geq$4 weeks after cRADKO induction. Longitudinal echocardiography for *in vivo* assessment was performed along with bulk RNAseq of hearts, isolated live cell ventricular cardiomyocyte Ca^{2+} imaging and sarcomere function, and whole-cell patch clamp recordings of $Ca_v1.2$ current. Beta-AR agonist was used to test beta-AR responsiveness *in vivo and* in cardiomyocytes. Cardiomyocyte size and fibrosis were assessed in histological sections and/or isolated cells.

Results: cRADKO of MLPKO (double KO, dKO) mice improved ejection fraction, after 1 month in males and females. Left ventricle dilatation was reversed significantly in dKO mice. Transcripts associated with HF (e.g., *Nppb, Acta1, Myh7, Xirp2*) were downregulated significantly in dKO. Whole-cell patch clamp recordings of cardiomyocyte demonstrated modulated $I_{Ca,L}$ in dKO. Cardiomyocytes from dKO mice showed significant improvements in Ca^{2+} handling, notably in transient amplitude and decay kinetics. In parallel, sarcomeres showed significantly improved contractility and rates of contraction and relaxation.

Conclusions: RAD ablation resulted in positive inotropy and lusitropy at the cellular level. *In vivo* systolic function was significantly improved, and pathological remodeling was attenuated. This study implicates a new therapeutic target for treating DCM and expands understanding of Ca^{2+} handling in heart failure.

Deficiency of Plasminogen Activator Inhibitor-1 Augments Angiotensin II-induced Cardiac and Thoracic Aortic Pathologies

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Background:
Thoracic aortopathies, including aneurysm, dissection, and intramural hemorrhage, pose grave risks due to potential ruptures and limited treatments. Angiotensin II (AngII) is a critical contributor to aortopathy. Plasminogen activator inhibitor-1 (PAI-1) is the most elevated protein in thoracic aortas of mice infused with AngII for 3 days, prior to overt pathology. Underscoring its key role, PAI-1 increases drastically in aortic walls of human thoracic aortopathies, yet its contribution to the disease remains unknown.

Methods and Results:
To determine the contribution of AngII infusion to plasma PAI-1 levels, wild type mice were infused with saline (control) or AngII (1,000 ng/kg/min) for three days. AngII infusion led to increased plasma PAI-1 concentrations (median 6.7 ng/mL; range 0.9-9.4 ng/mL) compared to controls, in which plasma PAI-1 was not detected ($P = 0.03$ by Mann-Whitney U test). To investigate the role of PAI-1 in thoracic aortopathy, whole-body PAI-1 deficient mice (PAI-1-/-) or their wild type littermates (PAI-1+/+) were infused with AngII for 28 days. External and luminal diameters in the thoracic aorta were similar between genotypes. However, in male mice, PAI-1 deficiency exacerbated aortic remodeling, defined histologically. Medial thickening and adventitial hemosiderin deposition, indicative of intramural hemorrhage, were pronounced in PAI-1-/- mice infused with AngII. Additionally, pathology was grossly evident by discoloration occupying 20% of the aortic surface in PAI-1-/- mice compared to 3% in PAI-1+/+ mice ($P = 0.002$ by Mann-Whitney U test). PAI-1 deficiency also resulted in a 35% decrease in ascending aortic wall distensibility, an inverse measure of vascular stiffness, determined by ultrasound ($P = 0.035$ by Student's t-test). Next, to investigate the acute phase of aortopathy, PAI-1-/- mice were infused with AngII for 7 days. Intramural hemorrhage severity was increased in both male and female PAI-1-/- mice, defined by overt hemorrhage in the aortic wall. Unexpectedly, PAI-1-/- mice also displayed an increased incidence of hemorrhage in ventricular tissue at 7 days of AngII ($P = 0.002$ in males and $P < 0.001$ in females by Fisher's exact test) and increased ventricular tissue remodeling defined by overt discoloration at 28 days of AngII infusion. Additionally, systolic blood pressure was decreased by 24 mmHg (median) in female PAI-1-/- mice infused with AngII, compared to PAI-1+/+ mice ($P = 0.04$ by Mann-Whitney U test). To determine the source of PAI-1 in thoracic aortopathy, PAI-1 was deleted in smooth muscle cells (SMCs), the most abundant aortic cell type. SMC-specific PAI-1 deficient mice were infused with AngII for 28 days. Notably, measures of dilatation and tissue remodeling were similar between genotypes.

Conclusions:
PAI-1 deficiency exacerbated AngII-induced hemorrhage and remodeling in both ventricular and thoracic aortic tissues in mice. SMC-specific PAI-1 deficiency did not alter aortic or cardiac tissue remodeling, indicating that aortic-derived PAI-1 is not a key regulator of AngII-induced pathology. Future research will investigate the source of pathologically elevated PAI-1 and the mechanisms driving aberrant tissue remodeling in PAI-1-/- mice.

Cardiomyocyte-Restricted Deletion of RAD Improves Cytosolic Calcium Levels and Abnormal Calcium Release Events

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Background: About 6.1 million adults in the United States have HF, and this contributes to about a third of cardiovascular disease deaths. Dilated cardiomyopathy (DCM) is the most common type of cardiomyopathy, and it results in systolic heart failure with a reduced ejection fraction, enlarged chamber, and dilated ventricles. DCM is characterized by hypocontractility and this is due to abnormal Ca^{2+} levels. RAD is a protein which inhibits calcium current through the $Ca_v1.2$ channel, and it has recently been shown that cardiomyocyte-restricted deletion of RAD (cRADKO) improves systolic function and calcium levels in healthy mice. This study expands to focus on the understanding of RAD's affect in diseased mice.

Hypothesis: The main hypothesis is that the deletion of RAD in a DCM model will improve systolic function by increasing trigger Ca^{2+}.

Methods: A DCM murine model of HF was used; this model is known as the muscle lim protein knockout (MLPKO) mouse. At 10 weeks, RAD was knocked-out of cardiomyocytes in the diseased mice. Calcium transients and sarcomere function of dispersed adult heart cells were measured at various stimulus frequencies; additionally, cardiomyocyte size was analyzed. The hypothesis for calcium transients is that deletion of RAD in the DCM model will increase peak amplitude and cytosolic calcium levels. Additionally, sarcomere fractional shortening is predicted to increase, leading to overall improved systolic function. Non-stimulated calcium release events were also analyzed. The hypothesis for cell sizing was that deletion of RAD in the DCM model will decrease cardiomyocyte length and increase width relative to disease state. A trained AI and filtering algorithm was used to calculate differences in cell width, length, and area.

Results: In the MLP cRADKO, calcium transient amplitude significantly increased by 34% at 0.1Hz, and significantly increased by 49% at 2Hz. Calcium transient baseline significantly increased by 36% at 0.1Hz, and significantly increased by 27% at 2Hz. Calcium transient departure velocity significantly increased by 37% at 0.1Hz, and significantly increased by 45% at 2Hz. Calcium transient return velocity significantly decreased by 39% at 0.1Hz, and significantly decreased by 44% at 2Hz. There were also significant results in sarcomeres. The disease state model was showed significantly more non-stimulated calcium release events overall.

Conclusion: Rad-reduction in the setting of DCM increased myocardial calcium homeostasis to promote positive inotropy. Potential arrhythmogenic non-stimulated calcium release was abrogated by cRadKO on the DCM background. This study determines a new therapeutic target for systolic heart failure and DCM with a mechanistic basis focused on regulation of excitation-contraction coupling.

Early life stress increases the IL-17a secretory capacity of white blood cells in obese female mice

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Early life stress has been established as an independent risk factor for cardiovascular and metabolic disease later in life, potentially mediated by chronic low-grade inflammation. Several studies have reported that adult subjects who were exposed to early life stress in the form of adverse childhood experiences display increased inflammatory markers, such as high levels of the pro-inflammatory cytokine Interleukin 17a (IL-17). Increased levels of IL-17 are linked to the pathophysiology of hypertension, obesity and diabetes in humans and mice. IL-17 is primarily secreted by T helper 17 (Th17) cells, a pro-inflammatory subtype of T-cell. Previously, we have shown that female mice exposed to a model of early life stress called maternal separation and early weaning (MSEW) display high blood pressure in response to an obesogenic diet, which is associated with increased plasma levels of IL-17, and TNFa and INFg. Therefore, this study aimed to test whether obese female mice exposed to MSEW show an exacerbated capacity to produce IL17 in response to the stimulation of white blood cells (WBCs) with *PHA*-L (Phytohemagglutinin-L), a potent mitogen inducing activation and proliferation of lymphocytes.

MSEW was performed by separating the pups from the dams in a thermoregulated incubator for periods of 4-8 hours, from postnatal day 2 to 16, followed by an early weaning. Litters of normally reared mice served as controls. At weaning, female mice were placed for 16 weeks on a high fat diet (60% kcal from fat). At the endpoint, mice were euthanized and whole blood collected in heparin. WBCs were isolated from whole blood by treatment with red blood cell lysis buffer (BioLegend) and viable cells were resuspended in 500 microliters of RPMI media and counted using Trypan blue exclusion (Invitrogen). The resuspended cultures were divided into two 250 microliter aliquots, one unstimulated and the other stimulated with PHA-L (5 ug). Cultures were allowed to incubate in sterile conditions for 24 hours. Then supernatant and cells were collected for IL-17 quantification by ELISA (invitrogen) and normalized by cell count (pg IL-17/ 10^6 cells).

Unstimulated WBCs from obese control mice generated 13.5±9.6 pg IL17/ 10^6 cells vs. 22.3±16.5 pg IL-17/ 10^6 cells in response to PHA, while unstimulated WBCs from obese MSEW mice generated 6.9±2.3 pg IL-17/ 10^6 cells vs. 49.3±23.8 pg IL17/ cells x10^6 ($P<0.05$ vs. control) in response to PHA. Thus, MSEW mice showed a greater fold increase in IL-17 production vs. control (7.2 vs. 1.6-fold increase, $p<0.05$, respectively). Taken together, these results indicate that in obese female mice, MSEW enhances the innate responsiveness of Th17 cells to an immune challenge, although future studies will investigate whether differences in the number of Th17 resident in different tissues may contribute to increase circulating levels of this cytokine.

Deletion of Rad Elevates L-type Calcium Channel Facilitation

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Background: Calcium dependent facilitation (CDF) is defined as progressive increases in $I_{Ca,L}$ amplitude in response to a depolarization train. CDF is dependent on Ca^{2+} activation of calcium/calmodulin dependent kinase II (CaMKII) phosphorylation of LTCC.CDF is believed to be adaptive, counteracting accumulated inactivation at higher heart rates, but it can also help promote arrhythmia, possible because of slowed $I_{Ca,L}$ decay kinetics. Rad, an RGK protein, inhibits current through the L-type calcium channel (LTCC) at rest and loss of Rad results in tonic modulated $I_{Ca,L}$. Phosphorylation of Rad by protein kinase A (PKA) downstream of the β-adrenergic activation also results in $I_{Ca,L}$ modulation. Modulated $I_{Ca,L}$ has a larger maximal conductance (Gmax), negative-shifted activation midpoint (V1/2), and faster decay kinetics. Late-phase calcium current promotes arrythmia, and consequently faster decay kinetics and subsequently decreased late-phase calcium current can be protective against development of arrythmia.
Hypothesis: Deletion of Rad promotes elevated CDF in ventricular myocytes. Mechanistically, elevated Ca^{2+} flux is responsible for increased CDF in the absence of Rad.
Methods: We used the whole-cell configuration of the patch clamp technique and several voltage protocols to evaluate CDF (and voltage-dependent facilitation) in cRadKO and WT littermates dispersed ventricular myocytes.
Results: Deletion of Rad elevated CDF in ventricular myocytes. Replacement of Ca^{2+} with Ba^{2+} as the charge carrier abrogated CDF in the absence of Rad. Exposure to isoproterenol elevated CDF in WT ventricular myocytes. STZ treated mice (ox-CaMKII model) had elevated CDF comparable to cRadKO.
Conclusion: Deletion of Rad contributes to calcium dependent facilitation secondary to increased Ca^{2+} flux in the heart. Alterations in CDF have been shown to have potentially arrhythmogenic consequences; however, in the absence of Rad arrhythmias were not observed suggesting that Rad speeding of $I_{Ca,L}$ decay kinetics is a dominate determinant of Ca^{2+}-handling triggered arrhythmogenesis.

The effects of platelet secretion in a novel model of ischemic stroke

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Abstract

Stroke is a major cause of injury and mortality, but the role of platelet secretion in stroke remains unclear. Genetic ablation of the cargo sorting protein, NBEAL2, in mice is known to inhibit α-granule cargo packaging and thus its secretion from platelets. Deletion of NBEAL2 appears to be protective in an acute intralumenal transient Middle Cerebral Artery occlusion (tMCAO) model of ischemic stroke (Deppermann et al. 2013). We have confirmed this using a similar intralumenal tMCAO model, suggesting that platelet α-granule cargo contributes to the pathologic sequelae of ischemic stroke that occur in the first 24 hrs. To better understand this role in stroke, we examined the role of NBEAL2 in a different stroke model, the tandem Common Carotid Artery/Middle Cerebral Artery (CCA/MCA) occlusion model with generates a different ischemic injury that takes longer to evolve. Following the surgery, infarct size, vessel architecture, and blood-brain barrier permeability were assessed. There was no significant difference in injury size between WT and NBEAL2$^{-/-}$ animals after 72 hrs ($p = 0.8$). As a possible explanation for these observations, we examined the vascular branching and morphology in the brains of NBEAL2$^{-/-}$ mice compared to wild-type. NBEAL2$^{-/-}$ brains had less branching from major vessels and larger vessel diameter even in naïve animals, but there was no difference in vessel permeability as measured by Evans Blue perfusion. Taken with the intralumenal tMCAO findings, this work suggests a differential role for platelet secretion in proximal and distal vessel occlusions. Characterization of the tandem CCA/MCA model provides a novel approach to studying these contextual effects, which will inform clinical treatment of ischemic stroke.

Sleep and Eating Rhythms are Associated with Metabolic Risk in Postmenopausal Women

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Postmenopausal women are vulnerable to metabolic dysfunction. Compelling evidence suggests that this is because they lack the protective effect of estrogens. We have shown that circulating estrogens regulate daily eating and sleep-activity rhythms in female mice and protect them from obesity and type 2 diabetes. However, few studies have investigated whether postmenopausal women have disrupted eating and sleep-activity rhythms that could contribute to their metabolic dysfunction. The purpose of this study was to investigate the relationship between eating rhythms, sleep, and metabolic risk in postmenopausal women. We studied overweight postmenopausal women without diabetes, who were not taking hormones (estrogens ± progestin) for a period of 7 days. Sleep timing was assessed by actigraphy and sleep logs. Times of first and last calorie intakes each day were collected from participants with a texting system. Body composition (DXA), BMI, and waist circumference were collected as markers of obesity. Lipid metabolism and glycemic control were assessed by fasting lipid panel and HbA1c as well as oral glucose tolerance test. Insulin sensitivity was estimated using the Matsuda Index and HOMA-IR. Forty-five postmenopausal women (mean ± SEM; 57.6 ± 0.6 years) participated in the study. We found that later timing of sleep onset was associated with later calorie window, greater BMI, waist circumference and body fat percentage. In addition, longer daily calorie window and later time of last calorie was associated with decreased insulin sensitivity. Furthermore, later time of last calorie was also associated with greater waist circumference and BMI. These data suggest that interventions that shorten the daily calorie window and advance the timing of last calorie and sleep onset may improve metabolic risk in postmenopausal women.

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Identifying and categorizing mutation-specific differences between trafficking-deficient *KCNH2* mutations based on intracellular trafficking mechanisms.

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Objective: Congenital Long QT Syndrome (LQTS) is a heritable disease associated with an increased susceptibility to cardiac arrhythmia, syncope, and sudden cardiac death. Most symptoms of LQTS are manageable, however, diagnosing and identifying people at risk of LQTS before they experience a cardiac event has proven difficult. This is due to the variable expressivity and low penetrance exhibited by LQTS, where individuals with the same disease-causative mutation may experience different or no symptoms. Loss of function mutations in *KCNH2* are associated with congenital LQTS type 2 (LQT2). *KCNH2* encodes Kv11.1 channels that conduct the rapidly activating delayed rectifier potassium current (I_{Kr}), which is responsible for repolarization of the ventricular action potential. LQT2 arises as a result of reduced I_{Kr}, and the most common LQT2-causative mutations in *KCNH2* reduce I_{Kr} through impaired trafficking of Kv11.1 channels to the cell surface. Our collaborator, Dr. Yamuna Krishnan (University of Chicago) has developed a novel pH-sensitive K^+ reporter that will allow us to accurately measure the lumenal conditions that Kv11.1 channels experience as they traffic. With this reporter, our objective is to identify potential mutation-specific differences under these lumenal conditions that may be masked at the cell surface.
Methods: Wild type (WT) Kv11.1 channels were exogenously expressed in human embryonic kidney cells (HEK293), and patch-clamp recording was used to measure steady-state channel activation and inactivation. WT Kv11.1 HEK293 cells were kept in solutions mimicking lumenal pH and K^+ conditions measured in the trans-golgi network, early endosome, and recycling endosome, with standard extracellular solution used as a control.
Results: Preliminary data has shown that WT KV11.1 channels actively function under lumenal conditions, and we can measure steady-state activation and inactivation under each condition.
Conclusion: These data demonstrate that WT Kv11.1 channel activity can be measured under lumenal conditions. Moving forward, we can continue these experiments in known trafficking-deficient Kv11.1 channel mutations to determine if the lumenal conditions alter the steady-state activation or inactivation of trafficking-deficient channels in a mutation specific manner.

Shifts in Glycolytic Phenotype in Smooth Muscle Cells of Sporadic Aortic Aneurysms and Acute Dissections

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ABSTRACT

Introduction: Ascending thoracic aortic aneurysms (ATAA) and their progression to acute dissection (ATAD) are associated with high risk of mortality. Because metabolic pathways can regulate cell phenotype and disease progression, we investigated the transcriptomic profile of glyocoysis in smooth muscle cells (SMCs) in human aortic tissue and its potential involvement in promoting an inflammatory phenotype in SMCs of aortic aneurysms and dissections. We hypothesized that glycolytic activity in SMCs is elevated in ATAA and ATAD tissues compared to healthy control aortic tissues.

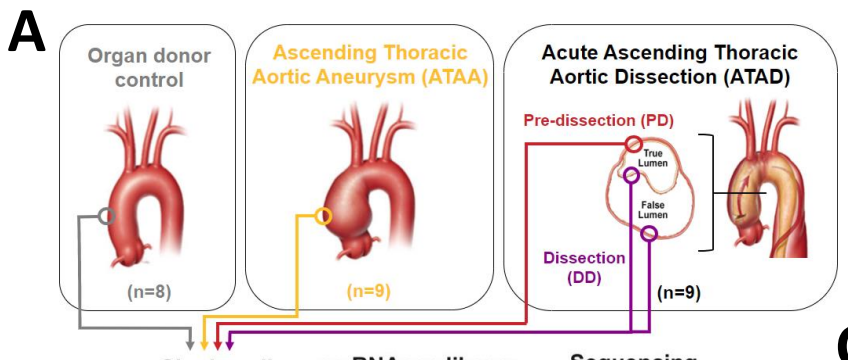
Methods: We performed single cell RNA sequencing (scRNA-seq) analysis of ascending aortic tissue from 9 patients with ATAA without dissection, 9 patients with ATAD (dissected and non-dissected areas collected separately), and 8 organ donor control subjects (Fig A). Within the SMC clusters analyzed (Fig B-C), we identified differentially expressed glycolytic genes between control, ATAA, and ATAD patients. Single-cell flux estimation analysis (scFEA) was performed to estimate metabolic flux variation in glycolytic activity in SMCs. scRNA-seq analyses and single-cell assay for transposase accessible chromatin using sequencing (scATAC-seq) were performed in ascending aortic tissues from wild-type (WT) mice infused with angiotensin II (AngII) or saline (control) and *Sting*$^{-/-}$ mice infused with AngII (Fig G-H, J-L).

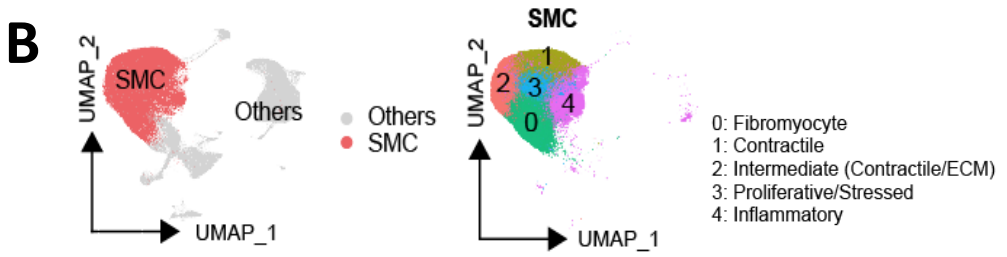
Results: Compared to control, glycolytic genes (e.g., *ENO1, HK1*) and glycolytic activity in SMCs were progressively upregulated from control to ATAA to ATAD (Fig D-F). We also observed progressive induction of lactate production gene *LDHA* from control to ATAA and ATAA to ATAD that was consistent with greater lactate accumulation in scFEA analysis (Fig D-F). In the angiotensin-induced AAD mouse model, AngII infusion increased the expression of glycolytic genes in SMCs (Fig I). Additionally, scATAC-seq analyses revealed elevated chromatin accessibility/gene activity of glycolytic genes (e.g., *Ldha*) in SMCs of Ang II-infused mice (Fig M-

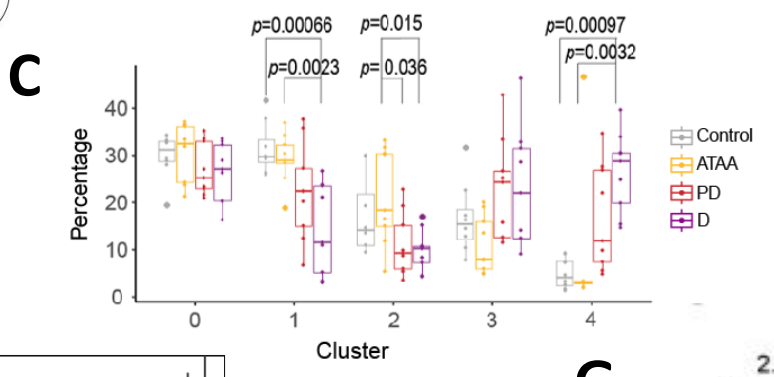
N), suggesting potential regulation at the epigenetic level. Furthermore, correlation analysis revealed that the activity of most glycolytic genes (e.g., *Ldha*) were positively associated with the motif activity of Nfkb1 and Nfkb2, which are well-established pro-inflammatory transcriptional factors (Fig O-P). Finally, knocking down stimulator of interferon genes (*Sting)*, an upstream trigger of NFkB, partially prevented Ang II-induced upregulation of glycolytic genes in SMCs (Fig I).

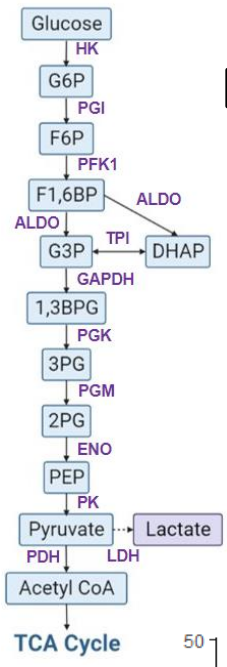
Conclusion: Our data suggest glycolytic activity and lactate production in SMCs is progressively increased from control to ATAA to ATAD. Activation of STING pathway and subsequent activation of NFkB pro-inflammatory signaling may play a critical role in epigenetic induction of these genes. Investigating the upstream and downstream regulators are key to understanding this metabolic shift in aortic disease progression.

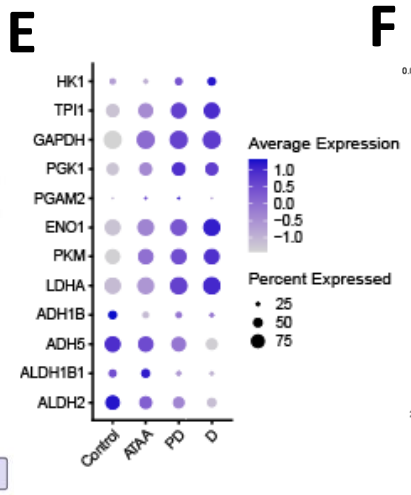
Figure:

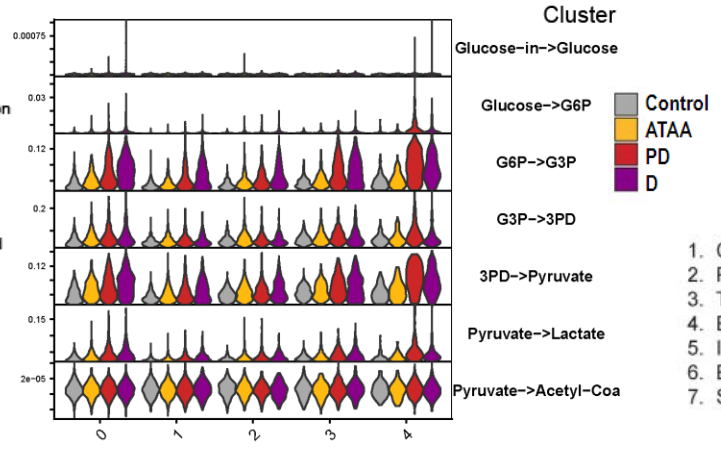

A
Organ donor control
(n=8)
Ascending Thoracic Aortic Aneurysm (ATAA)
(n=9)
Acute Ascending Thoracic Aortic Dissection (ATAD)
Pre-dissection (PD)
True Lumen
False Lumen
Dissection (DD)
(n=9)
Single-cell suspension
sc-RNAseq library
(Control/Disease)
Sequencing

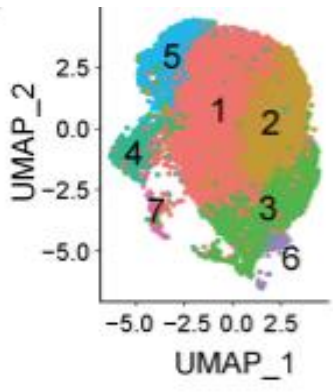

B
SMC
Others
UMAP_2
UMAP_1
Others
SMC
0: Fibromyocyte
1: Contractile
2: Intermediate (Contractile/ECM)
3: Proliferative/Stressed
4: Inflammatory

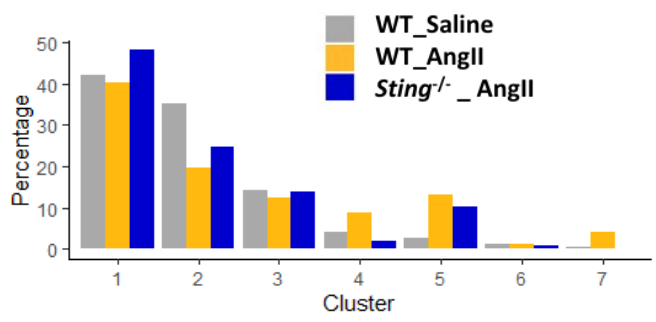

C
p=0.00066
p=0.0023
p=0.015
p=0.036
p=0.00097
p=0.0032
Percentage
Cluster
Control
ATAA
PD
D


D
Glucose
HK
G6P
PGI
F6P
PFK1
F1,6BP
ALDO
ALDO
G3P
TPI
DHAP
GAPDH
1,3BPG
PGK
3PG
PGM
2PG
ENO
PEP
PK
Pyruvate
Lactate
PDH
LDH
Acetyl CoA
TCA Cycle

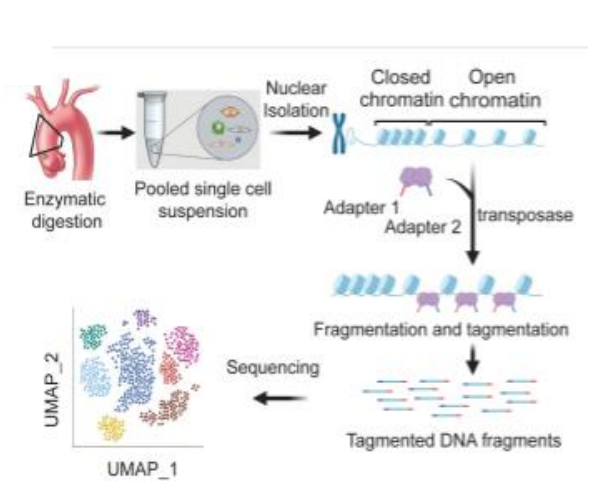

E
HK1
TPI1
GAPDH
PGK1
PGAM2
ENO1
PKM
LDHA
ADH1B
ADH5
ALDH1B1
ALDH2
Control
ATAA
PD
D
Average Expression
1.0
0.5
0.0
−0.5
−1.0
Percent Expressed
25
50
75

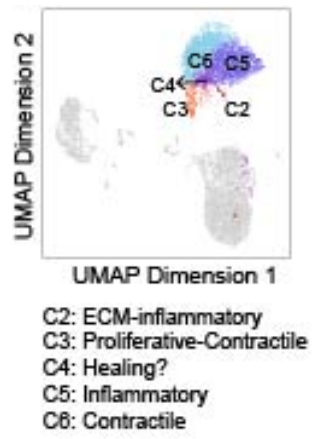

F
Glucose−in−>Glucose
Glucose−>G6P
G6P−>G3P
G3P−>3PD
3PD−>Pyruvate
Pyruvate−>Lactate
Pyruvate−>Acetyl−Coa
Control
ATAA
PD
D
Cluster

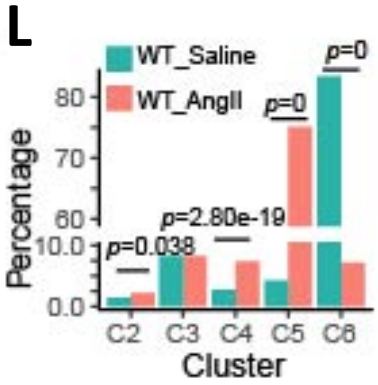

G
UMAP_2
UMAP_1
1. Contractile SMC
2. Proliferative SMC
3. Tnnt2+Contractile
4. ECM-Producing SMC
5. Inflammatory SMC
6. ECM Producing-Inflammatory SMC
7. Stressed SMC

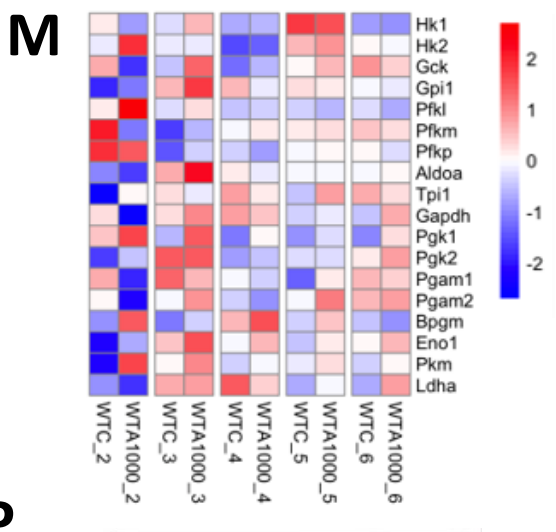

H
WT_Saline
WT_AngII
Sting-/- _ AngII
Percentage
Cluster

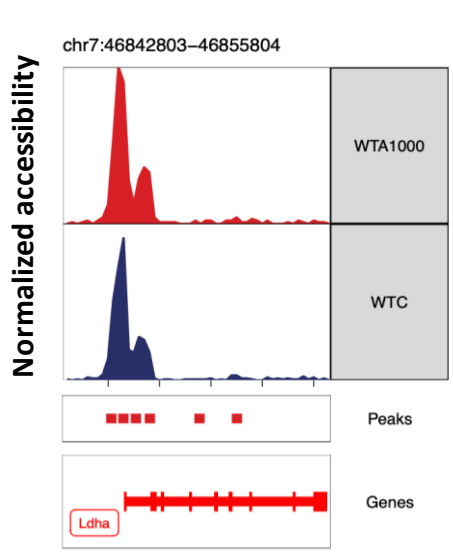

I
Hk1
Hk2
Gpi1
Pfkl
Pfkp
Aldoa
Tpi1
Gapdh
Pgk1
Pgam1
Bpgm
Eno1
Pkm
Ldha
Ldhb
Dlat
Pdha1
Adh5
Akr1a1
Aldh2
WTC
WTA2000
STING
Average Expression
Percent Expressed

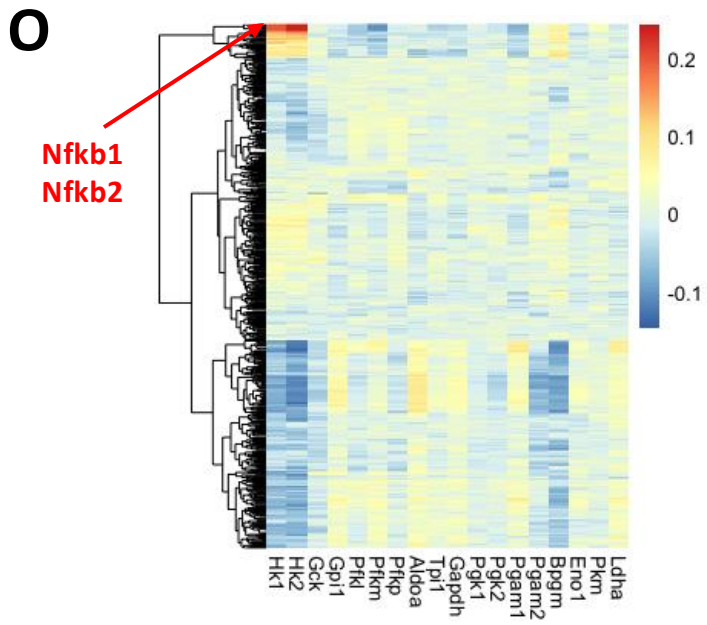

J
Enzymatic digestion
Pooled single cell suspension
Nuclear Isolation
Closed chromatin
Open chromatin
Adapter 1
Adapter 2
transposase
Fragmentation and tagmentation
Sequencing
Tagmented DNA fragments
UMAP_2
UMAP_1


K
C6
C5
C4
C3
C2
UMAP Dimension 2
UMAP Dimension 1
C2: ECM-inflammatory
C3: Proliferative-Contractile
C4: Healing?
C5: Inflammatory
C6: Contractile


L
WT_Saline
WT_AngII
p=0
p=0
p=2.80e-19
p=0.038
Percentage
Cluster


M
Hk1
Hk2
Gck
Gpi1
Pfkl
Pfkm
Pfkp
Aldoa
Tpi1
Gapdh
Pgk1
Pgk2
Pgam1
Pgam2
Bpgm
Eno1
Pkm
Ldha
WTC_2
WTA1000_2
WTC_3
WTA1000_3
WTC_4
WTA1000_4
WTC_5
WTA1000_5
WTC_6
WTA1000_6


N
chr7:46842803−46855804
Normalized accessibility
WTA1000
WTC
Peaks
Genes
Ldha


O
Nfkb1
Nfkb2
Hk1
Hk2
Gck
Gpi1
Pfkl
Pfkm
Pfkp
Aldoa
Tpi1
Gapdh
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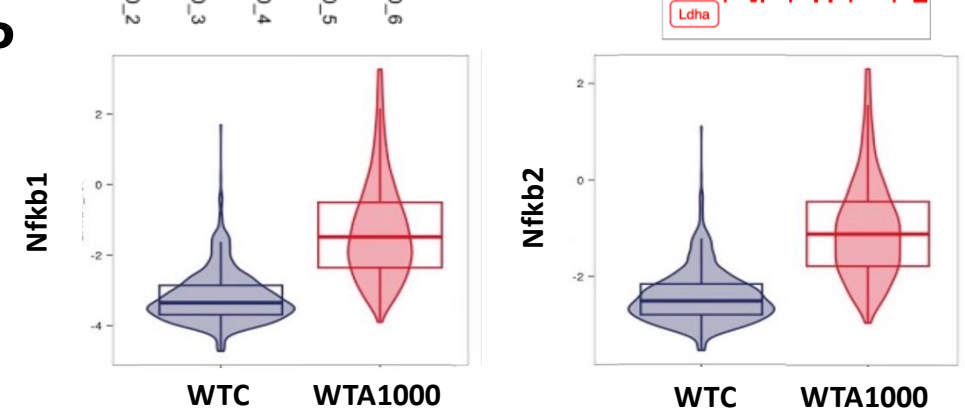
Pgk2
Pgam1
Pgam2
Bpgm
Eno1
Pkm
Ldha


P
Nfkb1
Nfkb2
WTC
WTA1000
WTC
WTA1000

Title: Exploring Altered Fatty Acid Metabolism in Human Hepatocellular Carcinoma

Authors: Garrett B. Anspach, Mikala Zelows, Nikki Dharanipragada, Gregory A. Graf, Robert N. Helsley

Background: Hepatocellular carcinoma (HCC) is the most common form of liver cancer worldwide, accounting for ~90% of all cases. By 2025, it is estimated that greater than 1 million individuals will be affected by liver cancer annually. While hepatitis B remains the most prominent risk factor for HCC, metabolic dysfunction-associated steatohepatitis (MASH) is becoming the fastest growing etiology of HCC, which is largely attributed to the parallel rise in obesity and diabetes mellitus. Thus, it is imperative we understand how fatty acid metabolism in the liver contributes to the development of HCC.

Methods: Human HCC tumor (n=10) and adjacent non-tumor samples (n=10) were obtained from the University of Kentucky Markey Cancer Center. RNA and protein were isolated and used for bulk RNA-sequencing and immunoblotting, respectively. Lipids were extracted using a Folch-based method and quantified using enzymatic assays. Data were analyzed using nonparametric analyses via a Wilcoxon or Mann-Whitney test, where appropriate.

Results: Human tumor samples showed altered expression of genes regulating fatty acid oxidation. While no differences were observed in *PPARa* gene expression, *ACADL*, *ACADM*, *ACADS*, *CPT2*, and *HADHA* were all significantly decreased in HCC tumor tissue. Consistent with other fatty acid oxidation genes, human tumors showed a significant decrease in *CPT1a* ($p=0.014$) at the mRNA level; however, CPT1a protein levels were elevated. Lipid profiling of human tumors revealed a significant reduction in free cholesterol ($p=0.0313$) and phosphatidylcholine ($p=0.0117$) levels, with a trend towards increased triglycerides ($p=0.0645$), as compared to adjacent non-tumor controls.

Conclusions: These results suggest that HCC tumors exhibit reduced fatty acid oxidation resulting in an accumulation of triglycerides, as compared adjacent non-tumor tissue.

Association of elevated serum aldosterone concentrations in pregnancy with hypertension

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Emerging evidence indicates a previously unrecognized, clinically relevant spectrum of abnormal aldosterone secretion associated with hypertension severity. It is not known whether excess aldosterone secretion contributes to hypertension during pregnancy. We quantified aldosterone concentrations and angiotensin peptides in serum (using liquid chromatography with tandem mass spectrometry) in a cohort of 128 pregnant women recruited from a high-risk obstetrics clinic and followed prospectively for the development of gestational hypertension, pre-eclampsia, superimposed pre-eclampsia, chronic hypertension, or remaining normotensive. The cohort was grouped by quartile of aldosterone concentration in serum measured in the first trimester, and blood pressure, angiotensin peptides, hypertension outcomes compared across the four quartiles. Blood pressures and body mass index were greatest in the top and bottom quartiles, with the top quartile having the highest blood pressure throughout pregnancy. Fur-ther stratification of the top quartile based on increasing (13 patients) or decreasing (19 patients) renin activity over gestation revealed that the latter group was characterized by the highest prevalence of chronic hypertension, use of anti-hypertensive agents, and intrauterine growth restriction. Serum aldosterone concentrations greater than 704 pmol/L, the 75th percentile defined within the cohort, were evident across all categories of hypertension in pregnancy, including normotensive. These findings suggest that aldosterone excess may underlie the development of hypertension in pregnancy in a significant subpopulation of individuals.

Regeneration of Elastic Fibers Following Aortic Dissections

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

Background

Aortic dissection (AD) is a life-threatening vascular disease displaying the disruption of the extracellular matrix (ECM). Elastic fibers are a major component of ECM and confer structural integrity to the aortic wall. Normally, elastic fibers are thought to not be synthesized in adulthood. However, it remains unknown whether and how elastic fibers are synthesized following ADs.

Methods and Results

AD was induced by administration of β-aminopropionitrile (BAPN, 0.5% wt/vol) to 4-week-old male C57BL/6J mice. Thoracic aortas were collected after 4 or 12 weeks of BAPN administration. False lumen formation lined with fresh hematoma was observed at 4 weeks of BAPN administration, indicating development of acute ADs. The false lumen developed striking remodeling by 12 weeks, suggesting AD progression to the chronic phase. RT-qPCR revealed that mRNA abundance of tropoelastin, the precursor of elastic fibers, was increased significantly during the chronic phase of ADs, but not during the acute phase. Of interest, in situ hybridization demonstrated the presence of tropoelastin mRNA in the vascular wall of the false lumen, and elastic fibers were formed in these lesions in chronic ADs, as evidenced by Verhoeff iron hematoxylin staining. Ultrasonography showed that aortas with AD lesions had higher elasticity compared to intact aortas. These data suggest that elastic fibers were newly synthesized during the progression of ADs, which provided mechanic functions to the vascular wall. Bulk RNA sequencing identified 7,264 differentially expressed genes (DEGs) between chronic ADs and control aortas. Among the1,173 DEGs, including tropoelastin, were increased in chronic ADs. Transcription factor enrichment analysis identified 120 transcription factors as potential regulators for the 1,173 DEGs. Alignment analysis further filtered these molecules and identified ETS variant transcription factor 5 (Etv5) which could be aligned in both the promoter and enhancer regions of tropoelastin. Of note, immunostaining showed that Etv5 was distributed in the vascular wall of the false lumen, which was spatially coincident with tropoelastin mRNA.

Conclusion

Chronic ADs exhibit vascular remodeling with new elastic fiber formation; the AD lesions are accompanied by enhanced Etv5 mRNA and protein.

Effects of HCM-associated Genetic Mutation G256E on the Contractile Function of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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[1]Department of Bioengineering, [2]Department of Molecular, Cellular, and Developmental Biology Pruitt Lab, University of California, Santa Barbara,[3]Department of Physiology, University of Kentucky, Lexington, KY, [4]Cardiovascular Research Area, University of Kentucky, Lexington, KY, [5]Department of Biological Sciences, Mississippi State University, Starkville, MS

Hypertrophic Cardiomyopathy (HCM) is a condition characterized by thickening of the heart muscle. In this study, we focused on the G256E mutation, a known genetic cause of HCM. Understanding the functional effects of this mutation is crucial for unraveling the underlying mechanisms of HCM.

We found that G256E-mutant hiPSC-CMs significantly increase contractile force compared to control hiPSC-CMs (.598 μN vs .251 μN, $p < 0.01$). When measuring contraction velocity, G256E-mutant hiPSC-CMs were significantly higher than control hiPSC-CMs (2.43 μm/s vs 1.75 μm/s, $p < 0.01$). G256E-mutant hiPSC-CMs were larger than control cardiomyocytes (cell area = ~1098 μm2 vs. ~816 μm2). Additionally, G256E-mutant hiPSC-CMs exhibited a higher overall sarcomere percentage shortening (~18.6% vs. ~14.9%). Preliminary qPCR results suggest significant alterations in genes crucial to sarcomere function and force generation (MYH7) and sarcomere organization (AnkrD1) in G256E mutant hiPSC-CMs compared to control hiPSC-CMs.

Our findings demonstrate that the G256E mutation may directly contribute to hallmark phenotypes of hypertrophic cardiomyopathy (HCM), such as irregular and overstimulated cardiomyocyte contractions. This is evidenced by the augmented contractile force output, contraction velocity, cell area, and sarcomere shortening observed in the mutant group. Moving forward, future research could focus on developing targeted therapies that address cardiomyocyte overstimulation as a potential treatment for HCM.

Nedd4-2 inactivation underlies the increase in Na^+/glucose cotransporter-1 in diabetic hearts

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

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

Methods/Results: We compared hearts from patients with and without T2D and hearts from rats with late-onset T2D caused by overexpression of human amylin in pancreatic β-cells versus hearts from wild-type littermates. SGLT1 protein level was significantly higher in both humans and rats with T2D hearts. In contrast, the SGLT1 mRNA levels were comparable in hearts from T2D and non-diabetic patients and rats. This result suggests that the T2D-induced increase in cardiac SGLT1 protein occurs at a post-transcriptional level. Co-immunoprecipitation and co-localization (via dual immunostaining) of SGLT1 with ubiquitin were reduced in diabetic hearts, which indicates that SGLT1 degradation is impaired. The E3 ubiquitin ligase Nedd4-2 is responsible for ubiquitination of several cardiac membrane transporters. Inhibition of Nedd4-2 expression (using si-RNA) or function (heclin) in HL-1 cardiomyocytes resulted in significantly higher SGLT1 protein levels, suggesting that Nedd4-2 also mediates SGLT1 degradation. Nedd4-2 is inactivated upon phosphorylation by several kinases, including the serum and glucocorticoid-regulated kinase-1 (SGK1). Using western blotting, we found increased Nedd4-2 phosphorylation in the hearts of humans and rats with T2D compared to their non-diabetic counterparts. Moreover, hearts from T2D patients exhibited higher levels of SGK1 and its activated phosphorylated form compared to their non-diabetic counterparts.

Conclusion: The increase in SGLT1 protein in the diabetic heart is regulated post-transcriptionally by an impairment in Nedd4-2 dependent ubiquitination and degradation of SGLT1.

Dim Light at Night Causes Sex-specific Differences in the Regulation of Blood Pressure in Mice

Dema Sami, Isabel Stumpf, Allison Ehlman, Tanya Seward, Wen Su, Ming Gong, Elizabeth A. Schroder, Brian P. Delisle, Abhilash Prabhat

Introduction: The advancement of technology has increased our continuous exposure to light. Studies have shown that dim light at night (dLAN) can affect the body's normal circadian rhythm, including blood pressure regulation. Arterial blood pressure follows a day/night rhythm that increases in the morning, peaks at midday, and decreases in the evening. The blood pressure increases in the morning, or morning surge, is correlated with an increased risk of cardiovascular events in people. We tested if mice's exposure to dim light at night affected the acrophase (peak) and periodicity of circadian rhythms for blood pressure and heart rate.

Methods: We implanted telemetry devices in wild-type female and male mice (n=5-6 per sex) to continuously monitor blood pressure, heart rate, activity, and body temperature. Mice were housed in thermoneutrality for a 12-hour light and 12-hour dark cycle (LD, 200 Lux: 0 Lux) with ad libitum access to food and water. We altered the mice's conditions from a 12-hour light and 12-hour dark cycle to a 12-hour light and 12-hour dim light at night cycle (dLAN, 200 Lux: 5 Lux). We used ClockLab to analyze the mice's acrophase and periodicity in the day/night rhythms of blood pressure, heart rate, activity, and body temperature before and after dLAN.

Results: The periodicity of the female and male mice maintained a 24-hour rhythm for diastolic blood pressure, systolic blood pressure, heart rate, activity, and body temperature before and after dLAN conditions. In female mice, the dLAN cycle caused the acrophase of the 24-hour rhythm to shift several hours earlier in diastolic blood pressure, systolic blood pressure, and body temperature ($p=0.01$, $p<0.05$). The female's acrophase of a 24-hour rhythm for heart rate ($p<0.05$) and activity ($p<0.05$) also peaked several hours earlier. In male mice with dLAN conditions, the acrophase of a 24-hour rhythm for diastolic blood pressure ($p=0.6$), systolic blood pressure ($p=0.4$), heart rate ($p=0.6$), activity ($p=0.1$), and temperature ($p=0.1$) did not display significant differences ($p>0.05$).

Conclusion: The data suggests that dim light at night affects the circadian rhythms of female and male mice differently to cause sex-specific changes in blood pressure regulation.

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

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

MS-NASH mice had higher overall weight and blood glucose levels. MS-NASH mice showed increased platelet activation markers, correlating with current studies in human obese patients. MS-NASH males showed significant increase in red blood cells ($p=0.0444$) and mean platelet volume ($p=0.0038$) without significant change in platelet count. This suggests a younger and more active platelet population. Interestingly, bleeding time was positively correlated with body weight in the MS-NASH mice due to rebleeding. Microfluidics showed a decrease in thrombus formation on collagen from the MS-NASH males, corresponding with the tail bleeding assay. Based on this preliminary study, there might be a dysfunction in platelet biogenesis and clot stability in the MS-NASH mice.

Impact of human amylin, sex and diet on glucose metabolism and heart function

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Introduction: Amylin is a pancreatic hormone co-secreted with insulin. It modulates insulin and glucagon secretion, induces satiety, and inhibits gastric emptying. Human amylin tends to form oligomers and aggregate when oversecreted whereas rodent amylin does not. Amylin oligomers result in amyloid deposits in the pancreas, which leads to type-2 diabetes, and peripheral organs, including the heart. This study is aimed to investigate the impact of human amylin knock-in on metabolic parameters and heart function in male and female mice that are long-term fed with high-fat diet.

Method: Male and female mice with knock-in expression of human amylin gene in pancreatic β-cells (HuAmy mice) and wild-type (WT) control mice were fed with either chow or a high-fat diet (60 kcal% fat) for 10-11 months, starting at 3 months of age. Body weight, fasting blood glucose, glucose tolerance, and heart function (using echo and electrocardiography) were studied at 13-14 months of age. Three-way ANOVA was performed to discern the influence of human amylin, sex, and high-fat diet on these parameters.

Results: Presence of human amylin, male sex, and high-fat diet were all associated with larger body weight. However, only human amylin and male sex were associated with impaired glucose metabolism, while high-fat feeding had no significant effect. Left-ventricular mass was significantly larger in male vs. female WT mice and not affected by diet. Presence of human amylin resulted in larger left-ventricular mass in females, to the extent that there were no differences between male and female HuAmy mice. Ejection fraction, fractional shortening, stroke volume, and cardiac output were higher in male vs. female mice and were not affected by human amylin and high-fat diet. ECG recordings revealed that human amylin, male sex, and high-fat diet were all associated with longer QTc intervals. Presence of human amylin resulted in longer QRS intervals and reduced heart rate variability, parameters that are associated with an increased risk of arrhythmias, in both male and female mice, irrespective of their diet.

Summary and conclusion: Presence of human amylin resulted in pro-arrhythmogenic ECG alterations in both male and female mice and larger left-ventricular mass in females. Somewhat surprisingly, long-term high fat feeding caused no significant alterations in glucose metabolism and heart structure and function, except for an increase in the QTc interval.

Relative expression of the N2B and N2BA isoforms of titin does not change markedly in heart failure with reduced ejection fraction in humans

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Titin is a giant sarcomeric protein that functions as a complex molecular spring. Two titin isoforms are co-expressed in human myocardium, N2B and N2BA. The N2B isoform is stiffer, so changes in the relative content of the isoforms modulates cardiac stiffness. We measured the relative content of the isoforms in samples procured from organ donors and patients who received heart transplants and/or Ventricular Assist Devices (VADs). The number of patients in each group (n) was organ donors, 30; dilated cardiomyopathy, 29; cardiac amyloidosis, 5; end-stage heart failure prior to VAD, 35; transplanted after mechanical circulatory support with a VAD, 35; titin truncation mutations, 8. Titin isoforms were separated using SDS-agarose gels and stained for total protein with Oriole fluorescent dye. Data are presented as mean ± SEM. The relative content of N2BA expressed in the middle transmural region of left ventricular samples from donors was 39.0 ± 2.0%. The content in left atrial samples was 58.8 ± 4.8% (p=0.0019, paired t-test). A linear mixed model compared the relative N2BA content in ventricular samples to that measured for organ donors. The only statistically significant difference was that the N2BA content was ~20% higher (i.e., relative content was 47.4 ± 1.9%) in the pre-VAD samples (p=0.047). To the team's knowledge, this is the largest analysis of titin isoform ratios in human hearts to date. The data suggest that the proportion of titin expressed as N2BA in left ventricular samples does not vary markedly in Heart Failure with reduced Ejection Fraction.

Uncovering a Role for Carnitine Palmitoyltransferase 1a in Adipocytes

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Background

Carnitine palmitoyltransferase 1 (CPT1) is the key rate-limiting enzyme in mitochondrial fatty acid oxidation (FAO), a mechanism that metabolizes long-chain fatty acids for energy. The Cpt1 isoforms, 1a and 1b, are thought to be primarily expressed in the liver and muscle, respectively. Our laboratory and others have shown that CPT1a is the most abundant CPT1 enzyme in both the liver and white adipose tissue (WAT) in mice and humans. Therefore, the objective of this study is to uncover a role for CPT1a in adipocyte biology.

Methods

Murine 3T3-L1 fibroblasts were used to study adipocyte differentiation. The induction of 3T3-L1 cell differentiation from fibroblasts into adipocytes were completed using a cocktail of 10 mg/mL insulin, 4mM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, and 1mM rosiglitazone in high glucose containing media with 10% fetal bovine serum. After 3-days of induction media, the cells were cultured in maintenance media containing 1 μg/mL insulin. Oil-Red O staining was completed at the beginning and end of 3T3-L1 adipocyte differentiation. Further, cells were harvested at two-day intervals using either Trizol or RIPA buffer for RNA and protein, respectively. Gene and protein expression was then measured by qPCR and immunoblotting.

Results

We confirmed that 3T3-L1 preadipocytes can differentiate into mature adipocytes *in vitro* by increased Oil Red O staining, a marker of neutral lipid accumulation. Further, RNA and protein levels of the classical adipogenic markers *Ppary*, *Cd36*, and *Atgl* were elevated. Fatty acid oxidation genes *PPara* and *Cpt2* had increased RNA and protein levels throughout differentiation. Intriguingly, C*pt1a* RNA decreased approximately 90% throughout differentiation, yet protein levels increased and stabilized throughout the differentiation process.

Conclusions

These results suggest that CPT1a may be controlled by posttranslational mechanisms in pre- versus post-differentiated 3T3-L1 cells. Our future directions will assess these modifications, and examine the role of *Cpt1a* in an adipose-specific knockout mouse model.

Autonomic Signaling shapes 24-h rhythm in HR in coordination with Core Body Temperature

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Introduction: Mammalian heart rate (HR) has a strong 24-h rhythm. This rhythm is part of a set of circadian rhythms in physiology that leads to anticipatory changes in central regulation to respond to predictable changes in the environment. One hypothesis is that the 24-h rhythm in HR (or RR interval) is controlled by a master circadian clock in the suprachiasmatic nucleus (SCN) via autonomic signaling. However, our modeling of the influence of core body temperature (T_b) on HR indicates the 24h-rhythm in T_b may also play a significant role in shaping the 24h-rhythm in HR. To measure autonomic signaling to the heart, we used heart rate variability analysis (HRV). HRV is based on fast fluctuations in RR (~1 sec) which are sensitive to autonomic blockade and are correlated with slow (~20 min) patterned fluctuations in HR (or RR interval). We developed a Linear Model to calibrate our measures of autonomic signaling and to integrate them with the influence of T_b on HR to reconstruct these slow patterned fluctuations in RR interval time series.

Methods: We analyzed mouse telemetry electrocardiogram (ECG) and core body temperature (T_b) data recorded from mice. Mice (n=5-6 per group) were housed in 12-hour light and dark cycles at room temperature (RT) with ad libitum access to food and water unless noted. We studied adult (3-6 mos. old) Wild Type (WT) mice housed at RT with ad libitum feeding (RT ALF), adult WT mice that had their time of feeding restricted to the light cycle only (inverse of normal feeding rhythm) (RT TRF), and adult WT mice housed in thermoneutrality with ad libitum feeding (TN ALF). To measure autonomic signaling to the heart, we follow a standard procedure for HRV analysis to calculate power in the high and low frequency bands: $P_{hf}(t), P_{lf}(t)$. To determine the reliability of these measurements, we calculated the cross-correlation of these derived time series and five-minute-averaged RR intervals $RR(t)$. Typically, we find robust positive correlations indicating these are surrogate and – redundant -- measures of parasympathetic signaling (PNA) to the heart. To enhance the detection of sympathetic signaling to the heart (SNA), we perform the same analysis on the reciprocal RR-interval time series. The resulting measures $S_{hf}(t), S_{lf}(t)$ have a negative correlation with $RR(t)$ supporting the utilization of these time series as measures of SNA signaling.

We used a Linear Model to calibrate our HRV measures of autonomic signaling and to integrate autonomic signaling with the influence of core body temperature on HR.

$$\mathrm{RR}_{\mathrm{fit}} = u + m_p\,(P_{hf}(t) + P_{lf}(t)) - m_s\,(S_{hf}(t) + S_{lf}(t)) + \mathrm{RR}_i,$$

where u represents the undetected tonal autonomic input to the heart and $RR_i(t)$ is obtained from an effective Q10 model.

Results: Statistical analysis demonstrates that going from RT ALF to RT TRF and to TN ALF, significantly increases RR and slows HR. Moreover, our recent autonomic blockade experiments show that the intrinsic HR set by core body temperature is relatively the same under these different experimental conditions. So, autonomic signaling likely plays an important role in generating the changes in RR we observe. By fitting the parameters of the Linear Model, we were able to reconstruct experimentally measured slow fluctuations in RR intervals with typical r-squared values greater than 0.80. However, to obtain these optimizations we had to fix parameters related to our Q10 model in accordance with experimental results from autonomic blockade. In particular, fitting the effective Q10 model to HR following autonomic blockade of certain animals, yields a Q10 of roughly 1.7. Importantly this value implies a two-degree variation in core body temperature(T_b) can result in a 50 bpm change in HR. So, because $T_b$has a robust 24-h rhythm, we infer that core body temperature may also play a role in shaping the 24-h rhythm in HR.

Conclusion: The changes in RR we observe under different environmental cues are likely mainly due to changes in autonomic signaling to the heart. To assess these changes, we used a Linear Model to calibrate and integrate our autonomic measures with the influence of T_b on HR. As a result we can visualize how both autonomic signaling and T_b shape the 24-h rhythm in HR.

Inhibition of β-Adrenergic and Muscarinic Receptors Cause a Loss in the Day/Night Difference in Heart Rate and Core Body Temperature

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Introduction: Basal heart rate (HR) exhibits a day/night rhythm. In mice, HR is higher during the night (i.e., dark cycle) when they are active and slowest when they are inactive (light cycle). The day/night difference in heart rate has been attributed to the daily changes in the autonomic regulation of the heart. This conclusion is based on heart rate variability measurements and pharmacological studies that inhibit muscarinic and β-adrenergic receptors. These studies were done in mice at room temperature and not thermoneutrality. Mice housed in thermoneutrality have HR dominated by parasympathetic tone. We tested whether the day/night differences in HR in mice housed in thermoneutrality are due to changes in autonomic regulation of the heart.
Methods: Four- to six-month-old male and female mice (n=6) were implanted with telemetry devices to continuously record HR (RR intervals) and core body temperature (Tb). Mice were housed under thermoneutral conditions (30^{o}C) in light and dark cycles with ad libitum access to food and water. Mice were injected twice daily for two consecutive days with propranolol and methylatropine to inhibit β-adrenergic and muscarinic receptor signaling. We tested for differences in the mean light and dark cycle HR or Tb before and after injections.
Results: There was a significant difference in the mean light cycle and dark cycle RR interval the two days before injection with propranolol and methylatropine ($p<0.0001$, n=6). Injecting mice with propranolol and methylatropine caused a loss in the difference between the light and dark cycle RR interval ($p>0.05$). The day/night difference in the light and dark cycle RR intervals returned the day after injection (($p<0.0001$). There was also a significant difference in the mean light and dark cycle Tb two days before injection with propranolol and methylatropine ($p<0.05$). Injecting mice with propranolol and methylatropine caused a loss in the difference between the light and dark cycle Tb ($p>0.05$). The day/night difference in the Tb returned the day after injection ($p<0.01$).
Conclusion: Pharmacological inhibition of β-adrenergic and muscarinic receptors caused a loss in the day/night difference in mean RR interval in mice housed at

thermoneutral temperatures. However, these medications also caused a loss in the day/night rhythm in Tb. These data raise the possibility that these medications may impact the day/night rhythms in RR interval by altering Tb.

Human left atria exhibit decreased crossbridge-attributable stiffness and length-mediated crossbridge recruitment compared to the left ventricle

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The force response of permeabilized human cardiac muscle preparations to step-like changes in length has been used to study the biophysical properties of muscle contraction and actin-myosin interactions. During a rapid lengthening, force increases to a peak (F_1) as the bound cross-bridges are strained. Force subsequently drops to a minimum value (F23) at a time (T23) before equilibrating to a new state-state force (F_{ss}). Here we examined the force responses to a series of lengthening and shortening movements in permeabilized muscle strips from the left ventricle and the left atria of organ donors at 37C at maximum Ca^{2+} activation. For the same length and Ca^{2+}, the slope of the relationship between F_1 and length change, a measurement proportional to the number of bound cross-bridges at the time of the length change, is larger in the left ventricle than in left atria. Similarly, the slope of the relationship between F_{ss} and the length change, a measurement of length-mediated recruitment of additional bound cross-bridges, is larger in the left ventricle than in left atria. The value of F23 is higher and the time T23 is shorter, in left atria compared to left ventricle. These differences in the force response are potentially manifestations of the increased proportion of alpha-myosin in the atria which are known to have faster cross-bridge cycling rates.

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Chronic circadian disruption, which is common in shift workers, is associated with increased risk of cardiometabolic diseases. The circadian system aligns 24h cycles of cycles of biological processes with the environmental light-dark cycle. Abrupt shifts in the timing of the light-dark cycle, such as occurs during transmeridian travel and shift work, misaligns the circadian system with the environment. This circadian disruption can be modeled in mice by shifting the light-dark cycle earlier, to approximate eastward travel, or later to approximate westward travel. Previous studies reported that females re-synchronize faster than males when the light-dark cycle is advanced (moved earlier), but the mechanism underlying this sex difference is unknown. The objective of this study was to investigate the circadian mechanisms that mediate the sex difference in the rate of re-synchronization to shifted light-dark cycles. We studied wheel-running activity rhythms as a marker of circadian rhythms in C57BL/6J adult male and female mice. We phase-advanced the light-dark cycle by 6h to simulate eastward travel. Female mice re-synchronized to the new light-dark cycle 3 days faster than males. We next studied the mechanisms underlying the accelerated re-synchronization in female mice. If females had faster, shorter period circadian clocks than males, then this could account for faster re-synchronization. However, we found that the period did not differ between male and female mice. Females could also re-synchronize faster if they had larger instantaneous phase advances (moving the circadian clock earlier) to light in the late night. To determine the magnitude of the phase advances in males and females, we housed mice in constant darkness and then exposed them to a 15-minute light pulse during the late night. We found there was no significant sex difference in the magnitude of the phase advance induced by a light pulse given during the late night. Overall, we found there were no differences in period or magnitude of phase advances that could account for faster re-synchronization to abrupt changes in the light-dark cycle in females. Future studies will investigate the role of estrogen signaling in regulating the sex difference in re-synchronization to shifted light-dark cycles. Uncovering the mechanisms underlying sex differences in phase shifting can used to develop new strategies to alleviate jet lag and shift work symptoms.

Single Cell RNA Sequencing Reveals a Lineage-specific Response to Angiotensin II in Smooth Muscle Cells in the Ascending Aorta of Mice

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Background:
Ascending thoracic aortic aneurysm is a life-threatening disease with no options for pharmacologic treatments. The major cell type populating the media of this region is smooth muscle cell (SMC). SMCs in this region are derived from 2 embryonic origins; cardiac neural crest (CNC) and second heart field (SHF). SHF-derived cells play a vital role in the pathophysiology of angiotensin II (AngII)-mediated thoracic aneurysm. The role of CNC-derived cells and functional differences between the two origins remain unknown.

Methods and Results:
Mef2c-Cre +/0 mT/mG mice were infused with AngII (1,000 ng/kg/day) for 3 days and ascending aortas were harvested. Aortic samples were also harvested from Mef2c-Cre +/0 mT/mG mice without AngII infusion as a control. After tissue digestion to create a single-cell suspension, FACS sorting was performed to separate cells based on their origin using mTotamto and mGFP signals. mGFP proteins were present on Mef2c-Cre positive cells indicating the cells were derived from the SHF, while cells with mTomato signal were not derived from the SHF (nSHF). After sorting cells by origin, single-cell RNA sequencing was performed to determine transcriptomic differences between origins. The “Seurat” R package was used to integrate the sequencing data and a two-way ANOVA analysis for the interaction between origin and infusion. This analysis identified 3,703 differentially expressed genes (DEGs) and hierarchical clustering found 4 major subclusters among these DEGs. Gene ontology analysis revealed that these subclusters were associated with cellular respiration, mitochondria function, p53 signaling, and RNA splicing. One of the clusters exhibited a unique transcriptional response to AngII infusion for cells derived from the SHF. In this cluster, there were 295 DEGs, all of which had significantly higher abundance in the SHF versus nSHF origin in response to AngII infusion. The most upregulated gene was *Tnnt2*, which regulates muscle contraction. The top 10 upregulated genes also included *Loxl2*, a key enzyme for elastic fiber development. Of note, other LOX family members, such as *Lox* and *Loxl1* were downregulated in SHF-derived SMCs compared to nSHF-derived SMCs in response to AngII infusion.

Conclusions:
AngII infusion alters the transcriptome of aortic SMCs in a lineage-specific manner. AngII upregulates genes related to muscle contraction and extracellular matrix development specifically in SHF-derived SMCs

Postdoctoral Fellow

Optimal Timing and Duration of BAPN Intervention to Augment Angiotensin II-induced Aortopathies in Adult Male C57BL/6J Mice

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Background: Infusion of angiotensin II (AngII) leads to modest dilatations of the ascending aorta and a low incidence of abdominal aortic aneurysms (AAAs) in adult male C57BL/6J mice. β-aminopropionitrile (BAPN), a lysyl oxidase (LOX) and LOX-like protein inhibitor, induces aortic aneurysms and rupture in 3-4-week-old C57BL/6J mice. In this study, we examined the effects of BAPN intervention at selected intervals on aortic aneurysms and rupture in adult male C57BL/6J mice infused with AngII.

Methods and Results: Male C57BL/6J mice (~11 weeks of age) were randomized to 4 groups (N=10/group) that were all infused with AngII (1,000 ng/kg/min) for 4 weeks. The groups were: (1) No other drug, (2) BAPN co-administration for the first 2 weeks [AngII+BAPN(1-2)], (3) BAPN co-administration for the last 2 weeks[AngII+BAPN(3-4)], and (4) BAPN co-administration for the entire 4 weeks [AngII+BAPN(1-4)]. During the study, no mice died in Group 1. One of 10 mice in AngII+BAPN(1-2) group and 6 of 10 mice in AngII+BAPN(1-4) group died of aortic rupture, as confirmed by necropsy (P=0.04; Log-Rank test). Three of 10 mice in AngII+BAPN(3-4) died of aortic rupture. However, 2 mice died prior to BAPN administration. Maximal diameters of the ascending, descending thoracic, and suprarenal abdominal aortic regions were measured after termination. The maximal diameters of the ascending aorta were significantly larger in AngII+BAPN(1-2) and AngII+BAPN(1-4) groups, compared to Group 1 (P=0.01 and 0.02, respectively; Kruskal-Wallis one-way ANOVA on Ranks followed by Dunn's method). No differences were found in the maximal diameters of the descending thoracic region among the 4 groups. The maximal diameters of the suprarenal aorta measured using ex vivo images were significantly larger in AngII+BAPN(1-2) group (P=0.001) or AngII+BAPN(1-4) group (P=0.004), compared to Group 1 (Kruskal-Wallis one-way ANOVA on Ranks followed by Dunn's method). The maximal diameters of both the ascending and suprarenal aortic regions were not different between AngII+BAPN(3-4) and Group 1 and between AngII+BAPN(1-2) and AngII+BAPN(1-4) groups.

Conclusion: BAPN administration throughout 4 weeks augmented AngII-induced aortic rupture. BAPN administration during the first two weeks attenuated aortic rupture, despite prominent dilations of both the ascending and suprarenal aortic regions.

Solving the Mystery of an Unknown Bleeding Disorder

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Background: A family was recently identified with an inherited bleeding disorder of unknown cause, characterized by a prolonged bleeding time but otherwise normal hemostatic parameters. Genetic analyses identified the Tyr242Cys mutation in fragment 2 of prothrombin, a region removed upon its activation to thrombin. Thrombin is the serine protease responsible for making blood clots, and thrombin deficiency is incompatible with life. Coagulation factor Xa cuts prothrombin at two specific sites (Arg271, Arg320) to generate thrombin. We hypothesize that addition of thrombomodulin will reduce anticoagulant dependence on thrombomodulin.
Methods: Calibrated Automated Thrombography (CAT) was utilized to measure thrombin activation in plasma from affected family members and healthy controls. Purified proteins were used to determine clotting rates in family members and controls.
Results: Thrombin generation was reduced in platelet-poor and platelet-rich plasma in the samples of all patients after tissue factor was added or contact pathway initiation. The variant is very close to the Arg271 activation site, and therefore we hypothesized impaired release of the activation fragments resulting in limited clotting. Thrombin generation is corrected through the mixture of Prothrombin-Wauwatosa plasma with plasma depleted of either PC or the cofactor protein S. In control samples, addition of thrombomodulin reduced thrombin generation samples containing Prothrombin-Wauwatosa, consistent with reduced anticoagulant dependence on thrombomodulin.

Conclusions: Findings support a novel mechanism in which Prothrombin-Wauwatosa activation results significant anticoagulant activity and reduced clotting time.

Feeding Behavior and Ambient Temperature Modify Heart Rate, Core Body Temperature, and Heart Rate Variability in Mice

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Introduction: The vagus nerve regulates the beat-to-beat variability in heart rate (HR). Breathing causes the beat-to-beat change in HR to oscillate in a wave-like pattern. Graphing the size of the oscillation as a function of the frequency of the heartbeats via a Fourier transform generates a power spectrum with a distinct peak in the high frequency (HF) domain that reflects the frequency of breathing. A second peak in the low frequency (LF) domain reflects autonomic outflow via baroreflexes. To better understand autonomic regulation of the HR, we studied changes in the HF and LF domains measured from mice under different conditions that alter autonomic regulation of the heart.

Methods: Mouse telemetry electrocardiogram (ECG) and core body temperature (Tb) data recorded from mice were analyzed. Mice (n=5-6 per group) were housed in 12-hour light and dark cycles at room temperature (RT) with ad libitum access to food and water unless noted. We studied adult (3-6 mos. old) Wild Type (WT) mice housed at RT (control mice), adult WT mice that had their time of feeding restricted to the light cycle only (inverse of normal feeding rhythm), Aged (18 mos. old) WT mice, adult transgenic mice with altered cardiac electrophysiological properties (LQT3 mice), and adult WT mice housed in under thermoneutral conditions. We measured the mean HR (RR interval), Tb, HF power (PHF), and LF power (PLF) across the 24-hour cycle and during the light and dark cycles separately.

Results: Restricted feeding to the light cycle and housing mice under thermoneutral conditions significantly impacted the mean RR interval ($p<0.05$). Compared to the control, restricted-fed mice had increased RR intervals, decreased Tb, and increased PHF and PLF during the light cycle ($p<0.05$). Compared to the control, housing mice under thermoneutral conditions increased the mean RR interval during the light and dark cycle ($p<0.05$). The increase in the RR during the light cycle correlated with a decrease in Tb and an increase in the HF power. Aged mice showed reduced Tb ($p<0.05$) without changes in RR interval, PHF, or PLF($p>0.05$). LQT3 mice showed no differences in RR, Tb, HF, or LF compared to control mice ($p>005$).

Conclusion: The data demonstrate that feeding behavior and ambient temperature are potent modifiers of basal HR, Tb, and autonomic regulation of the heart. Whether or not the changes in the mean HR during the 24-hour cycle reflect changes in Tb, autonomic outflow, or a combination of the two requires additional investigation.

Selected Abstract

Dynamic Phenotypic Transition of Smooth Muscle Cells During Human Ascending Thoracic Aortic Disease Progression: From Compensation in Aortic Aneurysm to Decompensation in Aortic Dissection

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Abstract

Introduction: The regulation of progression from ascending thoracic aortic aneurysm (ATAA) to dissection (ATAD) remain poorly understood. We examined the dynamics in smooth muscle cells (SMCs) of aortic tissues from ATAA and ATAD patients and further examined mechanisms in a mouse model.

Methods: Single-cell RNA sequencing (scRNA-seq) analyses were performed in ascending aortic tissues from individuals without aortic disease (n=8), patients with ATAA (n=9), and acute ATAD (n=9). Further, scRNA-seq and scATAC-seq were performed in ascending aortic tissues from mice infused with angiotensin II (AngII); scRNA-seq analyses were performed on SMC-specific *Tgfbr2*$^{-/-}$ mice.

Results: Six SMC subtypes were identified in human aortic tissues. We observed a profound SMC transition from contractile in control to extracellular matrix (ECM)-contractile phenotype in ATAA, however, was reduced in ATAD tissues. Instead, ATAD showed significant SMC transition to inflammatory phenotype. Consistently, many genes in ECM organization and muscle contraction, collectively termed compensatory response, were upregulated in ATAA but downregulated in ATAD tissues compared with controls, whereas inflammatory response and cell death pathway genes were significantly upregulated in ATAD tissues. Cell-cell interaction, regulatory network, and motif analysis suggested that TGF-β-MEF2C signaling contributed to the induction of the ECM-producing SMCs. MEF2C^{+} SMCs exhibited transition towards ECM-producing SMCs in both mouse and human aorta. SMC-specific *Tgfbr2*$^{-/-}$ mice showed compromised SMC transition to ECM-producing phenotype.

Conclusions: Our study reveals SMC phenotypic transitions to ECM-producing SMCs (a compensatory response) in ATAA and to inflammatory and pro-death SMCs (a decompensatory status) in ATAD. TGF-beta-MEF2C promoted the compensatory response in SMCs of the ascending aorta.

Selected Abstract

Title: The Role of Cysteine String Protein-α in Platelet Exocytosis

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Background: Platelets use SNARE-mediated exocytosis to maintain vascular integrity via the release of three types of granules: dense, α, and lysosomal. To understand how the process of exocytosis is regulated, we probed the role of Cysteine String Protein-α (CSPα), a potential t/Q-SNARE chaperone and assessed how it affects thrombosis and hemostasis. This abundant protein is the only detectible isoform from its respective family in platelets.

Objective: To address the role of CSPα in platelets.

Methods: We have examined the phenotype of CSPα$^{-/-}$ mice using an IDEXX hematology analyzer to measure platelet count and size. Platelet activation and morphology were examined using cytometry. Hemostasis was assessed using tail bleeding and a BioFlux microfluidics system. The levels of platelet SNARE machinery were quantified by western blotting. Protein localization was examined by 3-Dimensional Structured Illumination Microscopy (3D-SIM) and subcellular fractionation. Biochemical studies were done to examine protein acylation and association with lipid rafts in platelets.

Results: Hematology data indicates that CSPα$^{-/-}$ mice have normal platelet size and counts, but erythrocytes and leukocytes counts were lower in CSPα$^{-/-}$ mice. CSPα$^{-/-}$ platelets have defective α and lysosomal granule release, but integrin activation was unaffected. Tail bleeding in CSPα$^{-/-}$ and CSPα$^{+/-}$ mice was significantly increased compared to wild-type littermates. Thrombus formation was also defective in CSPα$^{-/-}$ mice at low-shear rates in microfluidics assays. Microscopy experiments suggest that CSPα is localized to a granule population, but it also is cytoplasmic as well. CSPα is acylated and is found mostly in triton soluble membrane fractions. Further experimentation is underway to better determine how CSPα interacts with elements of the platelet secretory machinery.

Conclusions: These experiments indicate that CSPα has a role in platelet secretion and thus in thrombosis and hemostasis. These data fill gaps in our knowledge of CSPα's physiological role and our understanding of how platelet exocytosis is controlled.

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Selected Abstract

Xufang Mu

- **Objective** - Male sex is a well-established risk factor for abdominal aortic aneurysms but the underlying mechanisms remain to be fully understood. Our lab reported aldosterone and high salt (Aldo-salt) induced aortic aneurysms in an age-dependent manner in male mice but whether it shows sex dimorphisms like humans is unknown. The current study tested the hypothesis that male mice are more susceptible to Aldo-salt induced aortic aneurysms through androgen mediation and investigated the potential mechanisms.

- **Approaches and Results** - Ten-month-old male and female mice were used in all experiments. We first demonstrated the sex differences in the Aldo-salt model with 70% incidence in male mice (7/10) compared to complete protection in female mice (0/9). Next, we showed androgen and androgen receptor (AR) mediated the process by several independent experiments: 1) orchiectomy provided strong protection (13.3%, 2/15) compared to sham control (69.2%, 9/13); 2) Exogenous DHT administration to orchiectomized mice restored the aneurysms formation (55%, 6/11); 3) inhibition of AR function by drug decreased total incidence compared to control (2/11 vs. 7/11). To dissect the mechanism, we conducted bulk RNA sequencing in aortas from 3 groups of mice given Aldo-salt 1) intact male mice; 2) orchiectomized mice; and 3) orchiectomized mice with DHT. Through bioinformatic analyses, we found PD-1 signaling pathway was significantly enriched in orchiectomized mice compared to control and reversed by DHT addition. Next, we found a significantly higher expression of PD-1 in spleen from orchiectomized mice than sham mice with Aldo-salt presence, at both protein and mRNA levels. Mechanistically we showed AR could bind to PD-1 promoter region and repressed PD-1 expression. Finally, we injected αPD-1 antibody or control IgG antibody to orchiectomized mice during the Aldo-salt administration to confirm phenotypes. Results showed that neutralization of PD-1 partially abolished the orchiectomy protection (0/8 vs. 5/12).

- **Conclusions** - Our results demonstrated that male mice are more susceptible to aldosterone and high salt-induced AAA. Androgen and its receptor mediate the high susceptibility in male mice partially through PD-1 signaling pathway.

Nicotine Promotes the Susceptibility of Female Mice to AngII-Induced Abdominal Aortic Aortopathies and Augments the Severity of Aortopathies in Both Male and Female Mice

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Background: Cigarette smoking is an irrefutable risk factor for abdominal aortopathies (AAs) with a pronounced and dose-dependent relationship with both smoking duration and quantity. However, some studies suggest that smoking is a stronger risk factor for aortopathy development in females than males. We examined the impact of nicotine on the formation and severity of angiotensin II (AngII)-induced AAs in male and female *Ldlr*$^{-/-}$ mice. Moreover, we defined the role of sex hormones on nicotine induced regulation of aortopathies in both sexes.

Methods: Male and female *Ldlr*$^{-/-}$ mice (8-12 weeks of age) underwent sham surgery or gonadectomy (GDX) two weeks prior to feeding a Western diet (Teklad TD88137). For males, we removed either one or both testes. Mice of each sex and treatment (GDX) were infused with AngII (1,000 ng/kg/min) in the absence or presence of nicotine (4 mg/kg/day by osmotic minipump) for 28 or 56 days. Aortopathies were quantified by ultrasound (AA internal diameter), external maximal AA diameters, and aortic rupture rates.

Results: Nicotine had no effect on body weight, blood pressure, or serum cholesterol concentrations in male or female mice. Plasma cotinine levels (nicotine metabolite) were higher in males than females infused with nicotine. Nicotine increased aortopathy incidence in females (from 10 to 30%) and males (from 68 to 85%), with more pronounced increases in aortic rupture rates in males (from 16 to 40%) than females (from 0 to 10%). Prolonged exposures (for 56 days) to AngII and nicotine increased aortic rupture of males (to 50%). Paradoxically, GDX of female mice reduced the incidence of AAs in response to nicotine (from 60 to 30%). In male mice co-infused with AngII and nicotine and undergoing GDX, AA incidence decreased depending on the number of testes removed (control, 100%; one testis removed, 70%; two testes removed, 40%). The number of testes similarly influenced effects of nicotine on AngII-induced maximal AA diameters and aortic rupture rates. The number of testes were inversely related to AA measures in response to AngII with nicotine.

Conclusions: Nicotine augmented the formation and severity of AngII-induced AAs in both sexes, with pronounced effects to promote aortic ruptures of males. Removal of female sex hormones paradoxically ameliorated effects of nicotine on AAs. The number of testes was inversely related to nicotine's augmentation of AA incidence and severity. These results suggest nicotine's actions to promote aortopathies are influenced by sex hormone status.

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