CARDIOVASCULAR RESEARCH DAY
BILL GATTON STUDENT CENTER
SEPTEMBER 20, 2019

Abstract Book

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SCHEDULE FOR THE DAY

Friday, September 20
University of Kentucky
Gill Heart and Vascular Institute
Cardiovascular Research Day
Bill Gatton Student Center
Grand Ballrooms

8:30 am
Guest Check–In Begins | Registration Desk

Morning Session | Ballrooms A&B

9:00 am - Welcoming Comments

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

9:05 am - Trainee Presentations I

Kurtis Mann
Graduate Student | Jonathan Wenk Lab | Mechanical Engineering
Recruitment from Myosin OFF State Steepens ESPVR in Finite Element Model of Left Ventricle

Smita Joshi, PhD
Postdoc | Sidney Whiteheart Lab | Molecular & Cellular Biochemistry
Fine Tuning Platelet Secretion to Modulate Hemostasis

9:30 am - Faculty Presentation
"Impact of microRNA-33 antagonism on atherosclerosis stabilization"

Ryan Temel, PhD
Associate Professor
Saha Cardiovascular Research Center and Physiology

10:00 am – BREAK

10:15 am - Gill Award Recipient

"The Discovery of the RAGE/DIAPH1 - Highlighting a Signaling Platform Implicated in the Pathogenesis of Obesity and Diabetic Complications"

Ann-Marie Schmidt, MD
Gill Heart and Vascular Institute Outstanding Contributions to Cardiovascular Research Award
The Iven Young Professor of Endocrinology
Professor of Medicine
Director, Diabetes Research Program
NYU School of Medicine

11:00 am - 90 Second Poster Pitches

Brooke Ahern | University of Kentucky
Jeff Chen | University of Kentucky
Lisa Green | University of Cincinnati
Masayoshi Kukida | University of Kentucky
Xian Li | University of Kentucky
Shayan Mohammadmoradi | University of Kentucky
Satoko Ohno | University of Kentucky
Martha Sim | University of Kentucky
Samuel Slone | University of Cincinnati
Nirmal Verma | University of Kentucky

11:15 am - Poster Session I

Odd numbered posters presented and judged

Lunch Session | Ballroom C

12:00 pm - LUNCH | BALLROOM C

12:30 pm - Lunch Speaker

"The Art of Scientific Communication"

Ushma Savla Neill, PhD
Vice President, Scientific Education & Training
Memorial Sloan Kettering Cancer Center
Editor at Large, The Journal of Clinical Investigation

1:30 pm - BREAK

Afternoon Session | Ballroom A&B

1:45 pm - 90 Second Poster Pitches

Kelsey Conrad | University of Cincinnati
David Henson | University of Kentucky
Malina Ivey | University of Cincinnati
Megan Jay | University of Cincinnati
Hannah Russell | University of Cincinnati
Velmurugan Gopal Viswanathan | University of Kentucky
Chia-Hua Wu | University of Kentucky

2:00 pm - Poster Session II
Even numbered posters presented and judged

2:45 pm - Trainee Presentations II

Maria Kraemer, PhD
Postdoc | Susan Smyth Lab | Internal Medicine
Loss of Endothelial Cell-Derived Lipid Phosphate Phosphatase 3 Exacerbates Calcification of Aortic Valves

Yanming Li, PhD
Postdoc | Scott LeMaire Lab | Baylor College of Medicine
Single-cell transcriptome analysis of human ascending thoracic aortic aneurysms reveals a cell atlas of the aortic wall

3:15 pm - Distinguished Alumni Speaker
"Determining how day-night rhythms confer risk for sudden cardiac death"

Brian Delisle, PhD
Associate Professor
Department of Physiology
University of Kentucky

3:45 pm - BREAK

4:00 pm - Gill Award Recipient
"Treating Cardiometabolic Disease MacGyver-Style: with a bag of sugar and a bottle of pesticide"

Babak Razani, MD, PhD
Gill Heart and Vascular Institute Translational Early Career Award
Associate Professor of Medicine
Professor of Pathology & Immunology
Center for Cardiovascular Research
Washington University School of Medicine

4:45 pm - Networking Session | Ballroom C

5:30 pm - Dinner & Awards | Ballroom C

GILL AWARD RECIPIENT

Gill Heart Institute Outstanding Contributions to Cardiovascular Research Award

Ann Marie Schmidt, MD

The Iven Young Professor of Endocrinology
Professor of Medicine
Director, Diabetes Research Program
NYU School of Medicine

Ann Marie Schmidt earned her B.A. in biology and history summa cum laude from NYU's Washington Square College and her M.D. degree with honors from NYU School of Medicine. She remained at NYU to complete her medical residency and chief residency, as well as fellowship in hematology and medical oncology, then moved to Columbia University, joining the department of physiology and cellular biophysics as a Post-doctoral Fellow of the Juvenile Diabetes Research Foundation (JDRF). From 2003 to 2010, she served as the Chief of the Division of Surgical Science and the Gerald and Janet Carrus Professor of Surgical Science. Effective July 1, 2010, Dr. Schmidt returned to NYU as the Director of the Diabetes Research Program at New York University Langone Medical Center, New York and the first Iven Young Professor of Endocrinology at NYU. She is a Professor of Medicine, Pharmacology and Pathology. Dr. Schmidt's laboratory discovered "RAGE," (receptor for advanced glycation end-products), a cell-surface receptor that exacerbates inflammation and damage when activated, to heart disease-related vascular injury, particularly in diabetes and its complications. She has studied RAGE and its relationship to inflammatory and immune disorders, peripheral nerve injury and regeneration, and neurodegenerative diseases. Her laboratory has recently discovered the intracellular interactions of the RAGE cytoplasmic domain with diaphanous-1, a member of the formin family. This discovery forms the basis for the identification of a new class of RAGE inhibitors for the treatment of diabetes and its complications and RAGE-related disorders. Dr. Schmidt is a Scholar of the JDRF and received the David Rumbough Research Award from the JDRF in 2010.

GILL AWARD RECIPIENT

Gill Heart Institute Translational Early Career Award

Babak Razani, MD, PhD

Associate Professor of Medicine (Cardiology)
Associate Professor of Pathology & Immunology
Center for Cardiovascular Research
Washington University School of Medicine

Dr. Razani received his B.S. in Electrical Engineering and Computer Science and B.A. in Molecular and Cellular Biology from the University of California at Berkeley. He then went on to earn M.D. and Ph.D. degrees as part of the MSTP at Albert Einstein College of Medicine. After completing Internal Medicine residency and Cardiovascular fellowship at Washington University School of Medicine, he has stayed on as faculty, recently promoted to the rank of Associate Professor. Dr. Razani has conducted seminal work in the atherosclerosis and metabolism fields. He was the first to establish that the autophagy-lysosome system becomes dysfunctional in atherosclerosis and described the pathogenic relevance of cytotoxic protein aggregates that accumulate as a result. His most recent work on stimulating the transcription factor TFEB as a means of driving autophagy-lysosomal biogenesis shows the potential power of this pathway in ameliorating cardiovascular and cardiometabolic diseases. Dr. Razani's efforts have been recognized by the scientific community receiving several prestigious early investigator awards at national forums including the Irvine Page (ATVB), David Williams (Kern), and Springer (NAVBO).

FEATURED SPEAKER

Ushma Savla Neill, PhD

Vice President, Scientific Education & Training,
Memorial Sloan Kettering Cancer Center
Editor at Large, The Journal of Clinical Investigation

Dr. Ushma Neill is the Vice President of Scientific Education and Training at Memorial Sloan Kettering (MSK), where she oversees the academic curriculum for pre- and postdoctoral researchers who train in laboratories across MSK. Her areas of focus include enhancing the career and professional development opportunities available to trainees as well as designing courses in areas such as rigor and transparency, statistics, computational biology, and imaging. A passionate advocate for scientific communication, Ushma is also deeply committed to developing platforms that allow trainees to enhance the efficacy of their communications to fellow scientists and to the lay public.

Her graduate education began at Northwestern University where she studied pulmonary mechanics and mathematical modeling. After completing her doctoral degree in biomedical engineering, Ushma used a Marshall Scholarship to study vascular permeability as a postdoctoral fellow at Imperial College London. Following her postdoc, she spent two years as a manuscript editor at Nature Medicine, and then joined the Journal of Clinical Investigation (JCI) as Executive Editor. During her nine years in this role, Ushma led academic editorial boards at Columbia University College of Physicians and Surgeons, University of Pennsylvania School of Medicine, and Duke University/University of North Carolina in identifying, soliciting, and peer reviewing high-quality manuscripts. She also oversaw the writing, commissioning, and editing of front matter, including Editorials, News, Commentaries, Reviews, and Perspectives.

Ushma first came to MSK in 2012 as Director of the Office of the President, serving as the Chief of Staff to President and CEO Craig B. Thompson. In this role, she worked closely with Dr. Thompson and other MSK leadership to shape and manage a strategic agenda. Ushma continues to serve as Editor-at-Large for the JCI, acting as the chief interviewer for the JCI video series “Conversations with Giants in Medicine.”

FEATURED SPEAKER

2019 Distinguished Alumni Speaker

Brian Delisle, PhD

Associate Professor
Department of Physiology
University of Kentucky

Brian Delisle, PhD, is an Associate Professor of Physiology at the University of Kentucky. For over 20 years his research focus has been on drug-induced, acquired, and inherited arrhythmia syndromes. Specifically, his laboratory studies how perturbations in cardiac ion channel function impact the risk for life-threatening cardiac events. His research program is currently funded to understand how disruptions in daily rhythms and circadian signaling in the heart modify cardiac ion channel function and the risk for sudden cardiac death.

FACULTY PRESENTATION

Ryan Temel, PhD

Associate Professor
Saha Cardiovascular Research Center
Department of Physiology
University of Kentucky

Ryan was raised in the suburbs of Pittsburgh, PA and attended Allegheny College in Meadville, PA where he earned a B.S. in Chemistry in 1995. With Dr. David Williams as his mentor, Ryan researched the roles of SR-BI and apoA-I in HDL selective cholesterol uptake and earned a Ph.D. in Biochemistry and Molecular Biology from SUNY Stony Brook in 2001. He did his post-doctoral training with Dr. Larry Rudel at Wake Forest University where he studied pathways involved in the storage, trafficking, and elimination of cholesterol. After earning a NIH Pathway to Independence Award, Wake Forest University promoted him to instructor in 2006 and to assistant professor in 2008.

He was recruited to the University of Kentucky in 2013 and became a member of the Saha Cardiovascular Research Center and the Department of Pharmacology and Nutritional Sciences. He was promoted to associate professor in 2015 and moved his academic appointment to the Department of Physiology in 2018. His lab is currently researching mechanisms of and therapies for non-alcoholic steatohepatitis and atherosclerotic vascular disease of the heart and brain. The Temel lab is one of the few in the world that uses monkeys to study metabolic and cardiovascular diseases.

SAHA AWARDEES

The Saha Cardiovascular Research Center is pleased to announce the recipients of the 2019 Saha Awards for Cardiovascular Research and Education. The Saha Awards are given to encourage and support staff and students with an interest in and dedication to cardiovascular medicine. Each award includes an unrestricted $1000 prize and a certificate.

The 2019 Recipients are:

UK MD, PhD Student Award – ***Brandon Farmer***

UK Healthcare Nursing Student Award – ***Chin-Yen Lin***

Paula Fritz Patient Education Award – ***Linda Clements***

Medical Student Award - ***David Carter***

EVENT SUPPORTERS

Gill Foundation of Texas

●

The Saha Fund for Cardiovascular Research and Education

●

The Estate and Family of Mrs. Hager Koostra

●

Mr. and Mrs. Bob Allen

2019 POSTER JUDGES

Douglas Andres
Babak Bazrgari
Brad Berron
Lisa Cassis
Cherry Croft
Alan Daugherty
Florin Despa
Sanda Despa
Vic Ferraris
Ming Gong
Scott Gordon
Gregory Graf
Zhenheng Guo
Sangderk Lee
Xiangan Li
Zhenyu Li
Analia Loria
Hong Lu
Andrew Morris
Gia Mudd-Martin
Ushma Neill
Barbara Nikolajczyk
Fredrick Onono
Phillip Owens
Julie Pendergast
Martha Peterson
Babak Razani
Jonathan Satin
Ann-Marie Schmidt
Ying Shen
Mary Sheppard
Ying Shen
Preetha Shridas
Samir Softic
Brett Spear
Steven Steinhubl
Venkateswaran Subramanian
Lisa Tannock
Ryan Temel
Sean Thatcher
Michael Tranter
Sudhakar Veeranki
Vincent Venditto
Qingjun Wang
Chris Waters
Sidney Whiteheart
Jeremy Wood
Frederique Yiannikouris

2019 POSTER PARTICIPANTS

2019 Abstracts

1

Staff

Himi Tripathi[1], Ahmed Al-Darraji[1], Hsuan Peng[1], Lakshman Chelvarajan[1], Bryana Levitan[1], Erhe Gao[2], Gabriela Hernandez[1], Andrew Morris[1], Susan S. Smyth[1] and Ahmed Abdel-Latif[1]

[1]Gill Heart Institute and Division of Cardiovascular Medicine, University of Kentucky and the Lexington VA Medical Center, Lexington, KY, USA | [2]The Center for Translational Medicine, Temple University School of Medicine, Philadelphia, PA, USA

Autotaxin Inhibition Reduces Cardiac Inflammation and Mitigates Adverse Cardiac Remodeling After Myocardial Infarction: Potential Therapeutic Targets for Ischemic Heart Disease

Introduction: The increased inflammatory response following (MI) can have harmful effects on cardiac function. This pathological inflammation aggravates tissue damage and is correlated with the development of heart failure. Lysophosphatidic acid (LPA), produced by autotaxin (ATX), regulates monocytosis and promotes inflammation. Signaling of lipid substrate including LPA is dephosphorylated and terminated by Lipid phosphate phosphatase 3 (LPP3). The role of ATX/LPA signaling nexus in cardiac inflammation is poorly understood which incited us to investigate the possible role of LPA to understand inflammation, a condition promoting cardiac adverse remodeling post-MI.

Methods and Results: Acute myocardial infarction (AMI) patients peripheral bloods were used to measure LPA, ATX and inflammatory cells. Following MI, LPP3 specific inducible knock out (Mx1-Plpp3Î", Plpp3fl/fl) mice and C57BL/6 mice were used for the study. Wild type mice were treated with pharmacological inhibitor PF-8380 twice a day for 7 days post AMI. AMI patient's plasma concentration showed increased LPA, ATX and inflammatory cells in compare to control and baseline and there was correlation between higher concentration of LPA and inflammatory cells subsets. MI was associated with increased number of cardiac neutrophils, inflammatory monocytes, macrophages and increased number of bone marrow and spleen myeloid progenitor cells during peak post-MI inflammation in Mx1- Plpp3Î" and reduction in inflammatory and progenitor cells in ATX inhibitor treated mice, as assessed by flow cytometry. Moreover, Mx1- Plpp3 mice cardiac functional recovery was reduced and increased infarct size as assessed by echocardiography and Mason Trichrome staining while cardiac recovery, scar size and angiogenesis was improved in ATX -inhibitor administrated mice.

Conclusion: LPA plays an important role in modulating inflammation and targeting ATX/ LPA signaling can represents a novel therapeutic target for coronary heart diseases.

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Postdoc

Hisashi Sawada[1], Hideyuki Higashi[3], Bradley C Wright[1], Debra L Rateri[1], Jessica J Moorleghen[1], Deborah A Howatt[1], Lang H Lee[3], Sasha A. Singh[3], Masanori Aikawa[3], Mark W Majesky[4], Alan Daugherty[1, 2]

[1]Saha Cardiovascular Research Center, University of Kentucky, KY | [2]Department of Physiology, University of Kentucky, KY | [3]Center for Interdisciplinary Cardiovascular Sciences, Brigham and Womens Hospital, Harvard Medical School, MA | [4]Departments of Pediatrics and Pathology, University of Washington, WA

LRP1 Deletion in Second Heart Field-derived Smooth Muscle Cells Promotes Angiotensin II-induced Thoracic Aortic Aneurysm

Objective: Thoracic aortic aneurysms (TAAs) display a gradient of pathology across the media that is most pronounced in the outer elastin layers of the ascending aorta. The smooth muscle cells (SMCs) in this region are derived from the second heart field (SHF). Low density lipoprotein receptor-related protein 1 (LRP1) is an important regulator of aortic wall structure, as its deletion in pan-smooth muscle cells (SMCs) exacerbates angiotensin II (AngII)-induced TAAs. The aim of this study was to determine whether deletion of LRP1 in SHF-derived SMCs augmented AngII-induced TAA.

Methods and Results: LRP1 floxed mice were bred to mice expressing Cre under the control of the Mef2c promoter to delete LRP1 in SHF-derived SMCs. The aortic dimensions were measured by ultrasonography for one year, but no apparent aortic dilations were detected. To determine effects on TAAs, AngII (1,000 ng/kg/min) or saline was infused for 4 weeks into male mice expressing Cre controlled by the Mef2c promoter or non-transgenic littermates. There was no difference in blood pressure between the two genotypes in response to AngII. However, AngII-induced thoracic aortic rupture was augmented by LRP1 deletion in SHF-derived SMCs (4 vs 38 %, $p < 0.05$, n = 21 40). In addition, AngII-induced thoracic aortic dilation was greater in SHF-specific LRP1 deleted mice than in wild type littermates (1.64± 0.05 vs 1.96 ± 0.07 mm at 4 weeks, $p < 0.05$). AngII-induced aortic dilation was not observed in the descending aorta of either genotype. To provide mechanistic insight, mass spectrometry-assisted proteomics was performed to profile the proteomes of ascending and descending aortas. A total 2,644 proteins were identified, and AngII infusion altered the abundance of 864 proteins in the ascending aorta of wild type mice, of which 4 of these proteins were augmented further by LRP1 deletion in SHF-derived SMCs. Comparison between ascending and descending aortas in response to AngII in wild type mice, 77 proteins were differentiated ($q < 0.1$). Collagen 12a1 was the only protein that was common between the 4 and 77 proteins, and it was upregulated by AngII and LRP1 deletion in SHF-derived SMCs ($q < 0.1$).

Conclusion: LRP1 deletion in SHF-derived SMCs increased the abundance of collagen 12a1 and augmented AngII-induced TAA formation.

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Staff

Robert N. Helsley[1], Anthony D. Gromovsky[1], Amanda L. Brown[1], Varadharajan Venkateshwari[1], Rebecca C. Schugar[1], Mohammad Nasser Kabbany[1], Rakhee Banerjee[1], Chase Neumann[1], Chelsea Finney[1], Preeti Pathak[1], Danny Orabi[1], Lucas Osborn[1], William Massey[1], Renliang Zhang[1], Brian E. Sansbury[4], Calvin Pan[5], Jessica Sacks[3], Richard G. Lee[9], Rosanne M. Crooke[9], Mark J. Graham[9], Madeleine E. Lemieux[8], Valentin Gogonea[10], John P. Kirwan[3], Daniela S. Allende[2], Mete Civelek[7], Lawrence L. Rudel[6], Aldons J. Lusis[5], Matthew Spite[4], and J. Mark Brown[1*]

[1]Department of Cellular and Molecular Medicine, Cleveland Clinic, Cleveland, OH 44195, USA | [2]Department of Anatomical Pathology, Cleveland Clinic, Cleveland, OH 44195, USA | [3]Department of Pathobiology, Cleveland Clinic, Cleveland, OH 44195, USA | [4]Center for Experimental Therapeutics & Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Womens Hospital, Harvard Medical School, Boston, MA 02115, USA | [5]Departments of Medicine, Microbiology, and Human Genetics, University of California Los Angeles, Los Angeles, CA 90095, USA | [6]Department of Pathology, Section on Lipid Sciences, Wake Forest University School of Medicine, Winston-Salem, NC 27157-1040, USA | [7]Department of Biomedical Engineering, University of Virginia, Charlottesville, VA 22904, USA | [8]Bioinfo, Plantagenet, ON K0B 1L0, Canada | [9]Cardiovascular Group, Antisense Drug Discovery, Ionis Pharmaceuticals, Inc.; Carlsbad, CA 92010, USA | [10]Department of Chemistry, Cleveland State University, Cleveland, OH, USA

Obesity-Linked Suppression of Membrane-Bound O-Acyltransferase 7 (MBOAT7) Drives Non-Alcoholic Fatty Liver Disease Progression

Objective: Recent studies have identified a common genetic variant rs641738 near two genes encoding membrane bound O-acyltransferase domain-containing 7 (MBOAT7) and transmembrane channel-like 4 (TMC4) that associates with increased risk of non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), alcohol-related cirrhosis, and liver fibrosis in those infected with viral hepatitis. Based on hepatic expression quantitative trait loci (eQTL) analysis it has been suggested that MBOAT7 loss of function promotes liver disease progression. The primary objective of this study was to identify the contribution of MBOAT7 in liver injury.

Approach and Results: To assess the role of MBOAT7 in liver injury, mice were fed chow or a high fat diet (60% kCal from fat) and intreperitoneally (IP) injected biweekly with 12.5 mg/kg of either a non-targeting control antisense oligonucleotide (ASO) or an ASO targeting murine MBOAT7 for 20-weeks. Mass spectrometry methods were used to analyze all phospholipid species in the liver, brain, adipose, pancreas, and plasma. RNA sequencing was utilized to assess gene expression changes in the liver after 20-weeks of feeding and ASO treatment. All hepatic lipid analysis, insulin signaling assays, and IP glucose and insulin tolerance tests were completed at 20-weeks of feeding with concurrent ASO treatment.

We demonstrate that MBOAT7 loss of function, but not TMC4, in mice is sufficient to promote the progression of NAFLD in the setting of high fat diet. MBOAT7 loss of function is associated with accumulation of its substrate lysophosphatidylinositol (LPI) lipids, and direct administration of LPI promotes hepatic inflammatory and fibrotic transcriptional changes in an Mboat7-dependent manner. We also show that hepatic MBOAT7 expression is dramatically reduced in obese humans and rodents, as well as in subjects with significant liver fibrosis. The hepatic steatosis seen with diminished MBOAT7 expression may in part be explained by alterations in the lipidome and proteome of cytosolic lipid droplets that favor lipid storage.

Conclusions: Collectively, these studies identify MBOAT7-driven acylation of LPI lipids as an important modulator of both liver disease progression and associated type 2 diabetes.

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Graduate Student

Megan Jay, Shannon M. Jones, Kelsey A. Conrad, Sarah Anthony, Michael Tranter, Joel Thompson, Mete Civelek, A. Phillip Owens III

Division of Cardiovascular Health and Disease, University of Cincinnati | SAHA Cardiovascular Research Center, University of Kentucky | Department of Biomedical Engineering, University of Virginia

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

Objective: In atherosclerosis, vascular smooth muscle cells (VSMCs) migrate from the media to the intima where they transdifferentiate into a macrophage-like state and are suggested to contribute 50-70% of atheroma foam cells. Protease activated receptor-2 (PAR2), known to augment inflammation upon activation, is highly expressed in VSMCs, up-regulated in atherosclerotic plaques, and is crucial for the initiation and progression of atherosclerosis via a VSMC-mediated mechanism. The objective of this study was to determine the role of PAR2 on VSMC phenotypic modulation in an atherosclerotic setting.

Methods and Results: To determine if PAR2 deficiency affects VSMC phenotypic modulation, mouse VSMCs that were Par2+/+ or -/- were treated with or without water-soluble cholesterol for 72 hours. Cells were either harvested to determine markers of VSMCs (α-actin or myosin heavy chain) and macrophages (CD68 and Mac-2) or incubated with latex beads to test for phagocytic activity. PAR2 deficiency attenuated loss of VSMC markers and gain of macrophage markers in cholesterol treated VSMCs. PAR2 deficiency attenuated phagocytosis of latex beads in comparison to PAR2 proficiency. Krüppel-like factor 4 (KLF4) is a zinc-finger transcription factor and is essential for VSMC phenotypic modulation and the progression of atherosclerosis. Activation of VSMCs with PAR2 agonist peptide results in a 2-log fold increase in Klf4 mRNA, which is ablated in Par-2-/- cells. Importantly, PAR2 is significantly correlated with KLF4 when comparing the atherosclerotic aortas of 101 different strains of mice in the University of California at Los Angeles (UCLA) hybrid mouse diversity panel (HDMP; top 50 gene; $P = 2.77 \times 10^{-17}$). Finally, we demonstrate that PAR2 is necessary for regulation of the mRNA stabilization protein human antigen R (HuR), which is speculated to bind and stabilize KLF4 mRNA.

Conclusions: Taken together, our results suggest that activation of PAR2 promotes VSMC phenotypic modulation through a hypothesized pathway of KLF4 upregulation and subsequent stabilization via HuR. Future studies will analyze the link between this trilateral group of proteins.

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Graduate Student

Shayan Mohammadmoradi [1,2], Deborah A. Howatt [1], Jessica J. Moorleghen [1], Huan Yang [4], Kevin J. Tracey [4], Hong S. Lu [1,3], Alan Daugherty [1,3]

[1]Saha Cardiovascular Research Center | [2]Department of Pharmacology and Nutritional Sciences, University of Kentucky | [3]Department of Physiology, University of Kentucky | [4] Center for Biomedical Science, The Feinstein Institute for Medical Research

Role of High Mobility Group Box 1 (HMGB1) in Angiotensin II-induced Abdominal Aortic Aneurysm

Background and Objectives: The nuclear protein, HMGB1, is an alarmin that exerts biological activities both intracellularly and by stimulation of cell surface receptors. Human studies show that abdominal aortic tissue of aneurysmal patients have increased HMGB1 protein abundance. Animal studies also indicate that HMGB1 contributes to vascular inflammation, remodeling and matrix metalloprotease production, all of which contribute to abdominal aortic aneurysm (AAA) development.

Methods and Results: Male and female LDLr-/- mice were fed a saturated fat-enriched diet (Diet # TD.88137, Harlan Teklad) for 2 weeks. After one week of feeding, mice were infused with either saline or AngII (1,000 ng/kg/min) by osmotic minipumps for 1 week. Western blot analysis of abdominal aortic tissue lysate demonstrated increase by 90% in HMGB1 in AngII-infused male group compared to mice infused with saline. AngII infusion in female mice did not show any HMGB1 protein accumulation. To characterize the localization of HMGB1 in aortic tissue, abdominal aortic sections of 14-day saline or AngII infused LDLR-/- mice were immunostained for HMGB1. This demonstrated a marked increase in aortic HMGB1 abundance in AngII-infused mice that is localized in the aortic adventitia. Since fibroblasts are a prominent cell type in the adventitia, cultured human aortic adventitial fibroblasts were incubated with AngII (1 μM) for 3 and 16 hours. Confocal microscopy of immunofluorescence stained cells and Western blot analysis of cellular fractions confirmed HMGB1 translocation and release. To determine the role of HMGB1 in AngII-induced AAAs, a monoclonal anti-HMGB1 antibody (2G7, mouse IgG2b, 50 μg/kg/every 3 days) or an isotype-matched control were intraperitoneally injected to 8-10 week old male LDLr-/- mice that were fed a Western diet and infused with AngII (1,000 ng/kg/day) for 28 days. Neutralizing HMGB1 with a low dose of 50 μg by every 3-day did not show a significant decrease in the abdominal aortic diameter of AngII infused mice.

Conclusion: Our findings indicate that HMGB1 is an important player in AngII-induced AAA formation.

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Postdoc

Carolina Dalmasso, Sophia Mounce, Jacqueline Leachman, Xiu Xu and Analia Loria
Department of Pharmacology and Nutritional Sciences, University of Kentucky

Acute Stimulation of Neuro-Adipose Connections Increase Blood Pressure Responses in Male Mice Exposed to Early Life Stress

Visceral adiposity increases sympathetic activation and the risk for obesity-induced hypertension. Experimental stimulation of afferent excitatory signals from adipose tissue contributes to increased sympathetic activation during obesity-induced hypertension as part of a sympatho-excitatory mechanism called the Adipose Afferent Reflex (AAR). Our lab has shown that male mice exposed to maternal separation and early weaning (MSEW), a mouse model that mimics early life stress in humans, display increased sympathetic tone and mean arterial pressure (MAP) when fed high fat diet chronically. Therefore, we hypothesize that the AAR could be implicated in the exacerbated obesity-induced hypertension observed in male MSEW mice. Litters were separated from the dam from postnatal days 2 to 16 and weaned early on day 17. Undisturbed controls (C) were weaned on day 21. After weaning, C and MSEW mice were fed a low fat (LF) or high fat (HF) diet for 16 weeks (10% and 60% Kcal from fat respectively, Research Diets). In mice implanted with carotid catheter for MAP measurements, subcutaneous (sc) or epididymal white adipose tissue (eWAT) were exposed for saline or capsaicin infusions (0.5 nmol/ul; 4 sites; 4ul/min; 2 min; bilateral; n=4-7). Saline infusion in scWAT or eWAT did not change MAP from baseline in any of the groups. Capsaicin infusion in scWAT decreased MAP response in both C and MSEW HF-fed mice compared to LF (p Diet<0.01), whereas infusion in eWAT increased the MAP response in HF-fed MSEW mice compared to C (7 ± 1 vs. -2.5 ± 3 mmHg; $p<0.01$). After MAP recordings, fat depots were removed to measure capsaicin-induced CGRP release (1 uM capsaicin). In scWAT, CGRP release was decreased in HF compared to LF similarly in both C and MSEW (p Diet<0.01); however, MSEW increased CGRP release compared to C (p MSEW<0.05). In addition, capsaicin infusion increased the number of Fos positive neurons in the paraventricular nucleus of the hypothalamus in MSEW mice fed a HF diet compared to saline (51 ± 6 vs. 26 ± 4; $p<0.05$), but not in C. Our results show that male MSEW mice fed a HF may display depot-specific afferent signals that influence acute MAP control. Moreover, the AAR could play an important role in the increased sympathetic tone and blood pressure observed in these mice.

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Postdoc

Xian Li, PhD, Xiaohong Song and Jeremy P. Wood, PhD
Saha Cardiovascular Research Center, University of Kentucky, Lexington, KY

Protein S-Activated Protein C synergize with Tfpiα to enhance inhibition of Prothrombinase

Background: Protein S (PS), a vitamin K-dependent plasma glycoprotein, functions as a cofactor for the anticoagulants activated protein C (APC) and tissue factor (TF) pathway inhibitor alpha (Tfpiα), which inhibit factors Va (FVa) and Xa (FXa), respectively. FVa and FXa form the prothrombinase complex, which generates the thrombin responsible for clot formation. Thrombin generation is characterized by a slow initiation phase, followed by a rapid propagation phase. The initiation phase is regulated by Tfpiα, which binds a regulatory acidic region (AR) in FVa, in addition to directly inhibiting the FXa active site, which may or may not require PS. However, the regulation of the propagation phase is unclear, as the FVa AR is rapidly removed by thrombin, and neither the PS/APC nor PS/TFPIÎ± system alone effectively inhibits prothrombinase. We hypothesize a synergistic relationship by which PS promotes both APC and Tfpiα to down-regulate thrombin production.

Methods and Results: To assess the effect of the combined PS/APC and PS/ Tfpiα systems on thrombin generation, we supplemented plasma with thrombomodulin, which promotes APC activation. In the absence of thrombomodulin, 5nM Tfpiα decreased peak thrombin by 55.1% (33.1±1.9 nM in the presence of Tfpiα vs. 73.7±39.9 nM in the absence) and endogenous thrombin potential (ETP) by 35.4% (475±42 nM*min vs. 735±189nM*min). In the presence of thrombomodulin, Tfpiα decreased these parameters by 65.7% (11.4 ± 2.6 nM) and 77.5% (107±22 nM*min), respectively, suggesting that APC makes Tfpiα a more potent inhibitor of thrombin generation. Consistent with our hypothesis that PS is not involved in regulating the initiation of coagulation, PS dose-dependently decreases both the ETP and peak thrombin in this assay, but has minimal effect on the lag time.

Tfpiα has 3 Kunitz-type inhibitor domains (K1, K2 and K3). The K2 domains bind and inhibits FXa, while the K3 domain binds PS to promote FXa inhibition. We identified an anticoagulant protein in the saliva of black flies, “black fly protease inhibitor” (BFPI), which contains the K2 and FVa-binding regions of Tfpiα, but lack the K3 domain. We hypothesized that BFPI is capable of binding FXa and the FVa AR but not PS, and thus is an inhibitor of the initiation phase, but not the propagation phase, of thrombin generation. Consistent with this hypothesis, BFPI inhibits FXa cleavage of a short peptide substrate similarly to Tfpiα, but PS does not promote this inhibition. In addition, BFPI inhibited prothrombinase assembled with FVa that contains the AR (IC50=4.9nM vs. 1.0nM with Tfpiα), but not thrombin-treated FVa, which lacks the AR.

We next used BFPI to study the effect of PS/APC on Tfpiα function, in the absence of a direct interaction between PS and Tfpiα. In a purified prothrombinase assay using thrombin-activated FVa, 5 nM BFPI had no impact on the maximal rate of thrombin generation, either in the presence or absence of PS. However, in the presence of APC and PS, 5nM BFPI decreased the maximum rate of thrombin generation by 17.3±3.3%. These data suggest that PS/APC-mediated degradation of FVa promotes BFPI-mediated inhibition of FXa, though the effect is reduced compared to Tfpiα.

Conclusions: BFPI mimics the ability of TFPIα to regulate the initiation of coagulation by binding FXa and the FVa AR, demonstrating that the K3/PS interaction is not required for this activity. However, the PS-APC system did promote inhibition of the propagation phase by either BFPI or Tfpiα. These data suggest that the PS-APC and PS-TFPIα systems cooperatively regulate thrombin generation by prothrombinase. While maximal inhibition requires that PS act as a cofactor for both APC and TFPIα, PS-APC independently promotes TFPIα function. Therefore, we propose a model in which PS-APC-mediated inhibition of FVa renders FXa susceptible to inhibition by TFPIα.

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Staff

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Adipocyte-derived serum amyloid A promotes angiotensin II-induced abdominal aortic aneurysms in obese C57BL/6 mice

Objectives: Obesity increases the risk for human abdominal aortic aneurysms (AAA) and enhances angiotensin II (AngII)-induced AAA formation in C57BL/6 mice. Obesity is also associated with increases in peri-aortic fat that expresses pro-inflammatory markers including serum amyloid A (SAA). We previously reported that deficiency of SAA significantly reduces AngII-induced inflammation and AAA in apoE-deficient mice, and have subsequently determined that AngII infusion significantly increases SAA expression in peri-aortic fat of obese C57BL/6 mice. In this study we investigated whether SAA plays a role in AngII-induced AAA in obese C57BL/6 mice.

Approach and results: The development of AAA was compared between 12-15 week-old male C57BL/6 mice (WT), C57BL/6 mice lacking SAA1.1, SAA2.1, and SAA3 (SAA-TKO); and SAA-TKO mice harboring a doxycycline-inducible, adipocyte-specific SAA1.1 transgene (SAA-TGfat; SAA expressed only in fat). All mice (n=12-15/genotype) were fed an obesogenic diet (60% kcal from fat) for 14 weeks, provided water containing 0.4 mg/ml doxycycline and 5% sucrose for the last 6 weeks to induce SAA transgene expression, and infused with AngII (1000 ng/kg/min) for the last 4 weeks via osmotic pump to induce AAA. Maximal luminal diameters of the abdominal aorta were determined by ultrasound before and after AngII infusion. Increases in body weight and fat mass were similar for all three groups of mice. At study termination, plasma SAA was 126 ± 22 and 41 ± 6 ï-g/ml in WT and SAA-TGfat mice, respectively. The maximal luminal diameters were significantly increased in WT (baseline: 1.20 ± 0.03 mm; post AngII: 1.51 ± 0.13 mm; p< 0.01) and SAA-TGfat mice (baseline: 1.14 ± 0.04 mm; post AngII: 1.53 ± 0.13 mm; p<0.01) but were not significantly different in SAA-TKO mice (baseline: 1.14 ± 0.03 mm; post AngII: 1.29 ± 0.06 mm).

Conclusions: We demonstrate for the first time that SAA deficiency protects obese C57BL/6 mice from AngII-induced AAA. SAA expression only in adipocytes is sufficient to cause AAA in obese mice infused with AngII.

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Graduate Student

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AT1a receptor deficiency attenuates thoracic aortic aneurysm progression in FBN1 C1041G/+ mice.

Objective:

Angiotensin receptor type 1 (AT1 receptor) activation has been implicated in thoracic aortic aneurysms (TAAs). Losartan, an AT1 receptor antagonist, has been consistently shown to attenuate TAAs in multiple animal models. However, recent studies concluded that Losartan's attenuation of Marfan syndrome associated TAAs is unrelated to AT1 receptor antagonism. To resolve this discrepancy, we determined the effects of AT1a receptor deletion on TAAs in the fibrillin-1 haploinsufficient (FBN1 C1041G/+) Marfan syndrome mouse model.

Methods and Results:

Aortas from wild type and FBN1 C1041G/+ littermates, that were AT1a receptor +/+ or -/-, were imaged from 1 to 12 months of age using a rigorously standardized ultrasound protocol and verified by direct visualization at termination. Male FBN1 C1041G/+ mice had increased aortic diameters at 1 month compared to wild type littermates (Ascending: 1.39±0.06mm vs 1.16±0.07mm; p=0.04. Root: 1.63±0.05mm vs 1.35±0.06mm; p<0.001). Dilation at 1 month was not attenuated by AT1a receptor deletion. Subsequent expansion of both the ascending aorta and the aortic root in male FBN1 C1041G/+ mice was attenuated by AT1a receptor deletion. This difference in FBN1 C1041G/+ mice with AT1a receptor +/+ vs -/- could be detected as early as 3 months (Ascending: 1.51±0.04mm vs 1.28±0.06mm; p=0.002. Root: 2.05±0.06mm vs 1.79±0.08mm; p=0.03) and persisted to termination. Conversely, aortic diameters in 12 month old female FBN1 C1041G/+ mice compared to their wild type littermates were minimal (Ascending: 1.50±0.06mm vs 1.36±0.06mm. Root: 2.06±0.13mm vs 1.77±0.13mm), limiting analysis of AT1a receptor deletion in female mice.

Conclusions:

Deletion of AT1a receptors attenuates TAA progression but not initial development in male FBN1 C1041G/+ mice. Minimal aortic expansion in female FBN1 C1041G/+ mice highlights the need to perform sex-specific analyses of TAA.

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Postdoc

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The role of activated fibroblasts during wound healing and regeneration

An acute injury to skeletal muscle results in a physiological fibrotic response where an increased amount of extracellular matrix (ECM) is produced and secreted to stabilize and protect the structural integrity until skeletal muscle stem cells regenerate the tissue at which time the excess ECM is eliminated. However, during chronic diseases such as muscular dystrophy, or upon a volumetric muscle loss (VLC) the compensatory fibrosis persists, stiffening the matrix, reducing function, and regeneration capacity that results in a permanent muscle loss. Therefore, despite the etiology of the muscle disease, therapeutic approaches aiding in skeletal muscle regeneration requires the control of fibrosis. Until recently, the main limitation has been the inability to study and manipulate the activities of fibroblasts, the cell type that produce the ECM upon activation, in vivo given a lack of cell type-specific genetic tools. To this end, our laboratory recently generated a novel mouse model that permits lineage tracing of all activated fibroblasts in virtually all the tissues after injury or stress stimulation. We have previously shown that periostin positive cardiac fibroblasts are necessary to maintain heart function after myocardial infarction. As cardiomyocytes are non-regenerative after injury, here we utilized a similar approach to study the role and fate of fibroblasts during skeletal muscle regeneration. Activated fibroblasts were lineage traced with an allele where tamoxifen-inducible MerCreMer cDNA is knocked in to the periostin locus (PostnMCM), along with a Rosa26-eGFP dependent reporter. PostnMCM x R26-eGFP mice muscle were injured by either intra-muscular cardiotoxin injection or with a mouse model of muscular dystrophy, along with tamoxifen administration to trace all activated fibroblasts. Mice were then sacrificed at various time points to determine the fate of the eGFP+ cells. The data show that periostin positive fibroblasts are the source of ECM and ablation of these cells reduces fibrosis. We are expanding our study to conduct single-cell RNA sequencing analyses to determine critical cellular players and transcripts in skeletal muscle disease and will compare these results to our existing cardiac fibrosis dataset.

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Graduate Student

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Brain Microvascular Injury and White Matter Disease Provoked by Diabetes-Associated Hyperamylinemia

Introduction: Diffuse brain white matter disease is highly prevalent in type-2 diabetes and has been clinically associated with vascular contributions to cognitive impairment and dementia in humans. However, the underlying mechanisms are not clearly understood. Recent data, including our work, suggest that mechanisms of diabetic brain injury in humans involve cerebrovascular accumulation of aggregated amylin, an amyloidogenic hormone synthesized and co-secreted with insulin by pancreatic β-cells.

Hypothesis: Circulating aggregated amylin accumulates in brain blood vessel walls and microvessels leading to chronic ischemia and white matter lesions.

Methods: Because amylin from rodents is non-amyloidogenic, we used rats expressing human amylin in the pancreas rats to mechanistically decipher pathophysiological processes associated vascular accumulation of aggregated amylin. Wild-type littermate male rats expressing rat amylin served as controls. To identify initial tissue targets of amylin deposition, we generated amylin knockout rats which were injected with aggregated human amylin. We carried out in vivo phenotyping, biochemical analyses of human brain tissues and studied the effects of the aggregated amylin on endothelial cells ex vivo.

Results: Amylin deposition in brain blood vessels is associated with vessel wall disruption and abnormal surrounding neuropil in patients with type 2 diabetes and dementia, in HIP rats, and in AKO rats infused with aggregated amylin. HIP rats have brain microhemorrhages, white matter injury, and neurologic deficits. Vascular amylin deposition provokes loss of endothelial cell coverage and tight junctions. Intravenous infusion in AKO rats of human amylin, or combined human amylin and apolipoprotein E4, showed that amylin binds to plasma apolipoproteins. The intravenous infusion of apolipoprotein E4 exacerbated the brain accumulation of aggregated amylin and vascular pathology in HIP rats.

Conclusion: These data identify vascular amylin deposition as a trigger of brain endothelial dysfunction that is modulated by plasma apolipoproteins and represents a potential therapeutic target in diabetes-associated dementia and stroke.

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Postdoc

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Lipid Phosphate Phosphatase 3 in Smooth Muscle Cells Regulates Dissecting Abdominal Aortic Aneurysm Formation

Lipid phosphate phosphatase 3 (LPP3), encoded by PLPP3, is a cell surface enzyme that regulates lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) availability and signaling. Genome wide association studies in humans identified heritable single nucleotide polymorphisms (SNPs) in the final intron of PLPP3 that independently predicted coronary artery disease (odds ratio, 1.17; P=3.81×10–19). The risk allele reduces gene expression by disrupting binding of CCAAT enhancer binding protein beta (CEBPβ). These observations have focused attention on the role of LPP3 in atherosclerotic vascular disease. Global loss of Plpp3 or smooth muscle cell (SMC) specific inactivation of Plpp3 promotes the development of experimental atherosclerosis or intimal hyperplasia in mice. Together, these results indicate that LPP3, perhaps in part due to its ability to degrade LPA and thereby limit LPA signaling functions to mitigate SMC proliferation and vascular inflammation. At present, little is understood about what regulates Plpp3 expression during vascular disease. We observed 72hr angiotensin II (AngII) treatment is a strong activator of PLPP3 expression in coronary artery human SMCs (caHSMCs), through pathways that involve NF-κB, as parthenolide, an inhibitor of IκBα degradation, blocked ATII induced PLPP3 expression. To understand the functional consequences of AngII promoted LPP3 expression, we infused AngII into mice with SMC specific inactivation of Plpp3 (SM22-Δ) or littermate controls (fl/fl). SM22-Δ mice displayed a significant reduction in abdominal aortic diameter and smooth muscle alpha actin (Acta2) gene expression compared to fl/fl mice. Histone 3 lysine 4 dimethylation (H3K4dime) within the myosin heavy chain 11 (Myh11) gene, a stable epigenetic marker of SMC lineage, was not significantly changed. These results indicate that smooth muscle cells from SM22-Δ mice may undergo phenotypic modulation and in support of this hypothesis we observed increased vimentin, a fibroblast marker, expression within the SMC medial layer of abdominal aortas in SM22-Δ mice. Overall these results demonstrate a novel pathway for regulation of LPP3 during vascular disease involving a NF-κB dependent pathway and in the absence of LPP3, SMCs assume a more dedifferentiated fibroblast like phenotype that is associated with protection against abdominal aortic aneurysm.

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Staff

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Prediabetic Hypersecretion of Amylin Alters Oxygen Sensing and Accelerates Aging

Capillary function and oxygen-carrying capacity of red blood cells (RBCs) decline in type-2 diabetes exacerbating the risk of hypoxia and organ malfunction. Amylin is a β-celhormone that forms pancreatic amyloid in patients with type-2 diabetes and its blood level is elevated in prediabetes. Given the amyloidogenicity of human amylin, we hypothesized that hyperamylinemia increases the risk of hypoxia by provoking microcirculatory disturbances. Using rats with pancreatic overexpression of human amylin (HIP rats) and transfusion with RBCs from diabetic HIP rats into normal rats, we show that the transition from prediabetes to diabetes is associated with amylin deposition in capillaries and RBCs, which increases RBC to endothelial cell adherence, decreases RBC hemoglobin and activates hypoxia-inducible factors in endothelial cells leading to arginase-nitric oxide dysregulation. Prediabetes-induced amylin dyshomeostasis accelerates aging in HIP rats with multi-organ impairments and increased mortality. Upregulation of epoxyeicosatrienoic acids, which are lipid mediators formed by endothelial cells, mitigates amylin deposition in capillaries and hypoxia. In humans, amylin deposition in RBCs increases with aging in association with type-2 diabetes, heart failure, cancer and stroke. Thus, prediabetes-induced amylin dyshomeostasis impairs capillary function and oxygen-carrying capacity of RBCs; amylin-loaded RBCs can initiate pathological processes that are involved in pathological aging

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Graduate Student

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Fibrinogen Depletion Attenuates Angiotensin II-induced Abdominal Aortic Aneurysm

Background: Fibrinogen and fibrin provide physical and biochemical support to a developing clot and is defined as one of the most crucial independent risk factors for cardiovascular diseases (CVDs). In addition to clot formation, fibrinogen promotes wound healing and powerful inflammatory and immune responses by engagement of leukocytes. Increased circulating fibrinogen and fibrin degradation products are correlated with increased diameter and progression of abdominal aortic aneurysm (AAA). However, a causal link between fibrinogen and AAA has not yet been established. The objective of this study was to determine the role of fibrinogen depletion in a mouse model of AAA.

Methods and results: To determine whether aneurysm resulted in a procoagulant environment, we examined plasma levels of thrombin generation by calibrated automated thrombography (CAT), thrombin anti-thrombin (TAT), and fibrinogen in control and AAA plasma from mice and humans. Patients and mice with AAA had significant elevations in thrombin generation, TAT, and fibrinogen versus saline controls (mice) and control patients (human). To determine the effect of fibrinogen, in vivo, low density lipoprotein receptor deficient (Ldlr-/-) mice were injected with scrambled anti-sense oligonucleotide (ASO) or Î²-fibrinogen ASO (30 mg/kg) 3 weeks prior to experimentation and throughout the study. Fibrinogen ASO treatment achieved > 90% depletion of fibrinogen. After 3 weeks, mice were fed a fat and cholesterol enriched diet (42% milk fat; 0.2% cholesterol) 1 week prior to and throughout infusion with angiotensin II (AngII; 1,000 ng/kg/day) for 28 days. Fibrinogen ASO attenuated abdominal diameter (33% decrease; P = 0.001), and inflammatory cytokines (>75% decreased IL-1 and IL-6; P = 0.001) versus scrambled ASO control. Further, fibrinogen depletion significantly attenuated aneurysm incidence and rupture-induced death (P < 0.05). Fibrinogen is known to promote inflammatory response by activating and recruiting leukocytes. To investigate the contribution of this effect in abdominal aortic aneurysm, FibÎ³390-396A mice carrying a mutant form of fibrinogen incapable of binding leukocyte αMβ2-integrin were fed a fat- and cholesterol-enriched diet (42% milk fat; 0.2% cholesterol) 1 week prior to and throughout infusion with angiotensin II (AngII; 1,000 ng/kg/day) for 28 days. Mutant mice showed attenuated aortic size and aneurysm incidence (P = 0.036) compared to controls.

Conclusions: Our results demonstrate that AAAs augment procoagulant markers in both humans and mice. Importantly, fibrinogen depletion attenuates AAA incidence, diameter, rupture-induced death, and inflammation, and impairing the inflammatory capabilities of fibrinogen reduces aortic size and aneurysm incidence. Therefore, reduction of plasma fibrinogen may be a novel treatment strategy in patients with AAA.

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Postdoc

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Mitochondrial Ca2+ is reduced and Na+ is unaltered in cardiac myocytes from diabetic rats

Introduction: Mitochondrial Ca2+ ([Ca2+]m) regulates mitochondrial energetics, oxidative state, rate of oxidative phosphorylation and ATP level. A reduction in [Ca2+]m slows down neutralization of the H2O2 produced in mitochondria, causing oxidative stress, which in turn increases the sarcoplasmic reticulum (SR) Ca2+ leak. Movements of Na+ across the mitochondrial membrane affect matrix protons and [Ca2+]m.

Hypothesis: (1) As myocyte Na+ concentration ([Na+]i) is elevated in type-2 diabetes (T2D), we hypothesized that mitochondrial Na+/Ca2+ exchanger (mNCX) is accelerated, resulting in lower [Ca2+]m, higher oxidative stress and cytosolic Ca2+ dysregulation in T2D hearts. (2) Increased rate of mNCX results in elevated [Na+]m in T2D myocytes.

Methods/Results: Rats expressing human amylin in the pancreatic β-cells (HIP rats) were used as a model of late-onset T2D and their wild-type littermates served as controls. The mNCX activity, measured as the rate of mitochondrial Ca2+ efflux in saponin-permeabilized, Rhod-2 loaded myocytes, was increased in T2D compared to control. In addition, the rate of mitochondrial Ca2+ influx through the mitochondrial Ca2+ uniporter (MCU) was significantly smaller in myocytes from T2D rats vs. control. These data suggest that [Ca2+]m is lower in T2D myocytes compared to control. The amount of Reactive Oxygen Species (ROS), measured from DCFA fluorescence, was higher in T2D myocytes compared to control. Both CGP-37157, a selective antagonist of mNCX, and mitoTempo, a mitochondria-targeted antioxidant, significantly lowered ROS levels in myocytes from T2D rats. In contrast, neither drug had a significant effect on H2O2 production in control myocytes. To determine the contribution of mitochondria-originated ROS to SR Ca2+ leak, we measured the effect of MitoTempo and CGP-37157 on Ca2+ spark frequency in myocytes from T2D and control rats. Both drugs significantly reduced Ca2+ spark frequency in T2D myocytes, but had no effect in cells from control rats. mNCX exchanges mitochondrial Ca2+ with Na+. In another set of experiments [Na+]m was measured during mCa2+ influx and efflux using SBFI in permeabilized ventricular myocytes. [Na+]m was comparable in myocytes from control and T2D both at high and at low [Ca2+]m, despite enhanced mNCX activity in T2D cells. Moreover, [Na+]m increased during Ca2+-loading of the mitochondria, which suggests activation of mNCX and/or mitochondrial membrane permeability transition pore (mMPTP).

Conclusions: mNCX activity is enhanced while MCU function is reduced in myocytes from T2D rats, culminating in lower [Ca2+]m. This reduction in [Ca2+]m may contribute to the cardiac complications of T2D by producing oxidative stress and exacerbating the SR Ca2+ leak.

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Graduate Student

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Does shift work-like circadian disruption exacerbate atherosclerosis in ApolipoproteinE-deficient mice?

Circadian rhythms are 24-hour oscillations of almost every biological process in the body. The circadian system synchronizes internal rhythms of physiology and behavior with external environmental cycles such as the light-dark cycle. Shift workers, who experience chronic circadian disruption, have an increased risk of cardiovascular disease. However, the mechanisms linking shift work and cardiovascular disease are largely unknown. In this study, we are investigating the effects of shift work-like circadian disruption on atherosclerosis in ApolipoproteinE-deficient (ApoE-/-) mice. Male and female ApoE-/- mice are housed in control 12L:12D or shift work-like conditions for 12 weeks. In the shift work condition, the light-dark cycle is advanced by 6 hours every week. This protocol approximates the highly variable sleep/work schedules that are experienced by shift workers. Our preliminary results show that shift work disrupts the daily rhythm of eating behavior and alters the molecular timekeeping of the liver circadian clock. We are now investigating the effects of shift work on cholesterol, inflammation, and atherosclerosis.

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Staff

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A Qualitative Study of Patient Expectations as a Barrier to Guideline Recommended Care for Syncope

Background: Syncope is a complex presenting symptom that requires thoughtful and efficient evaluation to determine the etiology of a patient's loss of consciousness. Estimates indicate that one-half of all Americans will experience syncope during their lives, with recurrence rates as high as 13.5%. A common symptom, approximately 1% to 3% of all emergency department (ED) visits, as many ED visits as atrial fibrillation, and up to 6% of all hospital admissions were due to syncope. Notably, experiencing syncope affects patients' quality of life. Adopting a standardized approach to syncope care based on the Evaluation and Management of Syncope Guideline offers an effective opportunity for delivering high value care to patients suffering this distressing and potentially dangerous event, however implementing a standardized approach in the hospital setting has barriers that exist at several levels: patient, provider, and organizational. In this study, we seek to identify the patient-level barriers and develop strategies to mitigate them. Additionally, we provide patients' suggestions for a better overall care experience.

Methods: We conducted focus groups, phone interviews, and interviews with patients in the Emergency Department at UK's Chandler Medical Center. Seven patients and six caregivers attended focus groups during November 2018-January 2019. Five patients and two caregivers completed one-on-one phone interviews with a researcher during March-April 2019, and 11 patients completed one-on-one interviews with a researcher in the ED during May-June 2019. All interviews followed a semi-structured interview guide and were transcribed verbatim. Transcriptions were coded using Nvivo, a qualitative analysis software, and followed a rigorous team-coding approach.

Analysis: Two independent coders blindly coded one focus group and one individual interview with a pre-set codebook developed by the research team. The coders then discussed disagreements and made necessary revisions to the codebook. Coders then met with a qualitative analysis expert who coded transcripts using a grounded theory approach to identify themes without access to the codebook. All three coders then discussed differences and similarities, and synergistically created one cohesive codebook. The two original coders then co-coded each transcript (n=21).

Results: Several themes and patient-level barriers emerged in qualitative coding. Most patients were unaware of the term syncope and claimed their provider did not use or explain this term. Patients prefer more information before, during, and after tests are conducted. Several patient-level barriers to implementation were identified. Of note, patients' expectations do not align with guideline recommended practices and patients' preferences for an aggressive care approach (i.e. over-testing) may cause more confusion than clarity. The majority of patients were given inconsistent or inappropriate educational materials upon discharge, which caused additional confusion and barriers such as polypharmacy.

Implications: To address barriers at the patient-, provider-, and organizational-level, the Project MISSION team is developing a multi-level multicomponent implementation strategy. Our results indicate that barriers at the patient-level can be addressed through patient educational materials in print and video form. Educating patients on syncope, what type of care and level of information to expect, and the potential dangers of an aggressive care approach may mitigate barriers. The Project MISSION team is working to refine Syncope-specific KRAMES materials to better align with the guidelines, and are creating a patient education video to inform patients on recommended practices and expectations

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Postdoc

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Diabetes-Related Amylin Dyshomeostasis Impairs Amyloid-Î² Clearance by miRNA-Mediated LRP1 Downregulation in brain capillaries

The brain blood vessels of patients with type-2 diabetes and Alzheimer's disease (AD) have deposition of amylin, an amyloidogenic hormone co-secreted with insulin. Here, we tested the hypothesis that vascular amylin deposition contributes to dementia by impairing the clearance of amyloid-β (Aβ) from the brain. We found that the incubation of primary rat brain microvascular endothelial cells (RBMVECs) with aggregated human amylin downregulated the expression of low density lipoprotein receptor-related protein 1 (LRP1), a key mediator of the transport of Aβ across the blood-brain barrier (BBB). The interaction of aggregated amylin with RBMVECs upregulated microRNA (miR)-103 and miR-107 expression, which were shown to bind at 3′ UTR region of LRP1 downregulating the protein expression post-translationally. Indeed, we found that the transfection of miR-103 and miR-107 in RBMVECs downregulated LRP1 expression. In functional in-vitro BBB model studies, we found that aggregated amylin reduced the trans-endothelial electrical resistance and decreased Aβ clearance from the brain side to blood side. Consistently, in the brain capillaries of rats that overexpress human amylin in the pancreas, the miR-103 and miR-107 expression was upregulated, whereas the LRP1 expression was downregulated. The human amylin-expressing rats accumulated more Aβ in the brain compared to wild type rats that express non-amyloidogenic rat amylin. In conclusion, amylin dyshomeostasis exacerbates Aβ accumulation in the brain through impairing the LRP1 expression. The results could help to identify novel therapeutic targets to reduce the BBB impairment in AD.

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Undergraduate

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Protein S Coordinates Anticoagulant Activity within Protein and Plasma-Based Systems

Background

The balanced regulation of thrombin generation is critical to cardiovascular health. In the coagulation pathway, the prothrombinase complex, a procoagulant enzyme comprised of factor Va (FVa) and factor Xa (FXa), activates thrombin which is ultimately responsible for clot formation. This coagulation cascade can also be initiated by tissue factor (TF). Thrombin generation is regulated by a series of anticoagulant proteins including activated Protein C (APC) and tissue factor pathway inhibitor (TFPI), which inhibit FVa and FXa, respectively. Protein S (PS), a third anticoagulant protein, functions as a cofactor for both APC and TFPI, and thus appears to serve as a master regulator. While it remains unclear whether PS interactions with APC or TFPI are most significant to its function, PS is undoubtedly critical, as heterozygous PS deficiency is associated with a 5-10 fold increased risk of thrombosis, and homozygous PS deficiency is associated with life-threatening thrombosis in neonates. In addition to its role as a cofactor for TFPI and APC, PS is also a cofactor for C4bp, a protein complex involved in the complement system. Approximately 60% of plasma PS circulates bound to C4bp; and C4bp prevents PS from binding to APC, thus preventing PS anticoagulant activity. According to the literature, PS binds APC with the PS EGF1-2 domains, and PS binds TFPI with the PS LG1 domain. As a result, we hypothesize that the EGF1-2 domains will allow for the investigation of specific APC and TFPI functions of PS in purified protein and plasma-based systems.

Method & Results

The EGF1-2 domains of PS were cloned and expressed in E.Coli, and confirmed by DNA sequencing, SDS-PAGE, and western blotting. Based on coomassie blue staining, the purity was approximately 16%. The function of EGF1-2 was first evaluated in a purified protein assay, which measures thrombin generation by prothrombinase. In this assay, APC inhibited the maximal rate of thrombin activation by 19.62±0.01% in the absence of PS and by 34.96±0.02% in the presence of 50nM PS. EGF1-2 dose-dependently reversed the effect of PS, with a 75% reversal observed upon the addition of 200nM EGF1-2, highlighting competition between EGF1-2 and PS function. In the absence of PS, 200nM EGF1-2 had no effect in the assay. At the same concentrations, EGF1-2 does not impact PS cofactor activity with TFPI. The function of EGF1-2 was next analyzed in plasma, a more complex biological system. Interestingly, plasma thrombin generation assays revealed EGF1-2 having the opposite effect; EGF1-2 greatly inhibited TF-initiated thrombin generation in the presence and absence of thrombodulin, the cofactor for thrombin that decreases blood coagulation. From these results, we hypothesized that the EGF1-2 domains are part of the C4bp binding site, and the addition of EGF1-2 results in the release of PS from C4bp and an increase in the PS pool. In support of our hypothesis, we found that EGF1-2 had no effect on thrombin generation in PS-depleted plasma that is also depleted of C4bp. When PS-depleted plasma was supplemented with 150nM PS, EGF1-2 had the expected procoagulant activity, increasing thrombin production from 50.4±19.9 nM to 90.4±6.0 nM.

Conclusions

Our data suggests that the PS-APC system regulates thrombin generation by prothrombinase, and the EGF1-2 domains compete against PS for binding to APC and thereby block PS anticoagulant activity. Our data further suggests that EGF1-2 is part of the C4bp binding site; therefore, the addition of EGF1-2 results in the release of PS, and has an anticoagulant effect within plasma-based systems.

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Postdoc

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CSF Amylin – Effect Modifier of the Aβ-AD Relationship

Background: Accumulating evidence from several laboratories indicates that patients with Alzheimer's disease (AD) have cerebral mixed deposits formed by β amyloid (Aβ) and amylin, a pancreatic hormone that crosses the blood-brain barrier and has amyloidogenic properties. In contrast to amylin from humans, rodents have non-amyloidgenic amylin, which does not accumulate in the brain or other tissues. Here, we speculated the difference in amyloidogenicity between human and rodent amylin species to test the hypothesis that chronically elevated levels of amylin in cerebrospinal fluid (CSF) promote mixed amylin-Aβ pathology worsening the behavior changes in a rat model of mixed amylin-Aβ pathology.

Methods: For studying amylin-Aβ pathology, we crossed rats expressing human amylin in the pancreas (HIP) rats with AD rats to generate ADHIP rats. Littermate AD and wild-type (WT) rats expressing the non-amyloidogenic rat amylin served as negative controls for brain amylin deposition. Behavior was tested in ADHIP, AD and WT littermate rats at 8 months of age (when all rats have normal behavior), at 12 months of age (when HIP and ADHIP rats develop amylin pathology) and at 16 months of age, by the Novel Object Recognition (NOR) and Morris water maze (MWM) tasks. Amylin-Aβ interaction in CSF was assessed by immunoprecipitation of amylin (1 ml CSF/rat; n=4 rats/group) followed by ELISA for amylin. The formation of mixed amylin-Aβ pathology in the brain was tested by immunohistochemistry.

Results: In ADHIP rats, behavior changes have developed at ~12 months of age, which was four months earlier than in AD littermate rats. Brain dysfunction in ADHIP rats correlated with elevated blood levels of aggregated amylin. The lower performance in ADHIP rats compared with age-matched rats in the other groups correlated with the development of mixed Aβ-amylin oligomers in CSF and mixed Aβ-amylin plaques in the brains of ADHIP rats.

Conclusions: Finding mixed amylin-Aβ oligomers in ADHIP rats indicates that CSF amylin level is effect modifier of the Aβ-AD relationship. The formation of "mixed" amylin-Aβ oligomers in vivo is consistent with in vitro and in silico studies showing that amylin-Aβ interaction can promote robust growth of mixed amylin-Aβ amyloids.

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Graduate Student

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Antiretroviral Therapy Does Not Correct Plasma PS Deficiency and Increased Thrombin Generation Associated with HIV

Background: Human Immunodeficiency Virus (HIV) infection is associated with an increased risk of thrombosis, and treatment with antiretroviral therapy (ART) does not decrease this risk. While the mechanism leading to thrombosis is unclear, HIV infection is associated with decreased plasma anticoagulant proteins, most commonly protein S (PS), and expression of tissue factor (TF) on monocytes. Despite the prevalence of PS deficiency, its pathologic consequences are unclear because PS concentration does not correlate with thrombin generation in the standard calibrated automated thrombography (CAT) assay. As increased plasma thrombin generation is a strong predictor of mortality in these patients, we sought to develop an assay that is sensitive to PS concentration and to determine which of the described procoagulant effects of HIV is associated with thrombin generation in this population.
Method: Citrated plasma was collected from 27 consenting HIV+ patients (16 naive samples collected from patients on first diagnosis and 11 samples from patients on ART) and 10 healthy controls.
Results: 63% (17/27) of HIV+ plasma samples were deficient in PS, compared to the controls, as determined by a total PS ELISA ($p=0.039$). PS is a cofactor for the anticoagulant enzyme activated protein C (APC), whose activation requires endothelial cell thrombomodulin (TM), which is not present in plasma. Thus, we established a modified CAT assay that is sensitive to PS concentration, by supplementing plasma samples with TM. In the standard CAT assay (citrated plasma incubated with TF and phospholipids), supplementing PS-deficient plasma with 150nM purified PS resulted in only a 12% decrease in total thrombin produced. However, if 20nM TM was included in the assay, PS supplementation resulted in a dose dependent decrease in thrombin generation, with a 75% decrease observed with 150nM PS.
In patient samples, plasma PS did not correlate with thrombin generation in the absence of TM, consistent with previous reports. In the presence of TM, there was a negative correlation, with decreased PS being associated with increased thrombin peak height ($p=0.0239$) and endogenous thrombin potential ($p=0.011$). Since 2.5% of total circulating PS is stored in platelets and released upon stimulation, we quantified platelet PS by immunoblotting, but found no correlation with plasma PS concentration or thrombin generation.
HIV is also reported to promote TF expression in monocytes, and monocytes can release TF-bearing microvesicles into plasma. Thus, we measured microvesicle TF, but found no difference between patients and controls. Microvesicle TF correlated with a shorter lag time for thrombin generation, but only when no exogenous TF was added ($p=0.0097$).
Treatment with ART resulted in undetectable viral load and decreased plasma markers of inflammation to the levels observed in controls. However, there was no effect of ART on plasma PS ($p=0.521$), platelet PS ($p=0.397$), or microvesicle TF ($p=0.712$). Unexpectedly, ART use did correlate with greater thrombin generation in the absence of TM, and thus the absence of PS activity (19% higher total thrombin, $p=0.0273$), suggesting a PS-independent procoagulant effect of ART.
Conclusion: PS deficiency in HIV patients correlates with higher thrombin generation when measured using an appropriate assay, and likely contributes to the thrombotic risk associated with this condition, while microvesicle TF is unlikely to be a contributing factor. ART treatment decreases viral load and inflammation, but does not restore plasma PS concentration and is not associated with any decrease in thrombin generation. Rather, one or more of the ART drugs appear to further increase thrombin generation through a PS-independent mechanism.

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Undergraduate

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The role of HuR in the transdifferentiation of fibroblasts and smooth muscle cells in atherosclerotic monkeys

Background: The onset of atherosclerosis in the coronary artery is initiated by monocyte infiltration, followed by an increase in the number of macrophages. In recent years, it has been discovered that vascular smooth muscle cells play an integral role in plaque formation. Most notably, they are thought to transdifferentiate into lipid engulfing macrophages known as foam cells. These differentiated cells proliferate and migrate, aiding in lesion formation within the artery. The role of smooth muscle cells in transdifferentiation is known, however the role of fibroblasts is thus far limited.

Aims: The purpose of this study was to determine the role of Human Antigen R (HuR) in fibroblasts migration and transdifferentiation into a macrophage like cell.

Methods and Results: Ten male African Green Vervet monkeys (Chlorocebus aethiops) were fed a fat and cholesterol enriched diet (37%, 0.3 mg/kcal cholesterol) for a period of 20 months, at which time a subset (n = 5) was euthanized and left anterior descending (LAD) coronary arteries were characterized (n = 5). The remaining monkeys (n = 5) were switched to a standard chow diet for 6 months, when LADs where characterized. Examination of the smooth muscle media in atherosclerotic monkey LAD coronary arteries shows strong colocalization between macrophage-like cells and activated fibroblasts. Human antigen R (HuR), an RNA binding protein involved in the transdifferentiation phenotype, is shown to localize to actin negative cells and activated fibroblasts. Further experimentation using a wound closure assay showed HuR inhibition prevented oxidized LDL-induced fibroblast migration; suggesting a role for HuR in fibroblast migration and conversion to macrophages. Myh11CreERT2 x Rosa26tdTomato/tdTomato x Apoe-/- VSMC lineage-tracing mice aortas were stained with HuR which showed strong colocalization between HuR and Myh11.

Conclusions: Our current work shows that HuR localizes to activated fibroblasts and macrophages in advanced monkey atherosclerosis while localizing to VSMCs in advanced mouse lesions. Future work will examine whether fibroblasts can transition to macrophage-like cells.

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Undergraduate

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Dietary Regulation of Lipoprotein Associated Lysophospholipid Mediators of Atherosclerosis

Lysophosphatidic acid (LPA) is a bioactive phospholipid that has been linked to atherosclerosis through studies with preclinical models and is associated with heritable cardiovascular disease risk in humans. LPA is formed by the cleavage of choline from lysophosphatidylcholine by the enzyme autotaxin (ATX). LPA accumulates in atheromas and can be visualized by mass spectrometry imaging in the lipid rich core suggesting the hypothesis that LPA associates with atherogenic low density lipoproteins. However, although LPA has been shown to be associated with low-density lipoprotein (LDL), nothing is known about the source, regulation and biological activity of LDL-associated LPA. We have used mouse models and mass spectrometry methods to examine effects of diet and genetically induced hyperlipidemia on circulating levels and distribution of LPA in plasma. While more than 90% of plasma LPA was associated with serum albumin in the blood of wild type mice fed either normal chow or a western diet we observed a 3-4 fold increase in LPA pools associated with low density (LDL and VLDL) pools in hyperlipidemic mouse models (LDLr-/- and PCSK9 treated C57Bl6) fed a western diet. Mass spectrometry analysis revealed diet dependent differences in the molecular species composition of these LDL and albumin associated LPA pools. These increases in LDL associated LPA were not observed in mice with genetic deficiency of autotaxin which is a secreted lysophospholipase D that is well established to be responsible for generation of plasma LPA in mice and humans. In human plasma, LPA was detected in LDL and LDL-LPA trends with plasma cholesterol. Interestingly, recombinant ATX was found to act on isolated LDL as a substrate. Taken together, these data support the hypothesis that LDL associated LPA could contribute to cardiovascular disease processes including atherosclerosis.

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Undergraduate

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Adipose Specific Deletion of HuR Leads to Cardiac Hypertrophy and Fibrosis

Introduction: Obesity is a heterogeneous metabolic disease characterized by an excessive accumulation of body fat, with more energy being taken in than is burned. Obesity is associated with several chronic diseases such as cardiovascular disease, diabetes, and cancer. Recent studies have suggested that adipose tissue may have a direct effect on multiple other organ systems via secreted factors. Human antigen R (HuR) is an RNA binding protein widely expressed throughout the body, including in both WAT and BAT, and has been shown to regulate many pathways involved in cellular metabolism, inflammation, and adipocyte differentiation. We generated mice with an adipocyte-specific HuR deletion (Adipo-HuR-/-) and examined markers of cardiac hypertrophy and fibrosis.

Conclusion: Deletion of HuR in adipocytes disrupts cardiac homeostasis and results in cardiac hypertrophy and fibrosis. Adipo-HuR-/-mice have increased levels of markers for fibrosis and hypertrophy in the heart.

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Staff

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Differential roles of tissue factor and protease-activated receptor 2 in the pathogenesis of abdominal aortic aneurysm

Background: Constitutively expressed in vascular smooth muscle cells (VSMCs), tissue factor (TF) is critical for the maintenance of hemostasis and serves as an activator of protease-activator 2 (PAR2) when complexed with factor VIIa (FVIIa) or FXa. TF expression is further increased in medial VSMCS in response to vascular injury in vivo and following angiotensin II (AngII) incubation in vitro. We and others have shown that TF/FVIIa-PAR-2 signaling induces proliferation and migration of VSMCs. Importantly, VSMC proliferation and migration is critical to the formation of abdominal aortic aneurysm (AAA). However, the importance of either TF or PAR2 in the etiology of AAA has not been examined. This study examines whether TF or PAR2 are associated with AAA pathogenesis using human samples and mouse models.

Methods and Results: Using histological analysis, TF and PAR2 are both highly expressed in human sections of AAAs localized in the tunica media. Microvessicle (MV) TF was significantly elevated in aneurysm patients (n = 250) versus age-matched non-aneurysm controls (n = 115) and was correlated with increased growth and diameter of the aorta. To examine the effects of TF, in vivo, we utilized an AngII infusion model of AAA. Male TF+/+ (n=15) or low TF (n=28; 1% of normal TF levels) mice, on a C57BL/6J background, were infused with AngII (1,000 ng/kg/min) for 28 days. Interestingly, low TF mice had increased luminal diameters of the suprarenal aortic region (+/+: 0.94 Â± 0.05; low TF: 1.58 Â± 0.09 mm; P=0.002), resulting in a 92% incidence of AAA compared to a 20% incidence in TF+/+ mice (P<0.03). Utilizing male VSMC-specific TF deficient mice on a low-density lipoprotein receptor deficient (Ldlr-/-) background, we determined this effect was VSMC-dependent (Cre+: 1.92 Â± 0.16 (n = 12); Cre-: 1.22 Â± 0.03 mm (n = 10); P = 0.001). Next, we determined the effects of PAR-2 deficiency in AAA. Ldlr-/-/Par2+/+ (n = 12) and Ldlr-/-/Par2-/- (n = 15) male mice were fed a fat-enriched diet and infused with AngII for 28 days. PAR2 deficiency attenuated abdominal aortic luminal diameters (Par2+/+: 1.78 Â± 0.06 mm; Par2-/-: 1.17 Â± 0.03 mm; P = 0.001) and incidence of AAA (84% versus 40%; P = 0.048) when compared to littermate controls. Utilizing bone marrow transplantation, we determine these effects were reliant upon hematopoietic cells. Total plasma cholesterol concentrations, lipoprotein cholesterol distributions, or increased systolic blood pressure was not different between groups.

Conclusion: These results suggest that TF limits AAA growth and incidence via VSMCs in a PAR-2-independent manner. To verify this hypothesis, studies are currently underway to examine Factor Xa (FXa) inhibitors in PAR2 deficient mice.

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Graduate Student

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Autotaxin Activity Predicts 30 Day Mortality in Sepsis Patients and Correlates with Platelet Count and Vascular Dysfunction

Objectives: We investigated whether platelet count was associated with biomarkers of endothelial function. Additionally, we aimed to identify novel predictors of outcomes in a cohort of patients with severe sepsis at a quaternary care academic medical center.

Design: Prospective, observational cohort

Patients: 86 sepsis patients admitted into intensive care units were prospectively enrolled into an on-site sepsis registry and biobank.

Measurements and Main Results: Platelet count, plasma angiopoietin-1 and angiopoietin-2 concentrations, and plasma autotaxin activity were determined for each patient at enrollment. Patient mortality was recorded up to 30 days following hospital discharge. Platelet count was significantly lower in patients that did not survive up to 30 days following hospital discharge. Angiopoietin-2 and the angiopoietin-2/1 ratio were significantly higher in patients that did not survive up to 30 days following discharge. Furthermore, plasma autotaxin activity was significantly higher in patients that did not survive up to 30 days. Interestingly, autotaxin activity correlated with platelet count and the ratio of angiopoietin-2/1 across our population.

Conclusions: Platelet count, the ratio of angiopoietin-2/1, and autotaxin activity all predicted 30 day mortality. Autotaxin activity within the plasma correlates with both platelet counts and vascular dysfunction biomarkers across both survivors and non-survivors indicating a possible involvement of autotaxin within sepsis.

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Postdoc

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Paracrine signaling from activated cardiomyocytes mediates cardioprotection after myocardial infarction

Background: Myocardial infarction (MI) is a major contributor to cardiac-associated death via large-scale cardiomyocyte (CM) loss and subsequent pathological remodeling in the compensating heart. Unlike zebrafish and neonatal mice, which have the ability to repair a cardiac injury, adult mammals lack this ability due to the non-proliferative nature of adult CMs. Recent studies have demonstrated the possibility of inducing cell cycle re-entry of adult CM by regulating the expression of senescence-inducing genes. Though most of these studies observed a limited induction of CM proliferation, they still showed improved cardioprotection, which suggests that there is an unknown mechanism that improves the heart function regardless of CM proliferation such as paracrine signaling. In the present study, we knock down two senescent-associated genes Rb1 and Meis2 to induce CM proliferation and determine alternative cardioprotective factors after MI. Methods and results: In vitro experiments were performed with adult CMs, isolated from 12 weeks old Fisher rats. We used 50nM each of siRb1 and siMeis2, designated as siRNA-cocktail, to knockdown Rb1 and Meis2, respectively. Results showed a significant increase in the adult CM cell number, a decrease in cell size, and an increase in mono-nucleated cells in the siRNA-cocktail treated group. Likewise, the immune-fluorescent analysis revealed a significant increase in proliferative markers EdU, PH3, KI67, and Aurora B in adult CMs receiving siRNA-cocktail. Finally, expression analysis for Rb1, Meis2, Cyclin D1, β-catenin, IL6, and p16 complimented the proliferative state of CMs. Subsequently, we performed siRNA mediated silencing of Rb1 and Meis2 in vivo by an intramyocardial delivery method utilizing a nanocomposite biocompatible hydrogel. Here, adult rats were subjected to a permanent LAD ligation surgery to create cardiac injury which was followed by simultaneous inhibition of Rb1 and Meis2. Results of this experiment showed that in knockout rats, the heart function was improved and the infarct size was reduced after injury. Interestingly despite the presence of CMs in cell cycle progression, the biggest physiological outcome was the increase in the vascularization and enhanced CM survivability in the peri-infarct area. Further in vitro interrogation of the cross-talk between CMs and endothelial cells identified a paracrine signaling from activated CM as the source of the cardioprotection after knocking down Rb1 and Meis2. Conclusion: Our study demonstrates a novel cardioprotective mechanism in induce adult CM cell cycle reentry to combat ischemic injury, revealing new avenues of therapeutics.

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Graduate Student

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Understanding the role of platelet bioenergetics in hemostasis and thrombosis

Platelets are abundant, anucleate cell fragments circulating in the bloodstream. They play a crucial role in maintaining vascular integrity and regulate hemostasis in response to vascular damage. Platelet activation initiates several processes including integrin activation, adhesion, shape change, aggregation, granule secretion, clot formation, and retraction. Each process appears to have different energy requirements, yet it is unclear which metabolic fuels and what energy-producing pathways are needed. Ravi et al. showed that platelets have metabolic plasticity (flexibility) and can switch between glycolysis and oxidative phosphorylation (OxPhos). However, the significance of this flexibility is uncharacterized. Recent literature indicates that platelet bioenergetics is altered in several diseases, including Alzheimer's, Parkinson's, traumatic brain injury, sepsis, and diabetes mellitus. While these alterations are being explored as disease biomarkers, it is unclear if metabolic dysfunction contributes to increased thrombotic risks associated with these diseases. Hyperactive platelets play a prominent role in thrombosis during myocardial infarction and stroke, which are major causes of death worldwide, while hypoactive platelets lead to pathologic bleeding. Thus, platelet reactivity contributes to hemostatic balance. Our overarching hypothesis in this proposal is that the metabolic flexibility of platelets is critical for their responses and function. Our preliminary results show that the energy dependence of clot retraction is greater than that for granule secretion or aggregation, making it the most sensitive process to platelet energy state. To measure the effects of altered platelet bioenergetics on their responses and functions, we developed an improved method to track clot formation and retraction with automated image acquisition, processing, and quantification (Prakhya et al. manuscript in preparation). We have shown that this new method can detect subtle alterations in clot formation and retraction kinetics at a superior temporal resolution, as compared to previous methods. With this tool, we will address which fuels and energy-producing pathways are needed/used to mediate the energy-intensive clot retraction process during platelet activation.

To further address the roles of bioenergetics in platelet function, we have generated a mouse strain with a lineage-specific deletion of transcription factor A, mitochondrial (TFAM), which is a transcription factor required for mitochondrial gene transcription and maintenance of the mitochondrial genome integrity. Although there are some in vivo models developed to probe the importance of glucose for platelet bioenergetics, e.g., the GLUT1/3-/- mice, there is no in vivo model designed to understand how mitochondria affect platelet functions. We hypothesize that deleting TFAM in a platelet-specific manner will disrupt the transcription of mitochondrial genes and thus affect the protein complexes required for OxPhos. This would disrupt energy metabolism in platelets. The purpose of our in vivo model is to explore the relationship between platelet mitochondrial bioenergetics and hemostasis. Furthermore, this model will be invaluable for future studies that address how mitochondrial dysfunction contributes to thrombosis in metabolic disorders like diabetes mellitus.

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Graduate Student

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HDL/SR-BI, not LDL/LDLr, mediates stress-induced glucocorticoid production

Objectives-

High stress conditions activate the hypothalamic pituitary adrenal axis and the adrenals secrete stress-induced glucocorticoids (iGC) as the physiological response to cope with stress. Previous study showed scavenger receptor class B type I (SR-BI) knock out mice failed to generate iGC in stress conditions, suggesting that SR-BI-mediated cholesterol uptake is a key regulator for iGC production in stress conditions. However, low density lipoprotein /low density lipoprotein receptor (LDL/LDLr) pathway can also provide cholesterol for iGC synthesis, and rodents have limited LDL levels in circulation. Therefore, the relative contribution of LDL/LDLr in iGC production remains be clarified. Here, we generated humanized SRBI-/-|ApoBtg mice with normal LDL levels in circulation to determine the relative contribution of HDL/SR-BI and LDL/LDLr to iGC production in stress conditions.

Methods and Results-

To obtain mice models with normal LDL levels in circulation, SRBI-/- mice were bred to ApoB transgenic (ApoBtg) mice, then the F1 SRBI+/-|ApoBtg mice were backcrossed to SRBI-/- to obtain the F2 SRBI-/-|ApoBtg and SRBI-/-|ApoBwt mice. We first examined the lipid profile in SRBI-/-|ApoBtg mice. The LDL levels were increased in the SRBI-/-|ApoBtg mice (5.7 folds increase in the LDL fraction of FPLC profile). There were no difference in the total and free cholesterol levels between SRBI-/-|ApoBtg mice and its littermate (SRBI-/-|ApoBwt mice). Thus, we successfully generated a mouse model with a human-like lipid profile. Then, we induced stress with adrenocorticotropic hormone (ACTH) and cecal ligation puncture (CLP) to evaluate iGC production. One hour after ACTH stimulation, both SRBI-/-|ApoBwt and SRBI-/-|ApoBtg showed no iGC production, while ApoBtg control mice produced iGC (15 folds)($p < 0.001$). Three hours after CLP treatment both SRBI-/-|ApoBwt and SRBI-/-|ApoBtg mice showed no iGC production, while ApoBtg control mice showed iGC production. In addition, we did not observe significant changes in the expression of genes involved in steroidogenesis.

Conclusion-

We generated SRBI-/-|ApoBtg mice with a human-like lipid profile but SRBI-/-|ApoBtg mice failed to produce iGC in stress conditions. These findings clarify that HDL/SR-BI, not LDL/LDLr, pathway is responsible for iGC production in stress conditions.

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Postdoc

Podium Presentation

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Loss of Endothelial Cell-Derived Lipid Phosphate Phosphatase 3 Exacerbates Calcification of Aortic Valves

Calcific Aortic Valve Disease (CAVD) is marked by calcium deposits within the aortic valve leaflets that results in aortic stenosis. CAVD has an 80% associated risk over 5 years of progression for heart failure, aortic valve replacement, or death. There are currently no effective treatment in preventing CAVD, and the incidence in individuals over the age of 65 has increased by 30% in the last 20 years. Modifiable risk factors such as diet and physical activity may be controlled to prevent disease; however, the pathophysiological development of CAVD is still incompletely understood. Autotaxin (ATX), the primary enzyme underlying synthesis of lysophosphatidic acid (LPA), has recently been detected in mineralized human aortic valves and shown to promote calcification and inflammation in human valve interstitial cells. Studies have suggested that ATX's association with human lipoprotein (a), a polymorphic lipoprotein linked to apoB by a disulfide bridge, may be important in mediating disease progression. Conversely, the expression and activity of LPP3, a phospholipid phosphatase involved in the degradation of LPA, was recently reported to be downregulated in CAVD. Although ATX and LPP3 have been identified as novel contributors to CAVD, how LPA regulation affects the disease progression remains poorly understood. Here we show that LPP3 is expressed in endothelial cells of the valve leaflet, and mice with endothelial cell specific knockdown of LPP3 exhibit a 1-2 fold increase in leaflet calcification. To determine the role of ATX, we quantified calcification of the valves from mice with a global or adipose-specific reduction in ATX. Our data indicate that global reduction in ATX enhanced calcification by 3 fold while reduction in adipose-derived ATX had no effect on early stage valve calcification. Our findings indicate that endothelial cell derived LPP3 regulates disease progression and that finite shifts in LPA regulation may be a critical factor in CAVD development. Furthermore, we potentially identified a novel separation between the role of adipose-derived ATX and other tissue sources of ATX in regulating valve calcification.

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Postdoc

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A Novel Model Cardiac Regeneration in Adult mammals

Background:

It is known that following cardiac injury in adult mammals, the contractile cells making up the heart, namely cardiomyocytes, are permanently lost and replaced by fibrotic scar tissue, severely affecting the heart contractile function and physiology. Acomys can shed up to 60% of its skin to avoid predators with remarkable ability to regenerate without scarring. Here, we investigated if this remarkable phenomenon of wound repair extends into the heart post myocardial infarction.

Method and Results:

We performed MI surgery on adult Acomys and Mus and evaluated mortality, cardiac function, infarct size, and macrophage polarization. There was a trend towards enhanced survival in Acomys and none of these mice experienced cardiac rupture. On the other hand, 100% of the mortality in Mus was due to cardiac rupture and occurred early. We observed significantly smaller infarct size in Acomys compared to Mus. Additionally, cardiac functional recovery and remodeling parameters stabilized after initial decline in Acomys but continued to deteriorate in Mus up to 50 days after MI. We evaluated macrophage response to investigate the mechanism of decreased infract size in Acomys after MI. Iba1+/CD206+ (anti-inflammatory macrophages) were higher and Iba1+/IL-1β+ (pro-inflammatory) macrophages were lower in Acomys compared to Mus in the first week after MI. The shift in macrophage balance coincided with enhanced angiogenesis in the peri-infarct region in Acomys.

Conclusions:

Our studies provide evidence that adverse remodeling is halted in Acomys after MI and this correlated with a shift towards reparatory M2-like macrophage and enhanced angiogenesis. This is the first mammalian model capable of innate cardiac regeneration and we are studying the mechanisms responsible for this phenomenon.

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Graduate Student

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Dietary choline contributes to abdominal aortic aneurysm (AAA) in a gut microbe-dependent manner

Introduction: The gut microbiota is a metabolically active, endocrine-like organ which contributes to cardiovascular health and disease. Carbon fuel sources from the diet (phosphatidylcholine, choline, L-carnitine) are metabolized by the gut microbiota to the waste product trimethylamine (TMA) which travels in the circulation to the liver and is rapidly oxidized to the molecule trimethylamine N-oxide (TMAO) by hepatic flavin-containing monooxygenases (FMOs). High circulating levels of TMAO have been associated with a variety of cardiometabolic diseases, including atherosclerosis, heart failure, and chronic kidney disease, however the role of the gut microbiota and specifically TMAO have never been explored in the context of abdominal aortic aneurysm (AAA). The purpose of this study was to investigate the role of the gut microbiota and TMAO in AAA.

Methods: Plasma samples from human AAA patients and controls were analyzed for plasma TMAO. To investigate the role of the gut microbiota in AAA, vehicle water or water containing antibiotics was provided to Ldlr-/- male mice. After one week, mice were fed either a control diet or a choline diet, each with 0.2% cholesterol. After one week of feeding, angiotensin II (AngII) was infused for 4 weeks. Aortas and plasma were evaluated for AAA disease and gut microbial metabolites TMA and TMAO, respectively. To investigate the role of the microbial enzyme CutC/D in AAA, vehicle water or water containing the CutC/D inhibitor CC199 was provided to Ldlr-/- male mice fed either a control diet or a choline diet. AngII was infused for 4 weeks and samples were processed for AAA disease and microbial metabolites. RNA sequencing (RNA-seq) was performed on the suprarenal abdominal aortas of control and choline fed Ldlr-/- mice infused with AngII for 3 days.

Results: Plasma TMAO was significantly elevated in a step-wise fashion with the rate of aneurysm growth in AAA patients versus control patients ($P < 0.001$). Plasma levels of TMA and TMAO were significantly elevated in choline-fed versus control-fed Ldlr-/- male mice ($P < 0.001$). Administration of broad-spectrum antibiotics significantly decreased abdominal aortic diameter ($P < 0.05$) and AAA incidence ($P < 0.05$) in choline-fed Ldlr-/- male mice. Similarly, administration of the CutC/D inhibitor CC199 significantly decreased abdominal aortic diameter ($P < 0.05$) and AAA incidence ($P < 0.05$) in choline-fed Ldlr-/- male mice. RNA-seq gene ontology clustering and enrichment analysis of 85 choline-specific transcripts shows that choline feeding with AngII infusion augments the expression of apoptotic process and attenuates autophagy and autophagosome assembly in 28 choline-specific downregulated genes. Importantly, Protein kinase R (PKR)-like endoplasmic reticulum kinase (Perk) was increased by 256-fold.

Conclusions: Increases in circulating TMAO augment the growth of aneurysms in human AAA patients as well as aortic diameter and AAA incidence in Ldlr-/- male mice in a gut microbe-dependent manner. RNA-seq demonstrates these effects are potentially due to increased apoptosis and decreased autophagy. Importantly, PERK has recently been found to be the putative receptor for TMAO.

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Postdoc

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Role of the Proteoglycan, Serglycin, in Platelets α-Granule Biogenesis

Occlusive cardiovascular diseases (e.g., strokes and myocardial infarctions, MI), a major cause of death and disabilities, are influenced by platelet secretion. Upon injury to a blood vessel, platelets activate and secrete cargo stored in membranous compartments, called granules. While secretion is needed for normal clotting, excessive release leads to occlusive thrombosis. Serglycin is an intra-granular, chondroitin sulfate proteoglycan produced by hematopoietic cells, including platelets. It was hypothesized that its negative charges are crucial for the storage of platelet granule proteins. Previous studies (Woulfe, DS et al, 2008) showed that Serglycin knockout (KO) mice exhibit a bleeding phenotype, defective aggregation, and α-granule packaging defects. These animals have been reexamined and showed that Serglycin KO platelets have α- granule secretion defects. Consistent with previous data, there is a defect in platelet factor 4 (PF4) levels but not in the α-granule membrane protein, P-Selectin. Antibody array analysis shows that physicochemical properties influence α-granule cargo packaging. Release of serotonin from dense granules and β-hexosaminidase from lysosomes were unaffected suggesting that Serglycin is only important for α-granules. Ultrastructural studies, by electron microscopy, show random and disperse α-granule cargo distribution in Serglycin KO platelets, while the wild-type platelets have a fibrous granular content. In sum, these studies are illuminating the physiological function of Serglycin in platelets and defining its role in granule cargo packaging and release.

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Undergraduate

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MicroRNA-33a/b Inhibition Attenuates Tissue Factor Activity and the Activation of Coagulation in Non-human Primates

Objective: Hypercholesterolemia is associated with increased cardiovascular morbidity and mortality via progression of atherosclerotic disease. Dyslipidemia is also associated with a prothrombotic state due to increases in the procoagulant protein tissue factor (TF), which is highly expressed in atherosclerotic plaques. We recently demonstrated that acute hypercholesterolemia can induce the activation of coagulation with time-dependent increases in monocyte TF activity and microvesicle (MV) TF activity in the circulation of both mice and monkeys. Our objective for the current study was to determine the effect of chronic dyslipidemia and subsequent correction of hypercholesterolemia with either standard chow or antagonism of the lipid metabolism regulating microRNA-33 (miR-33) on the activation of coagulation in non-human primates (NHPs).

Methods and Results: Thirty six male African Green Vervet monkeys (Chlorocebus aethiops) were fed a fat and cholesterol enriched diet (37%, 0.3 mg/kcal cholesterol) for a period of 20 months, at which time a subset was euthanized and atherosclerotic development characterized (n = 12). The remaining monkeys were switched to a standard chow diet and treated for a further 6 months with either saline (n = 12) or the miR-33a/b antagonist RG428651 (anti-miR-33a/b; n = 12). Blood was collected and citrated plasma processed at day 0 (baseline), 8 months, 20 months (peak experimental) as well as 22 and 26 months (intervention). Total plasma cholesterol was significantly elevated with 20 months of cholesterol enriched diet (491% increase compared to baseline; $P < 0.001$). Circulating MVs, MV TF, monocyte TF activity, thrombin antithrombin (TAT), and D-dimer were significantly augmented at 20 months compared to baseline ($P < 0.05$). After 6 months of intervention, vehicle saline and anti-miR-33a/b attenuated plasma cholesterol by 84% and 79% respectively and approached baseline cholesterol measurements ($P < 0.001$). Interestingly, anti-miR33 treatment significantly reduced all parameters past the original baseline control (day 0). Finally, anti-miR-33a/b also significantly decreased MV TF and TAT versus the chow regression diet ($P < 0.05$).

Conclusions: Our results demonstrate that chronic hypercholesterolemia augments circulating plasma MVs, plasma MV TF activity, monocyte TF activity, and coagulation variables (TAT and D-dimer) in NHPs. The prothrombotic state induced by hypercholesterolemia was attenuated by chow feeding with additional significant benefit derived from the miR-33a/b antagonist RG428651. Future studies will examine whether the benefit of anti-miR-33a/b is derived from decreased oxidized LDL activation of the CD36/TLR4/TLR6 heterotrimeric complex, increased plasma HDL, or decreased surface expression of phosphatidylserine in macrophages.

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Undergraduate

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Analysis of QT-RR Relation and Phenotypic Masking in a Mouse Model of Long QT Syndrome

Background: Sudden cardiac death (SCD) claims nearly 350,000 lives per year in the United States. Arrhythmia syndromes, in which the conduction system in the heart does not function normally, contribute to the incidence of SCD. Arrhythmia syndromes are diagnosed by an electrocardiogram (ECG) test that records the heart's electrical activity. ECG markers of interest include the RR- and QT-intervals. RR-I is the electrophysiological equivalent of heart rate: a long RR-I correlates with a slow heart rate. QT-I measures the ventricular repolarization time. QT increases as RR increases, so clinicians use a formula (typically Bazett's) to standardize the measurement (QTc). Congenital long QT syndrome (LQTS) may be diagnosed from an abnormally long QTc. LQTS type 3 (LQT3) is characterized by genetic mutations in sodium channels that prevent them from inactivating and cause current to "leak" out, lengthening the repolarization time. There are many mutations that cause LQT3, but our lab focuses on a three-amino-acid (KPQ) deletion in the gene SCN5A that causes autosomal dominant LQT3. Our lab found that in 45 patients with this mutation, 15 had normal or borderline QTcs and 2 of those had experienced a cardiac event. Thus, the phenotypic variation in QTc extends even to patients with the same genetic mutation. Problem: The variability in QTc measurement among LQTS patients makes it difficult to identify those who may be at risk for SCD or another cardiac event.

Hypothesis: Characterizing a mouse model of LQTS will provide a way to more accurately identify affected individuals.

Methods: An ECG telemetry study was performed on transgenic knock-in mice (n = 7) with the same mutation as the human study (Scn5aΔKPQ/+). From the telemetric data collected, RR-I over 3 days was averaged and approximately 300 individual heartbeats per animal were analyzed for RR and QT-I. QTc was calculated with a modified version of Bazett's formula adjusted for mouse heart rate. Additionally, the AHA, ACCF, and HRS recommend the use of linear regression functions to correct the QT-interval. I developed a novel approach for unbiased analyses of the QT-RR relation over a full range of RR-I.

Results: The average RR-I was slightly longer in Scn5aΔKPQ/+ animals (112.3±1.7 vs. 116.9±1.2 ms, p<0.05). The average QTc was 8 ms longer in Scn5aΔKPQ/+ animals (45.79±1.04 vs. 53.84±1.53 ms, p<0.05). There was a significant difference (p<0.05) in the slope of the best fit line between Scn5awt and Scn5aΔKPQ/+ mice on a plot of ln(RR-I/100) vs ln(QT-I), with the Scn5aΔKPQ/+ slope being nearly double that of Scn5awt (0.439±0.163 vs. 0.124±0.101). Preliminary results indicate that QTc does not change substantially as RR-interval increases for Scn5awt (48 ms at RR=90ms; 51 at RR=140) but dramatically increases for Scn5aΔKPQ/+ (53 at RR=90; 70 at RR=140).

Discussion: These results demonstrate that in this model of LQT3, measuring QT at a low RR-I may mask the phenotype. The dramatic lengthening of QTc at RR=140 ms was not reflected in the average QTc data. This indicates that a borderline QTc at a lower RR-I may become a significantly prolonged QTc at a high RR-I. In fact, many LQT3 patients experience cardiac events during sleep, when the heart rate is slowest. However, clinicians do not need to order a Holter monitor for every patient. Instead, they should be aware that certain conditions may cause QTc to change, so a patient with a variable QTc at different RR-intervals may be a candidate for additional testing. The conclusions here underscore the necessity for all clinicians to consider conditions that may bias biomarker measurements.

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Postdoc

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Soluble Prorenin Receptor Increases Blood Pressure in High-Fat Fed Male Mice

Obesity-related hypertension is a major public health concern. We recently demonstrated that plasma levels of the soluble form of the prorenin receptor (sPRR) were elevated in obesity-associated hypertension. Therefore, in the present study, we investigated the contribution of sPRR to blood pressure elevation in the context of obesity. High-fat fed C57BL/6 male mice were infused with vehicle or sPRR (30 µg/kg/day) via subcutaneously implanted osmotic minipump for 4 weeks. Blood pressure parameters were recorded using radiotelemetry devices. Male mice infused with sPRR exhibited higher systolic blood pressure (SBP, 24h. Veh: 132.9 ± 1.2 mmHg; sPRR: 141.0 ± 2.2 mmHg, P<0.05) and lower spontaneous baroreflex sensitivity than mice infused with vehicle (SBRS. Veh: 3.88 ± 1.19 ; sPRR: 2.14 ± 0.45 ms/mmHg). To define mechanisms involved in systolic blood pressure elevation, mice were injected with an angiotensin-II type 1 receptor antagonist (losartan), a muscarinic receptor antagonist (atropine), a Î²-adrenergic antagonist (propranolol) and a ganglionic blocker (chlorisondamine). Losartan did not blunt sPRR-induced elevation in systolic blood pressure. In male mice, our data suggested that sPRR mediated-elevation of blood pressure is independent of the RAS. Chlorisondamine treatment exacerbated the decrease in mean arterial pressure in male mice infused with sPRR (Î”MAP. Veh: -21.2 ± 2.9 mmHg; sPRR: -29.3 ± 2.6 mmHg, P<0.05). These results demonstrated that sPRR induced autonomic nervous dysfunction. Interestingly, plasma leptin levels were increased in high-fat fed C57BL/6 male mice infused with sPRR (Veh: 25.4 ± 6.2; sPRR: 41.7 ± 4.2 ng/ml, P<0.05). Since leptin has been reported to cause sympathetic activation leading to an increase in blood pressure, together our data suggested that plasma leptin mediates sPRR effect on sympathetic tone.

In conclusion, our results indicated that sPRR increased systolic blood pressure through an impairment of the baroreflex sensitivity and an increase in the sympathetic tone potentially mediated by leptin.

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Graduate Student

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Aberrant Trafficking of Granule Cargo in Megakaryocytes

Platelets maintain hemostasis by secreting storage granules that contain high concentrations of proteins and small molecules, affecting the local environment to stimulate clot formation, angiogenesis, inflammation, and immune responses. Platelets and their granules are produced by large progenitor cells, megakaryocytes (MK), that reside in the bone marrow. Disorders affecting the development of these granules are well documented and known as storage pool diseases (SPDs). These SPDs are the result of mutations that disrupt the intracellular trafficking of granule cargo, leading to either the lack of mature granules (ARC syndrome) or to granules that lack cargo (Grey platelet syndrome, GPS). The trafficking of cargo to these granules is integrated with the development of megakaryocytes from their stem cell precursors, and the major type of granule, the α-granule, also stores several growth factors that affect the hematopoietic stem cell pool. Many of the storage pool diseases mentioned above also exhibit myelofibrosis in the bone marrow, indicating that the aberrant trafficking of α-granule cargo is affecting the surrounding milieu of the bone marrow, particularly megakaryocyte development from hematopoietic stem cells.Thus, the development of megakaryocytes from stem cells appears to be influenced by the biogenesis pathway of the α-granule, a pathway that is not well understood. Understanding the trafficking of α-granule cargo and how this process influences hematopoietic stem cell development is relevant not only to diseases mentioned above, but also to in vitro platelet production, a technology that would increase our ability to provide consistent platelet transfusions to individuals in need. To address these gaps in the understanding, we will investigate the secretion and trafficking of two growth factors, Platelet Factor 4 and RANTES, stored in α-granules, that have documented effects on the development of MKs, and how their aberrant trafficking affects the development of the MK population. We hypothesize that errors in the trafficking pathway of α-granule cargo within MKs leads to an imbalance of PF4 and RANTES in the bone marrow environment, disrupting MK development and gross bone marrow homeostasis. We will evaluate this pathway by using primary MKs from mouse models that have been documented to have defects in either endocytosis or α-granule cargo retention. We will also use FACS-based assays to address the development profile of primary megakaryocytes from these mouse models. This will allow us to evaluate both the trafficking defects that lead to cargo loss, and the resulting effects this has on the population of stem cells in the bone marrow.

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Graduate Student

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Thrombin Activation of Platelet Protease-activated Receptor 4 Augments Atherosclerosis in Low-density lipoprotein receptor deficient mice

Objective: Platelet activation has been shown to play a critical role in both formation and propagation of atherosclerosis. Protease-activated receptors (PARs) 1 and 4 mediate signaling by the coagulation protease thrombin. While PAR1 and 4 mediate thrombin activation on human platelets, mouse platelets contain PAR4 with PAR3 acting as a cofactor. Recent studies have demonstrated direct thrombin inhibitors (DTIs) reduced atherosclerosis in hypercholesterolemic mice. A previous study found no difference with PAR4 deficiency in a apolipoprotein E deficient (apoE-/-) mouse model. In this study, we examined the effect of PAR4 deficiency on the development of atherosclerosis in low-density lipoprotein receptor deficient (Ldlr-/-) mice.

Methods and Results: Male Ldlr-/- mice (8-12 weeks old) that were on a Par4+/+ or Par4-/- background (n = 5 - 20 each group) were fed a fat and cholesterol-enriched diet for 12 weeks to induce hypercholesterolemia and chronic atherosclerosis. PAR4 deficiency attenuated aortic sinus (65% decrease; $P = 0.001$) and aortic arch (71% decrease; $P = 0.001$) atherosclerosis with no effects on total plasma cholesterol concentrations or lipoprotein distributions. Macrophage (CD68) and platelet (platelet factor 4) accumulation was also attenuated with PAR4 deficiency ($P < 0.05$). Bone marrow transplantations were utilized to examine non-marrow or marrow-derived effects (n = 15 for each of 4 chimeric groups). The reductions in atherosclerosis, macrophage accumulation, and platelet infiltration were attributable to hematopoietic-derived PAR4 ($P < 0.05$). To determine if PAR4 mediated all effects of thrombin signaling, Ldlr-/-/Par4+/+ or Ldlr-/-/Par4-/- mice were fed a fat and cholesterol-enriched diet, supplemented with placebo or the DTI dabigatran etexilate (30 g/kg diet) for 12 weeks. Dabigatran etexilate administration reduced atherosclerosis in Par4+/+ mice ($P < 0.05$) but not in PAR4-/- mice ($P = 0.75$). To determine the differences between platelet activation in the apoE-/- versus Ldlr-/- mouse strains, whole blood was collected from both strains (n = 4 each) into hirudin and multiple electrode aggregometry (MEA) performed. Platelets from apoE-/- mice demonstrated significant increases in both the maximum slope of aggregation and area under the aggregation curve compared to Ldlr-/- mice.

Conclusion: We demonstrated whole body and hematopoietic PAR4 deficiency resulted in significantly attenuated aortic sinus and root atherosclerosis, as well as decreased macrophage and platelet accumulation. Thrombin inhibition did not alter atherosclerosis in PAR4 deficient mice. Further, the hyperactivation of apoE-/- platelets may explain the differences between the apoE-/- and Ldlr-/- mouse strains with Par4-/-. Together, these results suggest that thrombin amplification of atherosclerosis in mice is likely due to activation of PAR4 on hematopoietic cells.

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Graduate Student

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The deletion of the prorenin receptor (PRR) in liver induces a counter regulatory increase of adipose sPRR and adipose remodeling

Our laboratory previously demonstrated that adipose prorenin receptor (PRR) KO mice have no adipose tissue (AT), elevated hepatic triglycerides (TG) and cholesterol contents. Interestingly, adipose PRR KO mice have a better ability to clear the glucose. Surprisingly, the deletion of PRR in AT increased hepatic PRR mRNA abundance and plasma soluble PRR (sPRR), likely originating from the liver. To determine the relative contribution of hepatic PRR to the above phenotype, PRR was deleted in liver using an AAV-TBG-Cre vector (Liver PRR KO). Body weight was assessed weekly in Control (C, n=5) and Liver PRR KO mice (KO, n=6). Results showed that after three weeks of injections, liver weights was significantly increased (C: 1.8 ± 0.1g, PRR KO: 2.4 ± 0.1 g, $P<0.05$). Hepatic PRR deletion decreased TG levels and PPARÎ[3] gene expression in liver suggesting that hepatic PRR participated in TG homeostasis. However, hepatic and plasma total cholesterol levels were significantly higher in liver PRR KO mice. This elevation was associated with increased SREBP-2 and HMGCoA-reductase gene expression in liver. Similar to adipose PRR KO, the deletion of PRR in liver elevated plasma sPRR levels (C: 5.9 ± 0.2 ng/ml, PRR KO: 8.9 ± 0.6 ng/ml, $P<0.05$). Total sPRR levels increased in AT of liver PRR KO (C: 36.2 ± 8.6 pg/mg of prot, PRR KO: 92.7 ± 22.3 pg/mg of prot, $P<0.05$) suggesting that elevated plasma sPRR originated from AT. Since previous data demonstrated that PRR deletion in adipose tissue inhibited adipogenesis, we investigated whether elevated adipose PRR and sPRR regulated adipocyte differentiation. Indeed, genes and proteins involved in adipogenesis, adipocyte differentiation and lipid trafficking were upregulated ($PPAR_{\gamma}$, perilipin, FABP4 and ATGL) in liver PRR KO mice. PRR gene expression was also significantly increased in AT suggesting that sPRR might regulate its own gene in a positive feedback loop mechanism. To test this hypothesis, 3T3-L1 cells were treated with sPRR. Our results showed that PRR and $PPAR_{\gamma}$ gene expression significantly increased, in a dose-dependent manner, in 3T3-L1 cells treated with sPRR compared with vehicle. Our results suggest a new paradigm shift in which sPRR could act as a hepato-adipokine to coordinate and/or disrupt lipid and glucose metabolism through inter-organ communication.

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Graduate Student

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Sex specific differences in a lipodystrophy-induced hypertension mouse model

Obesity rate is higher in women than in men and is more strongly associated with hypertension in extremely obese women. However, it is unclear whether the relationship of the adipose tissue, blood pressure control, and anti-hypertensive drug's efficacy is sex-dependent. Therefore, in the present study, we assessed the mechanism involved using a lipodystrophy-induced hypertension mouse model, nervous system inhibitors, and AT1R blockers (losartan).

The adipose prorenin receptor (PRR) KO male and female mice displayed lipodystrophy and elevated blood pressure. Adipose PRR KO and control littermates were fed a high fat diet for 20 weeks. Body weight was assessed weekly, body composition was measured monthly, and blood pressure was measured by radiotelemetry at the end of the experiment.

Results showed that the decrease in systolic blood pressure (SBP) induced by losartan was exacerbated in high fat (HF)-fed adipose PRR KO female mice compared with control female mice but not in male mice (Delta SPB: CTL male, -7.9±1.5 mmHg; KO male, -6.4±3.1 mmHg; CTL female, -5.3±1.9 mmHg; KO female, -14.2±0.9 mmHg). In contrast, the contribution of the autonomic nervous system, assessed via injections of propranolol, atropine and chlorisondamine, revealed that the tachychardic response was significantly greater in HF-fed adipose PRR-KO male mice compared with control, but not in female mice (Delta heart rate: CTL male, +114.2±12.1 bpm; KO male, +156.±11.1 bpm; CTL female, +84.8±10.1 bpm; KO female, +89.8±21.3 bpm). Interestingly, plasma sPRR levels, the soluble form of PRR, were higher in female than in male mice (CTL: Male: 2.8 ± 0.3 ng/ml; Female: 5.1 ± 0.5 ng/ml) and the deletion of adipose PRR exacerbated the difference (KO: Male: 7.1 ± 0.1 ng/ml; Female: 11.6 ± 2.4 ng/ml).

Together, our data indicated that SBP elevation was primarily mediated by an AngII-dependent mechanism in HF-fed adipose PRR-KO female mice whereas it was mainly driven by the sympathetic nervous system in HF-fed male adipose PRR-KO mice. The present study also suggested that the presence of an expanded adipose tissue decreased the ability of female to respond to ARBs whereas it decreased the ability of male to respond to autonomic nervous system inhibitors. Studies in our laboratory are currently investigating the potential role of sPRR in blood pressure regulation in both male and female mice.

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Graduate Student

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Endothelial scavenger receptor BI (SR-BI) protects against sepsis

Background: Sepsis is related to dysregulated host response caused by infection. Every year 30 million people are affected by sepsis and more than 20% people died of sepsis in the worldwide because of the unclear mechanism of sepsis. Previous studies revealed HDL receptor scavenger receptor (SR-BI or Scarb1) plays a markedly protective role in sepsis. SR-BI is a multifunctional receptor which is expressed in a variety of cells including endothelial cells. In this study, we explored function of endothelial SR-BI in sepsis using endothelial SR-BI specific knockout mice.

Methods and results: We bred the SR-BI floxed mice to VecadCre mice to generate VecadCreSR-BIfl/fl mice (endothelial cell specific SR-BI KO mice, the mice were backcrossed to C57BL/6J for 10 generations). We conducted cecum ligation and puncture (CLP) to induce polymicrobial sepsis. Seven-day survival rate was 73.9% vs 44% in SRBIfl/fl mice (n=23) and VecadCreSR-BIfl/fl mice (n=25), respectively (P=0.048). We then assessed the causes of death by quantifying bacteria load in tissues, leukocyte recruitment to peritoneal cavity, vascular permeability by Evans Blue, and cytokine, cholesterol, glucose levels in circulation. We found VecadCreSR-BIfl/fl showed lower cytokine levels and higher free cholesterol levels than SR-BIfl/fl mice at 20h post CLP.

Conclusion: Endothelial SR-BI plays a protective role in CLP induced sepsis death. But further experimental research is still needed to explore the mechanism of its protective effect.

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Graduate Student

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Elucidating the estrogen signaling mechanisms that regulate daily metabolic rhythms in female mice

The circadian system is a critical regulator of obesity in males, but its role in females is poorly understood. In our previous studies we found that estradiol regulates daily rhythms in females to confer resistance to diet-induced obesity. We are now investigating the mechanisms by which estradiol protects daily metabolic rhythms from disruption by high-fat feeding in female mice. Estrogen signaling via the classical estrogen receptor alpha (ERα) has been shown to regulate metabolism and obesity. Therefore, we hypothesize that estradiol regulates daily metabolic rhythms in females via ERα. To test this hypothesis, we are measuring the effects of high-fat diet feeding on daily rhythms in global ERα knockout (ERα KO) female mice. Consistent with previous studies, ERα KO female mice become obese and hyperglycemic when fed high-fat diet for 6 weeks, while wild-type females are resistant to diet-induced obesity. We are now investigating the effects of high-fat feeding on daily rhythms of eating behavior and locomotor activity and the molecular timekeeping rhythms in tissues in ERα KO mice. These studies may elucidate an estrogen signaling mechanism that regulates daily metabolic rhythms in females.

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Undergraduate

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Mice Exposed to Early Life Stress display sex-specific, depot-specific, fat-derived factors that worsen the endothelial function

Obesity-induced hypertension (HT), a major risk factor for cardiovascular disease (CVD), affects >70 million Americans annually. Epidemiological studies point to early life stress (ELS; abuse, neglect, or loss during the first decade of life) as an independent risk factor for negative health outcomes in adulthood, including increased body mass index and blood pressure. To study ELS, we use a mouse model of neglect, maternal separation early weaning (MSEW), that mimics the effects of ELS on the cardiovascular system in humans. Both males and females fed a HF diet saw an increase in fat mass; however, only females saw an additional increase in fat mass due to exposure to MSEW.

It is well known that adipose tissues store energy, but several studies have shown that it may also influence vascular function and metabolic processes by releasing hormonal factors. The purpose of this study was to identify fat-derived factors impact the vascular function in female mice exposed to MSEW. The aim of this study was to test the capacity of adipose tissue from mice stressed in early or adult life to modulate the endothelial function by performing dose-response to acetylcholine.

MSEW was performed by separating the pups from the dam for 4 to 8 hours during postnatal days (PD) 2 to 16 and weaned on PD 17. Control mice remained undisturbed and were weaned on PD 21. Female control and MSEW mice were fed a HFD for 22 weeks, whereas the last 6 week were exposed to restraint stress (RS). Then, perivascular adipose tissue (PVAT) and mesentery adipose tissue (MESAT) were isolated and incubated with DMEM media for 1 hr. We tested the effect of media explant on thoracic aortic rings from C57/B6J 6mos F mice. Rings were dissected and mounted in the myograph chambers for dose response to acetylcholine-induced vasorelaxation. A second dose response was performed in each ring after 30 min incubation with explant media.

Vascular relaxation was similarly impaired in aortic rings after incubation with MSEW PVAT explants (from 49.0+/-1.2 to 32.5+/-5.7 % relaxation) and control PVAT explants (from 49.7+/-6.2 to 42.3+/-5.7 % relaxation). The different in relaxation between DMEM and PVAT incubated rings were similar between groups (17.18+/-6.76 vs 19.45+/-4.719 Delta % relaxation). No statistically significant differences were found in vascular relaxation from rings incubated with MSEW MESAT explants (from 46.6+/-3.2 to 26.2+/-4.0 % relaxation) compared with control MESAT explants (from 51.0+/-4.4 to 36.5+/-3.7 % relaxation). However, the difference in relaxation between DMEM and MESAT incubated rings was greater in MSEW mice vs. control (22.08+/-3.924 vs. 11.75+/-2.734%, $p<0.005$, respectively).

The dose-response curves strongly suggest that MESAT explants from MSEW mice have a greater effect impairing relaxation, suggesting depot-specific signaling. Thus, fat-mediated vascular function may have a sex and/or depot-specific effects but have an overall negative impact on the vascular function, increasing the cardiovascular risk.

Keywords: Cardiovascular disease, early life stress, MSEW, myography, PVAT, mesenteric

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Graduate Student

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Early life stress modulates aldosterone-potassium homeostasis in a sex-specific manner

We have previously shown that mice subjected to maternal separation and early weaning (MSEW), a model of early life stress, display obesity-induced hypertension and reduced glomerular filtration rate (GFR) after 16 weeks on a high fat diet (HF). In this study, we hypothesized that an altered aldosterone and electrolyte homeostasis will precede the hypertension and renal dysfunction in HF-fed mice exposed to MSEW. MSEW was performed by separating the pups from the dam for 4 to 8 hours a day during postnatal days (PD) 2 to 16. Mice were weaned early on PD 17. Control mice remained undisturbed and were weaned on PD 21. Eight-week-old mice were fed on a HF (60 % fat Kcal) for 12 weeks. After 11 weeks, 24-hour urine was collected to measure proteins, creatinine, aldosterone and electrolytes. At week 12, transcutaneous GFR was measured in all mice and blood pressure were measured in male mice. Plasma was collected to measure aldosterone and electrolytes. Male MSEW mice displayed higher levels of plasma and urinary potassium compared to control mice ($n=10\text{-}12$, $p<0.05$), while aldosterone levels were increased in urine (721±169 vs. 316±65 ng/ml, $p<0.05$) but not in plasma (1564±196 vs. 1801±113 pg/ml). However, GFR and blood pressure were similar between groups. On the other hand, female MSEW mice displayed similar plasma and urinary potassium levels compared to control mice, while having significantly higher levels of aldosterone in urine (3617±746 vs. 1314±262 ng/ml, $p<0.05$) but not in plasma, while GFR was similar amongst the groups. Therefore, this data indicates that MSEW increases urinary aldosterone levels in both sexes; however, only male mice display hyperkalemia and kaliuresis, suggesting that males are compensating for the hyperkalemia by increasing aldosterone to excrete excess potassium in the urine. Based on previous findings, this data shows that a longer period on HF is needed for mice to develop the more robust decline in renal function and hypertension as previously observed in MSEW mice.

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Graduate Student

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Computation of Heart Rate from Video of the Face

Researchers have been working on non-contact measurement to improve telehealth and make vital monitoring accessible by a large number of people. One of the areas of development is the extraction of heart rate (HR) from images of the face captured as a part of video recording using digital cameras. Based on methods reported previously in the literature we implemented an algorithm to extract heart rate from the video of the subject's face. First, a region of interest (ROI) was manually selected and was tracked to minimize motion artifact from the movement of the subject. Then from the RGB image, the green frames were extracted from the video. A spatial mean of all the pixels within the ROI was computed for each frame to obtain a raw photoplethysmograph (PPG) signal. The raw PPG was band-pass filtered (0.02-0.7Hz) to produce final PPG. From the PPG, local peaks, i.e. beats, were detected which was followed by calculation of inter-beat intervals (IBI). The HR was calculated from the IBI. The HR calculated using the above-described method was validated against a contact measurement. Videos recorded during 40 one minute trials from one subject were analyzed. The video data was downloaded from â€œDEAP: A Database for Emotion Analysis using Physiological Signalsâ€. The results show that the mean HR within each of the 40 trials was similar to the mean HR computed from the contact PPG with an average difference of less than 5%. However, there were differences between the rates computed within each trial with the largest deviation of about 11% suggesting that while the approach does a reasonable job of estimating the HR, further refinement would be helpful.

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Graduate Student

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Effects of Cognition and Tempo of Music on Cardiac-synchronized Electrical Response of Brain

It is over 150 years ago that the intimate interaction between the heart and the brain was realized by Claude Bernard, and of all the organs in the human body, the heart is among the ones that have the most extensive neural connection with the brain. In this study, we used cardiac-synchronized EEG segments to investigate the effects of tempo and cognition induced by auditory stimuli. ECG and EEG were recorded from 14 subjects when they were listening to music and during silence (control). The 300-millisecond EEG segments ending at R-peaks of ECG were extracted. The frequency bands of Delta, Theta, Alpha, Beta, Gama and Gamma2 within EEGs were also analyzed to determine which bands were more sensitive to the induced changes. The eigenvalue analysis of covariance matrix of synchronized EEG showed that Alpha band in EEG from parietal zone was the most sensitive band among all other frequency bands to auditory stimuli. The cognition of song had much higher impact on Gamma and Gamma2 bands of EEGs from right hemisphere, which corresponds to music awareness, than tempo of song even though tempo is considered as the most impactful acoustic structural feature of music on physiological variables. The higher impact of local phase randomized version of known song than local phase randomized of unknown song on these bands also verifies the stronger impact of cognition relative to tempo.

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Postdoc

Podium Presentation

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Fine Tuning Platelet Secretion to Modulate Hemostasis

Globally, occlusive thrombotic events: e.g., heart attacks and cerebral strokes, cause >50% of total deaths attributed to noninfectious disease. However, aggressive attempts to limit thrombosis cause bleeding, which can be equally catastrophic. What is needed is a strategy to limit clot formation, but not prevent it. Platelets play a critical role in controlling bleeding by sensing vascular damage and releasing a host of components to seal breaches. This secretion process is mediated by Soluble N-ethylmaleimide Sensitive Factor Attachment Protein Receptors (SNAREs) and their regulators. To drive secretion, vesicle (v)-SNARE on granules and target (t)-SNARE on the plasma membrane (PM) form a trans-bilayer complex that mediates membrane fusion. Syntaxin 11 and SNAP-23 form the functionally relevant t-SNARE heterodimer. For v-SNAREs, platelets contain Vesicle Associated Membrane Protein (VAMP)2, 3, 4, 5, 7, and 8. We focused on how the platelet VAMPs influence secretion and whether modulating secretion can modulate clot formation. To address this goal, we genetically titrated the different VAMPs to define their roles in exocytosis and hemostasis.

We gathered global V3-/- and V8-/- animals. To overcome embryonic lethality of global VAMP2 deletion, we generated platelet specific V2âˆ†3âˆ† mice by using tetanus toxin which cleaves VAMP2 and VAMP3, and crossed them with V8-/- mice to create platelet-specific V2âˆ†3âˆ†8-/- mice. Structural analysis of wild-type and VAMP-deficient platelets showed that the Î± granule cargo solubilization/decondensation follows granule fusion. To define the structure of secretion, activation intermediates were fixed at various time points, post stimulation, and electron microscopy was performed. The data indicate that granule decondensation is time- and agonist concentration-dependent. Moreover, decondensation of granule cargo was VAMP-dependent. Three dimensional EM analyses indicate that VAMP8 plays a major role in compound, intra-granule fusion and also contributes to single, granule-PM fusion. Our structural data elucidate how platelet secretion occurs at the cellular level and offers an explanation for the complex secretion kinetics previously reported in activated platelets. To further measure the functional importance of the VAMPs, ex vivo secretion assays were used to monitor the kinetics and the extent of release from all three platelet granules (dense, Î±, and lysosomes). Only V2âˆ†3âˆ†8-/- platelets showed a robust defect in secretion (~70% decrease), more than observed for V8-/- platelets (~50%). When we studied the effects of secretion on hemostasis, only V2âˆ†3âˆ†8-/- mice showed significantly increased tail-bleeding times and delayed arterial thrombosis. V8-/- animals did show a delay in thrombus formation but no overt bleeding diathesis. Our data show that small differences in secretion kinetics alters hemostasis, thus by modulating platelet secretion, we can control thrombus formation without inducing pathological bleeding. These data identify the secretory machinery as a viable target to control occlusive cardiovascular diseases. Our work is the first comprehensive study showing how by targeting secretion we can achieve the long-sought balance between occlusive thrombosis and spurious hemostasis. Additionally, by titrating amounts and types of VAMPs in platelets we have created a valuable set of animals to precisely analyze role of platelet secretion in other vascular processes

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Graduate Student

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Adrenal insufficiency is a risk factor for pediatric sepsis-study in mice model

Background: Sepsis is a life-threatening condition that results from a dysregulated host response to infection. It is a devastating public health problem of children in worldwide. Generally, mortality rates for pediatric patients with sepsis range from 10%-20%. However, efforts toward consensus definitions, guideline development, epidemiologic surveillance quality assurance, and research funding for pediatric sepsis lag in time and attention behind adult sepsis. According to the reports, 25%-60% of septic patients suffer from relative adrenal insufficiency (RAI), which generally indicated by the insufficient inducible glucocorticoid (iGC) production in response to stress. Glucocorticoid (GC) therapy is frequently employed in sepsis treatment. The guidelines of the Surviving Sepsis Campaign 2012 recommend timely hydrocortisone therapy in children with fluid refractory, catecholamine resistant shock and suspected or proven absolute (classic) adrenal insufficiency (grade 1A). However, the current evidence does not definitively support the potential utility of GC in pediatric sepsis therapy. A lack of an RAI animal model presents a barrier to understand the effect of relative adrenal insufficiency and the efficacy of GC therapy in pediatric sepsis. Scavenger receptor class B type 1 (SR-BI), a multi-functional protein, abundantly expressed in steroid tissues and liver. Previous studies have shown that SR-BI Knockout mice display normal basal GC but lack iGC in cecal ligation and puncture (CLP)-induced sepsis. Here, we developed RAI mice model (specific depletion of SR-BI expression in the adrenal gland) to test our hypothesis that RAI is a risk factor in pediatric sepsis, the production of iGC is essential for the host response to septic stress. And a precision medicine approach should be used for GC therapy in sepsis-only applying GC to young mice with RAI.

Methods and results: Using SF-1Cre SR-BI fl/fl conditional knockout mice as RAI model (who exhibited specific depletion of SR-BI expression in adrenal gland), we found that 21 days SF1Cre SR-BIfl/fl young mice are susceptible to CLP-induced sepsis (86.67 % survival in SR-BIfl/fl mice versus 16.5% in SF1CreSR-BIfl/fl mice; p=0.001). Supplementation of hydrocortisone significantly improved the survival in SF1Cre SR-BIfl/fl mice with CLP treatment, which didn't show significant increase in survival rate of SR-BI fl/fl mice. To elucidate if the relative adrenal insufficiency in young mice affects the leukocyte recruitment during sepsis, we quantified peritoneal neutrophils and inflammatory monocytes with flow cytometry in SF1Cre SR-BI fl/fl and SR-BIfl/fl mice after 18h CLP surgery, the results showed that the SF1Cre SR-BIfl/fl mice have impaired inflammatory monocyte and neutrophil recruitment to the peritoneal cavity.

Conclusion: Using the unique adrenal insufficiency mice model, we demonstrated that RAI is a risk factor for pediatric sepsis, and GC treatment benefits young mice with RAI, the study results provided evidence that it is reasonable to use precision medicine approach for sepsis therapy-selectively applying GC therapy for a subgroup of patients with RAI.

Future study:

Study the potential mechanisms by which iGC affects sepsis

1. Investigating the phagocytosis of immune effector cells in spleen and blood (mainly assess the neutrophils, macrophages, T cells, and B cells.)

2. Studying the effect of GC on the sepsis-induced organ injury.

2. Studying the effect of GC on the regulation of inflammatory response.

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Graduate Student

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Periostin plays a critical role in cardiac fibroblast activity

Cardiac fibrosis is a consequence of almost all myocardial injuries. In myocardial infarction (MI), what starts as protective scarring to prevent ventricular wall rupture becomes a pathological remodeling process with the accumulation of excess extracellular matrix (ECM) proteins. Recently, cardiac fibroblasts (CFs) and their activated form, myofibroblasts (MFs), have emerged as potential therapeutic targets in preventing both acute and chronic cardiac fibrotic disease states. The secreted matricellular protein, periostin (Postn), appears to be expressed exclusively from MFs resident to the heart. Therefore, we generated a mouse model that permits lineage tracing of periostin expressing cells by inserting a tamoxifen inducible MerCreMer cDNA into the periostin locus. Here we show that all MFs in the heart after injury or stress stimulation can be lineage traced with a Rosa26-eGFP dependent reporter, allowing us to study new avenues of fibroblast biology. Upon injury, eGFP+ periostin lineage-traced myofibroblasts are αSMA positive and have an activated myofibroblast gene expression profile. However, when periostin protein is ablated with a whole body knockout approach, cardiac fibroblasts fail to activate, shown with the real-time periostin expression reporter, Postn-LacZ, allele after MI or Ang/PE infusion stimuli, suggesting a critical role for periostin during development and activation of cardiac fibroblasts.

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Graduate Student

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Association between ApoA-I (Apolipoprotein A-I) Immune Complexes and Adverse Cardiovascular Events

Apolipoprotein A-I (ApoA-I) is a target of IgG autoantibody induction in patients, but the role of these antibodies has not been fully elucidated. Anti-ApoA-I IgG antibodies targeting delipidated ApoA-I have previously been characterized as a biomarker of cardiovascular disease progression, but only moderate associations have been reported. We hypothesize that antibodies bound to ApoA-I as an immune complex are a critical and unexplored component of the antibody response to ApoA-I, which will better predict disease outcomes. To test this hypothesis we developed an ELISA assay to quantify IgG bound to ApoA-I as a soluble immune complex (IC) in sera samples. This ELISA assay was used to screen plasma from 359 patients with coronary artery disease (CAD). In Cox proportional hazard regression analysis, low levels of ApoA-I/IgG ICs were independent predictors of adverse cardiovascular outcomes (death, myocardial infarction and stroke) after adjustment for age, sex, diabetes mellitus, estimated glomerular filtration rate, presence of obstructive CAD, heart failure, total cholesterol, and HDL cholesterol. The adjusted hazard ratio between lowest and highest tertiles indicate an association between was determined to be 1.90 (95% CI: 1.03 - 3.49; p = 0.04). Additionally, Pearson correlation analysis failed to identify a correlation between ApoA-I/IgG ICs and 26 clinical characteristics. In a second cohort of 104 anonymized blood donors, ApoA-I/IgG IC levels in sera were compared to the concentration of the individual components. No significant relationship between ApoA-I/IgG ICs and total ApoA-I concentration (113± 44 mg/dL, r2 = 0.008) was observed, while a weak correlation was observed between ApoA-I/IgG IC level and total IgG concentration (12 Â± 4 mg/mL, r2 = 0.09). Antibody subclass composition of ApoA-I/IgG IC was then characterized in a subset of blood donor subjects and found to be enriched in the anti-inflammatory subclass, IgG4, as compared to percentage of IgG4 in total serum (36% versus 6%, p = 0.04). The enrichment of IgG4 in ApoA-I/IgG ICs relative to total IgG composition provides insight into potential mechanisms of the association between ApoA-I/IgG ICs and decreased adverse cardiovascular events in patients. Continued efforts to purify and characterize ApoA-I/IgG ICs from human plasma will enable us to elucidate the immunologic sequelae associated with their induction in vivo. These data and continued efforts to understand the functional implications of ApoA-I/IgG ICs will enhance the utility of ApoA-I/IgG ICs as a biomarker of cardiovascular progression.

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Graduate Student

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Fibroblast-specific deletion of Human Antigen R (HuR) reduces pressure overload-induced pathological cardiac remodeling

Background: Heart Failure (HF) is one of the leading causes of mortality in the United States and the prognosis is often worsened by adverse cardiac remodeling such as cardiac hypertrophy, dilation, and fibrosis. Preliminary data from our lab suggests Human Antigen R (HuR), an RNA binding protein, could be a target for reducing adverse remodeling. We have shown that a genetic deletion of HuR in the cardiomyocytes (iCM-HuR-/-) is protective in the preservation of cardiac function and reduction of cardiac fibrosis in a mouse model of HF (transverse aortic constriction (TAC). Interestingly, the changes in gene expression associated with the iCM-HuR-/- TAC vs. control TAC mice include many genes that are fibroblast-specific. Objective: Determine the fibroblast-specific role of HuR in the initiation and propagation of cardiac fibrosis. Methods and Results: We utilized a tamoxifen-inducible periostin-cre crossed with a HuR floxed mouse to induce deletion of HuR specifically in the in the activated fibroblasts. These myofibroblast-specific HuR deletion (mfHuR-/-) mice were then subjected to TAC or sham surgery. At 2 weeks post TAC, we isolated cardiac fibroblasts from control and mfHuR-/- mice and used qPCR to determine fibrosis-associated extracellular matrix (ECM) genes (eg. Periostin, MMP2, and fibronectin). We observed an increase in these pro-fibrotic genes in isolated fibroblasts from control TAC animals (n=2) that was blunted in the fibroblasts isolated from the mfHuR-/- mice (n=3). We also observed cardiac hypertrophy at 2 weeks post TAC in the control mice, indicated by increased heart weight to body weight ratio and ANF expression, that was decreased in the mfHuR-/- mice. At 8 weeks post TAC, we also observed decreased cardiac hypertrophy in mfHuR-/- mice (n=3) compared to controls (n=2). Conclusion(s): In summary, our preliminary data shows that a fibroblast-specific knockout of HuR mediates an early change in myofibroblast gene expression following TAC and is cardioprotective in the reduction of cardiac hypertrophy and fibrosis.

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Postdoc

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Active time-restricted feeding improved sleep-wake cycle in db/db mice

People with diabetes are more likely to experience sleep disturbance than those without. Sleep disturbance can cause daytime sleepiness in diabetic patients, which may impair their daytime performance or even lead to workplace injuries. Therefore, restoring the normal sleep-wake cycle is critical for diabetic patients who experience daytime sleepiness. Previous data on a diabetic mouse model, the db/db mice, have demonstrated that the total sleep time and sleep fragmentation are increased and the daily rhythm of the sleep-wake cycle is attenuated. Accumulating evidence has shown that active time-restricted feeding (ATRF), in which the timing of food availability is restricted to the active-phase, is beneficial to metabolic health. However, it is unknown whether ATRF restores the normal sleep-wake cycle in diabetes. To test that, we used a noninvasive piezoelectric system to monitor the sleep-wake profile in the db/db mice with ad libitum feeding (ALF) as a baseline and then followed with ATRF. The results showed that at baseline, db/db mice exhibited abnormal sleep-wake patterns: the sleep time percent during the light-phase was decreased, while during the dark-phase was increased with unusual cycling compared to control mice. In addition, the sleep bout length during both the light-phase and the full 24-hour period was shortened in db/db mice. Analysis of the sleep-wake circadian rhythm showed that the ATRF effectively restored the circadian but suppressed the ultradian oscillations of the sleep-wake cycle in the db/db mice. In conclusion, ATRF may serve as a novel strategy for treating diabetes-induced irregularity of the sleep-wake cycle.

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Graduate Student

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Intracranial atherosclerosis in nonhuman primates promotes neurovascular hallmarks of vascular cognitive impairment and dementia

Vascular cognitive impairment and dementia (VCID) is the second most common cause of dementia trailing Alzheimer's disease. Poor cardiovascular health facilitates poor brain health and improvements in vascular outcomes may potentially delay or prevent onset of VCID. Notably among the many vascular hallmarks of VCID, intracranial atherosclerosis (ICAS) is a public health concern for both its role in stroke and subsequent cognitive dysfunction. Reducing low-density lipoprotein (LDL) concentration with statins is a primary therapeutic approach to stabilize atherosclerotic vascular disease (AVD), but statins only reduces ischemic stroke risk by ~20% and do not appear to reduce VCID. This suggests that treatment of hypercholesterolemia alone is not an optimal approach for reducing VCID and additional therapies are likely needed to promote the regression and/or stabilization of ICAS. To date, there is paucity of suitable animal models that develop ICAS making it difficult to test co-therapies to reduce ICAS burden and downstream hallmarks of VCID. During a study to determine the impact of microRNA-33 (miR-33) antagonism on cardiovascular AVD, we fortuitously discovered that our nonhuman primate (NHP) model had ICAS and other neurovascular hallmarks of VCID. Indeed, evaluation of intracranial arteries revealed that after 20 months on an atherogenic diet over 50% of NHPs (n=63) developed ≥1 atherosclerotic lesions within the circle of Willis (COW), the main arterial network that supplies blood to the brain. Atherosclerotic lesions from sections of COW suggested progressive characteristics of AVD such as: necrotic cores surrounded by macrophages, smooth muscle cell migration into the intima with increased staining of picro Sirius red (PSR)+ collagen initiating the formation of a fibrous cap. Furthermore, the initial preliminary data collected on a subset of animals (n=12) presented, in addition to ICAS, evidence of neurovascular hallmarks of VCID such as: gross ischemic lesions, gross infarcts, brain arteriolosclerosis (B-ASC), microinfarcts and microhemorrhages. Moreover as a surrogate for direct measures of reactive gliosis, the density of positive immunohistochemistry (IHC) staining for IBA1+ microglia and GFAP+ astrocytes was assessed on brain sections from 2 animals: one animal with ICAS and a control animal with no AVD. We are currently working on analyzing intracranial arteries and brains from our NHPs in hopes of making an impact on the field of VCID research were currently no treatments or preventative approaches have been developed for clinical trials.

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Postdoc

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Rapid transitions between the OFF and ON states of myosin contribute to contraction-relaxation coupling in cardiac muscle

Abstract Hearts contract and relax during each beat, as they pump blood into the aorta and then refill. The maximum rates of contraction (dP/dt)max and relaxation (dP/dt)min are important indices of cardiac function and are frequently compromised in patients who have cardiovascular disease. Experimental data from isolated trabeculae and working hearts show that (dP/dt)max and (dP/dt)min are correlated and scale in proportion during experimental perturbations. This is described as contraction-relaxation coupling. The molecular mechanisms that underpin this behavior are unclear. We investigated contraction-relaxation coupling using a computer model (MyoSim) that simulates dynamically coupled thick and thin filaments and includes force-dependent transitions between the OFF and ON states of myosin. Initial calculations simulated twitch contractions at different muscle lengths and exhibited contraction-relaxation coupling. We then perturbed the model by systematically varying the values of parameters associated with each sarcomere-level process. This produced a wide range of twitch responses. Contraction-relaxation coupling was maintained in nearly every case. The only exceptions were perturbations which reduced the occupancy of the myosin OFF state. We conclude that rapid transitions from the ON to the OFF state of myosin are essential for contraction-relaxation coupling.

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Staff

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Serum amyloid A Mediates the Pro-atherogenic Effects of Cholesterol Ester Transfer Protein in Mice

Objectives: Serum Amyloid A (SAA) is predictive of cardiovascular disease (CVD) in clinical studies and plays a causal role in the development of atherosclerosis in mice. SAA demonstrates a number of pro-atherogenic effects in vitro. However, high-density lipoprotein (HDL), the major carrier of SAA in circulation, masks these pro-atherogenic effects. We recently demonstrated that remodeling of HDL by Cholesterol Ester Transfer Protein (CETP) results in the liberation of lipid-free SAA as well as the transfer of SAA to apolipoprotein B-containing lipoproteins, thus unmasking the protective effects of HDL. In this study, we investigated whether the previously described pro-atherogenic effect of CETP in mice is dependent on SAA. Approach and results: Atherosclerotic lipid accumulation was assessed in 16-18 week-old apoE-deficient (apoE-/-) mice and apoE-/- mice lacking SAA1.1, SAA2.1, and SAA3 (apoE-/- x SAA-TKO) injected i.p. with either an adeno-associated virus (AAV) expressing CETP (n=8-10) or a control AAV (n=5). All mice were fed a chow diet and sacrificed 16-weeks after AAV injection. At study termination, plasma CETP and SAA levels in mice were determined and atherosclerosis was quantified by en face analysis of the aortae. Plasma lipoprotein profiles were determined by Fast Protein Liquid Chromatography (FPLC) and SAA in the various lipoprotein fractions was analyzed by ELISA and western blotting. As expected, CETP was not detected in the plasma of mice injected with control AAV, as mice lack CETP. CETP activities were 74.3 ±9.4 nmol/ml/h and 65.03 ±5.7 nmol/ml/h in apoE-/- and apoE-/- x SAA-TKO mice respectively (p>0.05). There was no significant difference in plasma levels of IL-6, TNF-α or IL-1β between groups. As expected, expression of CETP significantly increased atherosclerotic lesion area in apoE-/- mice (11.13±1.92%) compared to control-treated apoE-/- mice (4.08±1.29%; p=0.03). However, CETP expression did not increase atherosclerosis in apoE-/- x SAA-TKO mice (3.40±0.77%), demonstrating that SAA is required for CETP-induced atherosclerosis. Whereas SAA was found exclusively on the HDL fraction in control apoE-/- mice lacking CETP, SAA was associated with VLDL and LDL as well as HDL in apoE-/- mice with AAV-mediated CETP expression. Conclusions: Our results indicate that SAA is required for the pro-atherogenic effects of CETP. Morever, the CETP-mediated effect is associated with a redistribution of SAA to apoB-containing particles.

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Graduate Student

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Translational control of mRNA via a novel, muscle specific, ribosomal protein

In mice, at birth, all ribosomes contain ribosomal protein L3 (RPL3) but as post-natal development progresses striated muscle ribosomes switch to using the paralog of RPL3, RPL3-like, while other organs continue expressing RPL3. RPL3-Like is highly evolutionarily conserved and specific to striated muscle. In the heart, we found that its expression is even more specific, with it only being found in the ventricles while the atria expresses the ubiquitous, RPL3. To determine the function of these ribosomal paralogs, we created a genetic knockout mouse. We found that when we knockout RPL3-like, expression of its paralog remained high in the ventricle even after post-natal development. We did functional testing of WT and RPL3-like KO hearts using both ECG and Echocardiography. We found no functional differences at baseline but saw a more rapid response to isoproterenol in KO when compared to WT. We hypothesized that the differences seen in isoproterenol response were due to changes in translation of ion channels or calcium regulatory mRNAs. We isolated translating ribosomes from both WT and KO mouse ventricles and performed RNA sequencing of the translating mRNA. We found that genes involved in actin cytoskeleton regulation were less translated in the KO ventricles. We hypothesize that disruption of actin polymerization is slowing beta adrenergic receptor internalization and therefore allowing signaling to continue longer. Future work will be needed to confirm cytoskeletal disruption of actin and its effects on beta adrenergic receptor activity and localization.

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Undergraduate

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Early-Life Changes in Peripheral Metabolism: A Study of APOE Genotype Effects Using Indirect Calorimetry

Apolipoprotein E (APOE) is present in both the periphery and the brain, and is associated with circulating lipoproteins, especially very low-density lipoprotein and high-density lipoproteins. APOE is well known for its connection to both Alzheimer's disease (AD) as well as cardiovascular diseases (CVD). In humans, there are three common isoforms of APOE: E2, E3, and E4. The E4 isoform, compared to E2 and E3, is associated with an increased risk for the development of both AD and CVD. It has become clear that AD is influenced by both metabolic and vascular factors – both of which precede and may contribute to dementia. Interestingly, E4 is associated with deficiencies in both areas; there is both decreased cerebral glucose metabolism and lower cerebral blood flow in E4 individuals. However, the precise mechanism by which APOE alters glucose metabolism is unknown, and E4's effects on fatty acid metabolism are also an understudied area. Therefore, we are conducting a human study in which we probe the effects of APOE on glucose and lipid utilization by measuring metabolic rate and respiratory exchange ratio (RER) – a reflection of energy substrate usage – using indirect calorimetry. Our preliminary findings in 85 subjects show increases in Respiratory Exchange Ratios (RER), as well APOE genotype specific effects on resting energy expenditure (REE). Additionally, a dietary glucose challenge resulted in a heightened increase in RER following ingestion in E4 individuals. Plasma blood glucose levels also show an APOE-dependent change pre- and post- glucose challenge, with E4 individuals demonstrating larger increases. These findings are an important step toward elucidating the precise mechanism(s) of APOE's effects on metabolism in the brain and the periphery in order to better understand the role of this important genetic risk factor on metabolism, vascular disease and dementia.

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Graduate Student

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Conserved Sequence in the Loop Region of Angiotensinogen Affects Plasma Angiotensinogen Concentrations But Has no Effects on Angiotensin II-mediated Functions

Background and Objective: Angiotensinogen (AGT) is the unique substrate of all angiotensin peptides. The loop region of AGT protein formed by residues 291-301 is highly conserved and contains strictly conserved hydrophobic residues W292 and V299 along with S298. In this study, we determined whether deletion of these conserved sequence in mouse AGT would affect AngII-mediated functions.

Methods and Results: Surface plasmon resonance analysis showed that mutation of W292 in AGT loop region decreased its binding affinity to megalin, a protein that is abundant in renal proximal convoluted tubules. To determine whether mutation of W292A affected AngII-mediated functions in mice, we used hepatocyte-specific AGT deficient (hepAGT-/-) mice that have ~10% of plasma AGT concentrations as their wild type littermates. AGT was repopulated in hepAGT-/- mice by infection with adeno-associated viral vectors (AAV) encoding the native or mutated protein (W292A). Repopulation of the mutated AGT increased plasma AGT concentrations, blood pressure, and atherosclerosis, to the magnitudes, which were comparable to those in hepAGT+/+ mice. Subsequently, we determined whether deletion of the entire conserved sequence (291-301) would affect AngII-mediated functions. Administration of AAV encoding mutated AGT by substitution of the loop region with GA linker only moderately increased plasma AGT concentrations, although mRNA abundance of AGT in liver was comparable with its abundance in hepAGT +/+ mice. Despite the much lower concentrations of plasma AGT, depletion of the conserved sequence in the loop region increased blood pressure and atherosclerosis to the magnitudes, which were equivalent as in hepAGT-/- mice repopulated with wild type AGT.

Conclusion: The highly conserved sequence in the loop region of AGT may affect AGT protein metabolism, but does not affect AngII-mediated functions.

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Postdoc

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Macrophage overactivation promotes aortic dissection associated with medial expression of Arf and impaired proliferation of smooth muscle cells in mice

Background and Objective: Aortic dissection (AoD) is a medical emergency with high mortality for which there is a poor understanding of the molecular mechanisms. We found that macrophage-specific deficiency of Socs3 (mSocs3-KO), a negative regulator of Stat3, in angiotensin II (AngII)-infused mice led to AoD following focal medial disruption at the aortic branches. Inflammatory differentiation of macrophages and impairment of reparative response in smooth muscle cells occurred only in mSocs3-KO, but not its wild type controls, before AoD. In the present study, we determined whether Socs3 affected cell proliferation in AngII-infused mice.

Methods and Results: We measured bromodeoxyuridine (BrdU) uptake and expression of cell cycle machinery, namely cyclin-dependent kinases (Cdks), cyclins and negative regulators, in mice infused with 1 g/kg/min AngII. In WT mice, AngII alone did not induce BrdU uptake before AoD, but the development of focal medial disruption at the aortic branches was associated with strong BrdU uptake in medial SMCs, expressions of Cdks and cyclins, which was followed by fibrotic healing. In mSocs3-KO, Ang II induced high expressions of Cdks and cyclins both before and after the development of focal medial disruption. Strikingly, aorta with focal medial disruption in mSocs3-KO mice showed diminished BrdU uptake and upregulation of Ink4a/Arf, negative regulators of cell cycle. Immunostaining confirmed the localized expression of Arf protein at the site of focal medial disruption. In cultured mouse SMCs, AngII induced protein expressions of Cyclin D1 and Arf, but not Ink4a.

Conclusion: Our findings implicate that premature proliferation of medial SMCs by AngII before development of focal medial disruption may trigger the expression of Arf, which is likely responsible for the impairment of SMC proliferation and repair response, contributing to aggravating the focal medial disruption to AoD in mSocs3-KO mice.

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Staff

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Adaptation of the American Heart Association's Healthy for Life 20 x 20 Program for Individuals with Disabilities and Caregivers: A Pilot Study

Purpose: Healthy for Life is a community-based program initiated by the American Heart Association (AHA) and Aramark, a US-based food service. One goal of this program is to increase consumption of fruits, vegetables and whole grains 20% by 2020. Methods: This pilot project used pretest-posttest quasi-experimental design to assess changes in eating behaviors while attending four experiential sessions in the Healthy for Life program. The Human Development Institute partnered with New Vista to recruit participants and locate an appropriate facility for the activities. A paper-based survey assessing eating behaviors was distributed prior to activities during week one and four. Existing AHA materials including presentations, videos, handouts, hands-on experiences, surveys and SMART goal planning were universally adapted to promote access of information for individuals of all abilities. Results: Twenty-nine participants started in the first session, and twenty-four ended the last session of the program. Participants were individuals with disabilities and caregivers with ages ranging from 18-65+ years old; ~60% female and 40% male; over 80% were Caucasian and ~15% Black American. Overall mean daily consumption of servings reported by participants increased 16% for fruit (P=0.08), 5% for vegetables (P=0.27) and 13% for whole grains (P=0.13). Changes in daily consumption of 2+ servings reported by participants from first to last session were: fruit increased from 59% to 77%, vegetables increased from 69% to 74%, while whole grains did not change. In terms of SMART goal planning for healthy behavior change, participants discussed goals during the sessions with 55% of participants completing at least one session's handout on their goals. Limitations: Self reporting of food consumption and behaviors, short duration of intervention, and no follow up on behavior change. Conclusion: Universal design in program adaptations allowed the AHA curriculum to be more broadly accessible to reach an under-served demographic. Using these results, more Healthy for Life experiential sessions could be universally adapted to support a long term program engaged in healthy lifestyle change for all.

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Graduate Student

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Maternal Exercise Protects Male Offspring Against Obesity and Diabetes Caused by Perinatal PCB126 Exposure

Accumulating evidence suggests perinatal toxic exposures to environmental contaminants, including polychlorinated biphenyls (PCBs), potentially contributes to the significant increase in the prevalence of diabetes and obesity. We previously demonstrated that maternal exercise during pregnancy improved offspring glucose homeostasis. The purpose of this study was to investigate glucose tolerance and body composition in offspring born to exercised dams exposed to PCB126 during the perinatal period. We hypothesized that maternal exercise would protect offspring against the long-term detrimental effects of perinatal PCB exposure. Sixty multiparous female ICR mice were equally divided into four experimental groups: Sedentary/Vehicle, Sedentary/PCB, Exercise/Vehicle, and Exercise/PCB. Dams in the exercise groups had voluntary access to a running wheel in the cage prior to and during mating, pregnancy, and nursing. Dams were exposed to 0.5 μmole/kg of PCB126 or vehicle via oral gavage during the perinatal period. Weaning occurred at postnatal day 21. Male offspring body weight was recorded weekly, while body composition and glucose tolerance were measured at 4, 12, and 23 weeks of age. The perinatal PCB treatment caused a significant increase in fat deposition in adult male offspring born to sedentary dams ($p < 0.05$), and maternal exercise was able to attenuate the increased fat mass in the PCB-treated group ($p < 0.05$). The perinatal PCB exposure caused a significant delay in glucose disposal compared to those offspring of vehicle-exposed animals ($p < 0.05$). This demonstrates that PCB perinatal exposure detrimentally affects glucose homeostasis. Importantly, maternal exercise improved glucose disposal in the PCB-treated offspring ($p < 0.05$). These data suggest that short-term maternal exercise could be an effective intervention against toxic exposures that occur during fetal and early postnatal development.

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Graduate Student

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Enhanced Autophagy in Smooth Muscle Cell Rich Aortic Medial Layer Profoundly Increases AngII-induced Abdominal Aortic Aneurysm Formation in Male and Female Mice

Background: Abdominal aortic aneurysms (AAAs) are permanent dilations of the abdominal aorta with greater than 80% mortality after rupture. The prevalence of AAAs is sexual dimorphic as males are at a 4-5 times greater risk of developing AAAs than females. The aneurysmal process relies on inflammation and degradation of the aortic wall. Autophagy, a cellular self-eating degradation process, which regulates cellular homeostasis by recycling damaged organelles and long-lived proteins, may be a culprit in AAA formation. Recent findings demonstrated that autophagy-related genes are markedly upregulated in human AAAs. However, its functional role in AAA development is still unclear.

Methods and Results: Male LDLr-/- (8 weeks old) were fed a fat-enriched diet (21% wt/wt fat; 0.15% wt/wt cholesterol) for 3 weeks. After 1 week of diet feeding, mice were infused with either saline or AngII (1000 ng/kg/min) by osmotic minipumps for 2 weeks. Western blot analysis demonstrated that AngII infusion strongly increased autophagy proteins, Beclin1, Atg7 and LC3B-II in the abdominal aortas. By using GFP-LC3 transgenic mice, we determined the cellular distribution of autophagy protein under AngII-infused condition in the abdominal aorta. Immunofluorescent staining using red fluorescence tagged anti-GFP antibody showed a strong increase in GFP-LC3B in the smooth muscle cell (SMC) rich aortic medial layer of AngII-infused aortas. Furthermore, to test whether autophagy induction influences AAA formation, male and female LDL receptor -/- mice (8 weeks old) were fed a fat-enriched diet (21% wt/wt fat; 0.15% wt/wt cholesterol) supplemented with or without an autophagy inducer, celastrol (10mg/kg/day) for 5 weeks. After 1 week of diet feeding, mice were infused with AngII (500 or 1000 ng/kg/min) for 28 days by osmotic minipumps. Ex vivo measurement of abdominal aortas showed that celastrol supplementation profoundly increased AngII-induced AAA formation in male mice, with an incidence of 90% (10/11) compared to 36% (4/11) incidence in control group. Interestingly, in female mice, which are normally resistant to develop AngII-induced AAAs, celastrol supplementation significantly increased AngII-induced AAA formation as similar to male mice, with an incidence of 80% (12/15) compared to 6% (1/15) incidence in control group. Since celastrol supplementation mediated autophagy activation abrogates sexual dimorphism of AngII-induced AAAs in mice, next we examined whether sex differences influence autophagy activation in the abdominal aorta of male and female mice. Cross-sections of abdominal aortas from male and female GFP-LC3 transgenic mice infused with saline and AngII were immunostained with an immunofluorescent anti-GFP antibody. Interestingly, in both saline and AngII conditions, LC3B protein abundance is profoundly higher in males compared to females in which, LC3B abundance is very low under both basal and AngII infused conditions.

Conclusion: These findings demonstrate that enhanced autophagy by Celastrol supplementation accelerates AngII-induced AAA formation, and contributes to sexual dimorphism of AAAs in mice.

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Graduate Student

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Adaptive mechanisms maintain sterol balance following disruption of biliary cholesterol secretion

Objective: Biliary cholesterol excretion, via ABC transporter heterodimers (ABCG5/ABCG8), is the primary route for excess cholesterol elimination. In the loss of biliary cholesterol secretion, by obstructive cholestasis or genetic inactivation of ABCG5/ABCG8, biliary cholesterol levels decrease by 80%; however, whole-body sterol balance is maintained. This indicates the presence of an adaptive indirect or non-biliary route of cholesterol elimination in the gastrointestinal tract. The present study aims to investigate the time course and degree of these adaptive changes in cholesterol efflux when ABCG5 and ABCG8 are inactivated specifically in the liver of adult mice.

Methods/results: Mice harboring lox-p sites at the Abcg5/Abcg8 locus alone or a second transgene encoding cre-recombinase with the albumin promoter (chronic ABCG5/ABCG8 deletion). A third group of Abcg5/Abcg8flox mice were administered an AAV encoding Cre-recombinase driven by a liver specific promoter at 8 weeks of age (acute ABCG5/ABCG8 deletion). The mice were maintained on standard rodent chow and feces were collected two days prior to and up to 28 days after AAV administration. At day 28; basal bile, plasma, liver and five segments of intestine of equal length were collected. No differences in plasma cholesterol were detected across the groups. Biliary cholesterol secretion was significantly reduced by 54% and 66% in mice modeling acute and chronic deletion, respectively. No differences in fecal neutral sterols or total fecal bile acids were detected at any time point after AAV administration.

Conclusions: Despite the marked reduction in biliary cholesterol elimination, no differences were observed in total fecal cholesterol elimination. Potential explanations include the adaptive changes happening too rapidly to be detected in the present study. Nevertheless, adaptive changes to maintain cholesterol elimination do take place upon disruption of the biliary pathway and future experiments include investigating where along the gastrointestinal tract the absence of biliary cholesterol secretion is accommodated for in cholesterol elimination.

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Graduate Student

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Androgen Mediates the High Susceptibility of Male Mice to Aldosterone and High Salt-induced Abdominal Aortic Aneurysm

Objective: Abdominal aortic aneurysm (AAA) has a high mortality rate when ruptured, and male sex is a non-modifiable risk factor for AAA in human. Our lab recently reported a novel mouse AAA model, in which aldosterone infusion in the presence of high salt intake induces an age-dependent AAA. However, it is unknown whether this AAA model shows a sex difference similar to human. In this study, we investigated whether the male mice are more susceptible to aldosterone plus high salt-induced AAA and whether the pathway is mediated through androgen.

Approaches and Results: To determine whether there is sex difference in aldosterone plus high salt-induced AAA, 10-month-old male and female wild-type C57BL/6 mice were infused with aldosterone (200ug/kg/d) and salt for 4 weeks to induce AAA. We found that female mice were effectively protected from the AAA induction: none of the nine female mice developed AAA. In contrast, 7 of the 10 male mice developed AAA independent of blood pressure level. The AAA in the male mice was associated with breakages of aortic elastin. Interestingly, the female mouse heart and kidney were also protected from aldosterone and high salt-induced fibrosis. To further investigate if the male sex organ plays a role in the formation and progression of AAA, ten-month-old male mice received sham (n=13) or orchiectomy (n=15) surgery and then infused with aldosterone in the presence high salt intake. Results showed that orchiectomy powerfully protected the male mice from AAA: only 1 of the 15 orchiectomized mice developed AAA. In contrast, 9 of the 13 sham-operated mice developed AAA. We then tested whether loss of the male sex hormone testosterone mediates the protective effect of orchiectomy. The following four groups of 10-month-old mice were administered with aldosterone and high salt: 1) normal male mice, 2) orchiectomized male mice, 3) orchiectomized male mice plus DHT pellet (10mg pellet/60 day release; 0.16mg/d); and 4) male mice administered with androgen receptor degradation enhancer ASC-J9 (50mg/kg/d). The results demonstrated that DHT restored the high AAA incidence in the orchiectomized male mice. The ASC-J9 mimicked the orchiectomy significantly decreasing the incidence of AAA.

Conclusions: Taken together, our results demonstrated that male mice are much more susceptible to aldosterone and high salt-induced AAA. Androgen and its receptor mediate the high susceptibility in male mice. These results suggest the aldosterone plus high salt-induced AAA is a useful mouse model for the study of mechanisms underlying the sex difference in AAA, and implicate androgen and its receptor as a potential therapeutic target in AAA treatment.

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Graduate Student

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Identification of Novel Therapeutic Strategies to Treat Long QT Syndrome

Introduction: Kv11.1 (KCNH2) channel proteins conduct the delayed rectifier potassium current (IKr) during the cardiac action potential (AP), which is critical for regulating ventricular repolarization. Loss-of-function mutations in the KCNH2 gene delay repolarization, and are a leading cause of the deadly pro-arrhythmic Long QT Syndrome (LQTS). Most loss-of-function KCNH2 missense mutations linked to LQTS generate Kv11.1 channel proteins that decrease the trafficking to the cell surface membrane.

Purpose/Hypothesis: Class III antiarrhythmics, i.e. dofetilide, are highly selective IKr blockers that result in drug-induced LQTS. Incubating cells expressing trafficking-deficient Kv11.1 channel proteins in dofetilide for 24-48 hrs increases Kv11.1 channel trafficking to the cellular membrane. Although dofetilide corrects for the congenital trafficking deficiencies long-term use causes drug-induced LQTS. The goal of this project was to test the hypothesis that incubating cells expressing trafficking-deficient Kv11.1 channel proteins in dofetilide and the IKr activator (LUF7244) can increase Kv11.1 channel trafficking and decrease dofetilide-mediated IKv11.1 block. Methods: LUF7244 was custom synthesized and applied to human embryonic kidney (HEK) cells stably expressing wild type (WT) Kv11.1 channel proteins and cells expressing trafficking-deficient LQTS-linked Kv11.1 missense mutation G601S-Kv11.1 channel protein. These cells were studied in control conditions (no drug) and with LUF7244 (10μM) with or without dofetilide (1μM). Kv11.1 currents (IKv11.1) were measured in the cells using the whole-cell patch clamp technique.

Results: In cells expressing WT-Kv11.1 or G601S-Kv11.1 channel proteins, LUF7244 acute application and long-term (24-48 hr) incubation increased IKv11.1 despite the presence of dofetilide. Compared to control cells expressing WT-Kv11.1 channel proteins, the peak IKv11.1 at 0 mV after dofetilide + LUF7244 application increased IKv11.1 from 23.9±3 pA/pF to 45.3±9 pA/pF (p<0.05, n=10 each). In G601S-Kv11.1 expressing cells, long-term treatment with LUF7244 and dofetilide increased IKv11.1 compared to control. The peak IKv11.1 at 0 mV increased from 1.6±0.3 pA/pF to 7.7±1.4 pA/pF (p<0.05, n= 10) from control to chronic dofetilide + LUF7244 treatment respectively. Surprisingly, LUF7244 application alone increased IKv11.1 23.5±2.8 pA/pF (p<0.05, n=6 each), three times more than cells chronically incubated.

Conclusion: LUF7244 application did not interfere in dofetilide-mediated trafficking of G601S-Kv11.1 channel proteins and increased IKv11.1. LUF7244 application alone increased IKv11.1 ~3-fold without dofetilide-mediated correction of trafficking deficiencies in G601S-Kv11.1 channel protein. This raises the possibility that IKv11.1 activators alone could be used to treat LQTS patients with KCNH2 mutations that encode trafficking deficient Kv11.1 channel proteins.

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Staff

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Prolonged Pharmacological Inhibition of Lysyl Oxidase Induces Heterogeneous Aortic Pathologies in Juvenile Male and Female Mice

Background and Objective: Aortic aneurysms and dissections are devastating cardiovascular diseases associated with high mortality. Disruption of elastin fibers is an important feature of aortic aneurysms and dissection. Lysyl oxidase (LOX) is the enzyme that initiates the irreversible covalent cross-linking of collagen and elastin in vascular tissues. In this study, we determined effects of inhibiting LOX on aortic pathology in both male and female mice.

Methods and Results: Four-week-old male and female C57BL/6J mice were administered β-aminopropionitrile monofumarate (BAPN; ~1g/kg/d in drinking water), a LOX inhibitor, or control (drinking water alone) for 12 weeks. In mice administered BAPN, during the first month, more males succumbed to aortic rupture than females (7 of 33 male and 1 of 34 female mice; Fisher exact test, P = 0.027). However, females had greater incidence of aortic rupture in subsequent months, with an equivalent overall occurrence over the 12 weeks of BAPN administration (11 of 23 males and 10 of 24 females; Fisher Exact test, P = 1). In mice with aortic rupture-induced death, necropsy showed that aortic dissection was either restricted to the thoracic aortic region or from the thoracic region extending to the abdominal region, and aortic rupture in male mice occurred predominantly in the abdominal region (thoracic versus abdominal: 14% versus 86%), whereas aortic rupture in female mice was more prevalent in the thoracic region (thoracic versus abdominal: 73% versus 27%; male versus female: P = 0.005). In mice that survived 12 weeks of BAPN administration, the maximal diameter of ascending, arch and descending thoracic aortas measured in situ was larger than controls in both male and female mice. The severity and location of aortic pathologies was highly variable for both sexes. No overt pathologies were observed in the abdominal aorta of mice subject to 12 weeks of BAPN administration. Accordingly, there were no differences in maximal diameters, measured ex vivo, of the suprarenal aortic region between mice administered control and BAPN.

Conclusion: These results demonstrate heterogeneity of thoracic aortic pathologies induced by inhibition of LOX, which also exhibits sexual dimorphism in onset of aortic dissection between male and female mice.

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Staff

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Nonhuman primates consuming high-fat/high-cholesterol diet have signs of non-alcoholic fatty liver disease

Excessive accumulation of lipids in the liver is a major risk factor for the development of non-alcoholic steatohepatitis (NASH), which can progress to liver cirrhosis, hepatocellular carcinoma, and ultimately death. However, there are no clinically approved therapies for the treatment or prevention of NASH and most preclinical studies have been limited to mouse models. In our current study, we analyzed liver samples from male cynomolgus monkeys before and after 20 months of high fat/high cholesterol feeding (HFHC diet: 0.4% cholesterol and 38% fat Kcal). As expected, NHPs on HFHC diet displayed severe hypercholesterolemia, which was characterized by significantly elevated VLDL and LDL particle number and cholesterol concentration. Compared to baseline samples, liver collected after HFHC diet had elevated total cholesterol and triglycerides levels. In addition to steatosis, the livers had increased inflammation. Massive accumulation of CD68 positive macrophages/Kupffer cells was observed in the liver of NHPs on HFHC diet. Pro-inflammatory markers such as serum amyloid A, C-reactive protein, and MCP-1 were also significantly increased in the liver of NHPs on HFHC diet. Based upon a significant increase in the mRNA of the necrosis marker FAS ligand and significant elevations in circulating AST and ALT levels, the HFHC diet induced hepatocyte death and consequently impaired liver function in monkeys fed the HFHC diet. However, the HFHC diet caused no change in the expression of genes associated with collagen deposition and liver fibrosis. Moreover, blinded assessment of liver sections by a pathologist revealed that due to insufficient hepatic inflammatory cell infiltration and fibrosis, the HFHC diet-fed monkeys had not developed NASH. Therefore, in a new cohort of NHPs, we are attempting to drive NASH formation by enriching the HFHC diet with fructose, which has been a critical dietary component for mouse NASH models.

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Postdoc

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Inhibition of Hippo-YAP Signaling Pathway Attenuates Angiotensin II-induced Ascending Aortic Aneurysms in Male LDL Receptor Deficient Mice

Objective: Hippo-YAP signaling pathway is well known to regulate cell proliferation and differentiation. Mammalian STE20-like protein kinase 1 (MST1), a core component of the Hippo pathway, promotes cell apoptosis and inhibits cell proliferation. Our preliminary Western-blot data demonstrate that angiotensin II (AngII) infusion enhances the expressions of Hippo-YAP pathway related proteins (MST1, P-MOB1, P-YAP, and total YAP) in the aortic wall. Recent studies showed MST1 inhibition, or activation of its downstream YAP signaling suppress vascular smooth muscle cell apoptosis. However, the functional role of Hippo-YAP pathway in the development of aortic aneurysms is still unknown. The purpose of this study is to examine the effect of Hippo-YAP signaling inhibition by XMU-MP-1 (inhibitor of MST1 and 2) on AngII-induced ascending and abdominal aortic aneurysm formation in mice.

Methods and Results: Male LDL receptor -/- mice (8-10 weeks old; n=16-17 per group) were fed a fat-enriched diet (21% wt/wt milk fat; 0.15% wt/wt cholesterol) for 5 weeks. The MST inhibitor, XMU-MP-1 (3 mg/kg/day) or vehicle was administered daily by gavage for 5 weeks. After 1 week of high fat feeding and MST inhibitor dosing, the mice were infused subcutaneously with AngII (1,000 ng/kg/min) by osmotic minipumps for 4 weeks. AngII increased systolic blood pressure similarly in both groups. XMU-MP1 administration had no significant effect on AngII-induced on abdominal aortic luminal dilation as measured in-vivo by ultra-sonography (pre-infusion- Vehicle: 1.13 ± 0.02 versus XMU: 1.13 ± 0.04 mm; post-infusion- Vehicle: 2.00 ± 0.15 versus XMU: 2.25 ± 0.23 mm; P = not significant) and external aortic expansion as measured by ex vivo diameter (Vehicle: 2.00 ± 0.18 versus XMU: 2.27 ± 0.29 mm; P = not significant). XMU-MP1 administration significantly suppressed AngII-induced ascending aortic dilation and aortic arch expansion compared to vehicle group (Ascending arch area: Vehicle: 13.02 ± 0.67 versus XMU: 10.84 ± 0.60 mm; P = 0.021; Aortic Arch Area: Vehicle: 21.26 ± 0.79 versus XMU: 18.77 ± 0.80 mm; P = 0.036, respectively). However, XMU-MP1 administration had no effect on atherosclerotic area of aortic arch (Vehicle: 4.62 ± 0.82% versus XMU: 6.57 ± 1.76%).

Conclusion: These findings suggest that Hippo-YAP signaling inhibition by XMU-MP-1 attenuates AngII-induced ascending aortic dilation, but did not influence abdominal aortic aneurysm formation in male LDL receptor -/- mice.

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Graduate Student

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Rapid Decay of Calcium Current in Myocardial Rad Deletion is the Result of Increased Calcium Dependent Inactivation of the L-type Calcium Channel

Background: The L-type Ca2+ channel current (ICa,L) provides trigger calcium to contribute to cellular calcium signaling. Disruption of channel inactivation can lead to increased cellular excitability and arrhythmias. L-type Calcium channels (LTCC) are inactivated by two mechanisms: calcium dependent inactivation (CDI) and voltage dependent inactivation (VDI). Rad GTPase associates with LTCC and serves as an endogenous inhibitor of LTCC activity. Overexpression of Rad blocks ICa,L; absence of Rad increases ICa,L. Using a cardiac-restricted inducible Rad knockout mouse, we found that ICa,L was increased without evidence of arrhythmias.

Objective: To test the hypothesis that myocardial Rad deletion increases early ICa,L without increasing late, arrhythmogenic ICa,L by accelerating inactivation kinetics, specifically CDI.

Methods: We used our cardiac-restricted inducible Rad knockout mouse (cRadKO). We examined ICa,L through the whole cell configuration of the patch clamp technique. Calcium was used as the charge carrier in the presence of calcium chelators EGTA or BAPTA to assess CDI. Barium was used as the charge carrier to assess VDI. Decay of current was measured at 30 milliseconds (ms) and 150 ms after peak.

Results: In the presence of EGTA and Calcium or BAPTA and Calcium, cRadKO ICa,L decay was faster than WT. ICa,L decay at 30 ms (At -10 mV, EGTA: cRadKO = 71 +/- 3%, n=15; WT = 34 +/- 5%, n=18; $p<0.0001$; BAPTA: cRadKO = 41 +/- 4%, n=7; Radfl/fl = 23 +/- 5%, n=9; $p=0.02$). No difference was found between models at 150 ms. When Barium was used as the charge carrier, there was no difference between cRadKO ICa,L decay and WT ICa,L decay.

Conclusion: VDI was not difference between the two models. Early cRadKO ICa,L decay was faster than WT ICa,L because of accelerated CDI. The calcium contributing to the inactivation does not come from calcium induced calcium release from the sarcoplasmic reticulum, but instead from calcium in the nanodomain of the LTCC. These new findings challenge the canonical assumption that CDI stems directly from the sarcoplasmic reticulum, and continue to support that myocardial Rad deletion is a promising cardiac inotropic therapeutic direction.

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Staff

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Free thiols effectively suppressed inflammatory activity of oxidized phospholipids in the vascular endothelial cells

Study Goal: Metabolic stresses, including overnutrition, obesity, and hyperlipidemia elevated reactive oxygen species (ROS) formation in the vascular system. ROS interacts with unsaturated acyl chains of phospholipids located on the LDL particles and in the cell membranes. The oxidation products of phospholipids (Ox-PLs) are electrophilic, proinflammatory, and atherogenic. In this study, we tested a hypothesis that the electrophilic property of Ox-PLs is essential for the inflammatory gene expression in the vascular endothelial cells.

Methods & Results: We prepared oxidation products of the phospholipids by 48-72 hour of air exposure of PAPC (1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine). The mixture of oxidation products of phospholipids contained the bioactive components (PGPC, POVPC, and PEIPC) previously identified in the minimally-modified LDL particles produced in vivo. The Ox-PAPC induced a broad and robust gene expression changes in the human aortic endothelial cells (HAECs) after 4-hour cell treatment at the dose of 50ug/ml. There were no cell-damage or induction of apoptosis by the Ox-PL treatment. The cotreatment of the nucleophilic free thiols with the Ox-PL effectively suppressed the bioactivity of the Ox-PLs. We applied a list of endogenous and exogenous free thiols for the comparison: i.e., glutathione, cysteine, beta-mercaptoethanol, dithiothreitol, and N-acetyl-L-cysteine. All free thiols showed similar suppression effects. To confirm the role of electrophilic property for the inflammatory gene induction, we tested N-ethylmaleimide, which induced IL-8 and HO-1 expression in the cells like Ox-PAPC. The gene induction by N-ethylmaleimide was also suppressed by free thiol GSH cotreatment. Normal high-density lipoprotein (HDL) has antioxidant properties, and we confirmed that the HDL also showed effective suppression of the Ox-PL-induced inflammatory gene expression in the HAECs.

Conclusion - The electrophilic property is required for the bioactivity of Ox-PLs in the vascular endothelial cells. Endogenous and exogenous free thiols effectively suppressed inflammatory gene expressions by the Ox-PLs. These results suggested that synthetic or natural products with free thiols may be applicable as tools to ameliorate the vascular inflammatory responses associated with oxidative and metabolic stresses in humans.

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Undergraduate

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Valsartan, but not sacubitril, is effective in lowering AAA formation in hyperlipidemic male mice

Objective: Abdominal aortic aneurysms (AAAs) are a sexual dimorphic disease where males have 4-10 fold higher prevalence compared to females. Earlier work in our lab has shown that male, vascular smooth muscle cells have higher amounts of neprilysin compared to female, vascular smooth muscle cells. Neprilysin is a metalloendopeptidase that can cleave multiple substrates including angiotensin II (AngII) and natriuretic peptides. Sacubitril is a known competitive inhibitor of neprilysin; however, its effects on AAAs are unknown. Valsartan is an angiotensin receptor blocker that has been shown to block AngII-induced AAAs in rodent models. The combination of sacubitril and valsartan has been utilized in the treatment of heart failure; however, it has not been tested against AAA formation. Therefore, we evaluated the effects of valsartan, sacubitril, and the combination on the formation of AngII-induced AAAs.

Methods and Results: Ldlr-/- male mice (8-12 weeks of age) were fed a Western diet (Teklad, TD.88137) for the duration of the study. Valsartan was given at 0.3, 0.5, 1, 6, and 20 mg/kg/day for seven days prior and during the 28-day AngII infusion. Sacubitril was given at 1, 6, and 9 mg/kg/day in the same manner. Vehicle-treated males were given solvent only (1:1 Propylene glycol/DMSO mix). Day 27 ultrasounds show that valsartan dose-dependently inhibited AAA formation when compared to the vehicle treated mice, except for the 0.3 mg/kg/day dose ($P<0.05$). Interestingly, sacubitril did not inhibit AAA formation at any doses studied ($P>0.05$). Combinational drug therapy using 0.3 mg/kg/day valsartan with 1 mg/day/day sacubitril or 0.5 mg/kg/day with 9 mg/kg/day sacubitril also showed no effect on internal abdominal aortic diameters by ultrasound ($P>0.05$). Atherosclerosis and other study endpoints are currently being examined in these studies.

Conclusions: Valsartan showed effective inhibition of AAA formation in a dose-dependent manner. However, sacubitril did not show any effect on AAA formation. Interestingly, combinational drug therapy also did not show any effect on AAA formation, but higher doses of valsartan may be required in order to influence AAA development.

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Staff

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Comparisons of Levels of Inflammatory Biomarkers in Serum and Saliva: Results from a Cardiovascular Disease Risk Reduction Study

Background/ Objectives
Systemic inflammation is associated with high risk for cardiovascular disease (CVD). The American Heart Association recommends that high-sensitivity C-reactive protein (hsCRP), an inflammatory marker most often measured in blood, be used to guide treatment decisions for patients at intermediate or high CVD risk. Upstream from hsCRP are interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) that may be better targets for therapeutic interventions than CRP. Although these markers can be measured in blood, it may also be possible to measure these in saliva which would provide a medium that requires a less invasive method of collection. The purpose of this study was to assess agreement and correlations between serum and salivary levels of IL-6, LI-1β and TNF-α and to examine whether the biomarkers were correlated with serum hsCRP.

Methods
Serum and saliva specimen were collected at baseline from 421 adults (mean age 51.4 ± 13.3 years, 73.9% female) who were at risk for CVD and participating in a risk reduction intervention. All specimen were collected at the same time during baseline data collection. To measure serum levels of the biomarkers, venous blood was drawn using a red top tube. Blood was immediately centrifuged and serum separated and frozen at -800C. For lab analyses, samples were run using a six-plex kit from Millipore and were read in a Luminex IS100 (Austin, TX). To measure salivary biomarkers, participants expectorated 5 ml of saliva into a vial that contained freeze-dried protease inhibitor. Undiluted samples were run using the same procedure as for serum. For statistical analyses, Bland-Altman analyses were conducted using standardized values to assess agreement between serum and saliva levels of IL-6, IL-1β, and TNF-α. Log transformations and Spearman's ρ were used for correlation analyses due to non-normal distribution of the biomarkers. All analyses were conducted using SPSS v.25. Results For the Bland-Altman analyses, there was a bias of 0 for each of the markers and most mean differences between serum and saliva biomarkers fell within the limits of agreement. For serum and salivary IL-1β, only 7 observations were outside the limits of agreement. For IL6 and TNF-α, only 27 and 12 observations, respectively, were outside the limits of agreement. Correlation analyses showed positive correlations between serum and saliva levels of IL-6 (ρ =.13, p=.006) and of TNFa (ρ =.10, p=.03). Only serum levels of IL6 were correlated with hsCRP (ρ =.14, p=.006)
Discussion and Conclusion
Our findings indicate that saliva may provide as accurate a medium as serum for determining levels of IL-6, IL-1β and TNF-α. Measuring inflammatory biomarkers in saliva, therefore, could potentially provide a less invasive alternative to measuring these in serum. It is of interest that only serum IL-6 was correlated with serum hsCRP. Although this may be attributed to the fact that IL-1β and TNF-α are further downstream from CRP than is IL-6, the lack of correlation between salivary IL-6 and hsCRP needs further exploration.

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Postdoc

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Effect of Ovariectomy on Peripheral and Central Period 2 Gene Expression in Mice

Objective
Oscillations in circulating estrogen levels is associated with the estrous cycle and is linked to circadian rhythms of clock genes, physiology, and behavior in female rodents. Circadian rhythms in clock gene expression, such as Period 2 (Per2), is observed not only in the suprachiasmatic nucleus (SCN) but also in other peripheral tissues. However, whether estrogen affects Per2 oscillation in peripheral versus SCN tissues is mostly unknown.

Approach and Results
We used the mPer2Luc female mice to address this critical question. The mPer2Luc mice express a Per2-luciferase fusion gene that allows real-time monitoring of the clock gene Per2 oscillations in various tissues in isolated tissues (ex vivo) and in living mice (in vivo). The mPer2Luc female mice (28-40 weeks old) were divided into two groups: sham (n=5) and ovariectomized (n=5). The locomotor activities were continuously recorded by ClockLab. The daily oscillation of mPer2 bioluminescence was recorded by LumiCycle in real-time in tissue explants and using the IVIS system in vivo. Our preliminary results showed that the body weight was increased but the uterus weight and the locomotor activities were decreased in ovariectomized mice compared to the sham groups of mice. Interestingly, the daily oscillation of Per2 in the liver didn't have phase changes after surgeries for two weeks, but the relative intensity of liver Per2 was lower in ovariectomized living mice than the sham group of mice. Moreover, whereas the daily oscillation of Per2 in mot peripheral tissues examined by Lumicycle did not change, the daily oscillation of Per2 in the SCN and the peripheral tissues related to metabolism, such as white adipose tissue, displayed 4.48±1.03 and 3.96±0.75 hours of phase delay, respectively, in ovariectomized mice compared to the sham group.

Conclusions
Ovariectomy differentially suppresses the daily oscillation of Per2 in the SCN, white adipose and liver. These findings suggest that the effect of estrogen on the circadian rhythm of central and peripheral clock genes is tissue-specific.

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Graduate Student

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Inflammasome activation triggers DVT through pyroptosis

Venous thromboembolism (VTE), including pulmonary embolism (PE) and deep vein thrombosis (DVT), is one of the most common causes of cardiovascular death worldwide. Tissue factor (TF) derived from myeloid leukocytes play a key role in the development of DVT. However, the mechanism of TF release from myeloid leukocytes that contribute to the development DVT is not well elucidated. In this study, we reported a critical role of inflammasome activation and pyroptosis in the development of DVT. Using a flow restriction-induced mouse DVT model in the inferior vena cava, we show that deficiency of caspase-1 protected against flow restriction-induced DVT. Flow restriction-induced DVT was inhibited by deficiency of Gasdermin D (GSDMD), an essential mediator of pyroptosis. After induction of DVT, fibrin was deposited in the vein as detected by Western blot with a monoclonal antibody that specifically recognizes mouse fibrin in the wild type mice, but not the caspase-1 deficient mice and GSDMD deficient mice. IL-1 was increased in tissue of vein following induction of DVT, indicating that inflammasome is activated during DVT, which was inhibited by caspase-1 or GSDMD deficiency. Our data reveal a critical role of inflammasome activation and pyroptosis in the development of DVT.

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Postdoc

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Inhibition of sodium-glucose cotransporter-2 prevents the progression of kidney injury and vascular calcification in a rat model

Objective: Vascular calcification is a critical health concern in patients with diabetes and kidney dysfunction. Since vascular calcification is linked to systemic mineral imbalance such as hyperphosphatemia, lowering serum phosphorus concentrations with nutritional therapy and administration of oral phosphate binders has been a major therapy for preventing vascular calcification. However, a decrease in serum phosphorus concentrations alone does not completely suppress vascular calcification. To determine a mode of enhanced suppression of vascular calcification, we examined whether, an anti-diabetic drug, canagliflogin, inhibited progressive kidney injury and vascular calcification.

Methods and Results: At 9 weeks of age, diabetes was induced in male Wistar rats by intraperitoneal administration of streptozotocin (STZ), and one week later CKD was induced by removal of the left kidney. One week after the surgery, rats were fed a high phosphate diet (2.0% wt/wt)) and intraperitoneal administration of paricalcitol (3 times a week), and were administered 10 mg/kg/day either canagliflogin or vehicle for a duration of 2 weeks. At termination, blood and urine were collected, and renal and thoraco-abdominal aortas were dissected. Plasma concentrations of urea nitrogen (BUN), creatinine (Cre), calcium, and phosphate were measured. Immunostaining was performed to evaluate kidney injury. Vascular calcification in aortas was evaluated by Von Kossa and Alizarin Red S staining, and measurement of calcium content. STZ induced diabetes and nephrectomy that provoked kidney damage with renal tubular injury, renal-enlargement, and vascular calcification in aortas. Inhibition of SGLT2 by canagliflozin suppressed renal tubular injury, renal-enlargement, and vascular calcification.

Conclusions: Our results support that selective inhibition of SGLT2 receptor improved the progression of kidney injury and reduced vascular calcification in diabetic and chronic kidney disease related conditions.

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Graduate Student

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Probing the Connections between Micro-Vasculature of Bone and its Mechanical Integrity and Pathology

The circulatory system spans across the entire human/animal body delivering a wide array of compounds that keeps our body functioning. While it is apparent that vasculature extents to vital organs, the network that resides within bones could hold more valuable information than we expect. The vasculature tree that exists within the bone serves as a key component in maintaining physiological functions and preserving health of a human or animal.

In our studies of sesamoid bones of racehorses, we noted the micro-vasculature tree inside the bone. This study seeks to establish possible interconnections between the micro-vasculature tree and the complexity of the loading conditions, microarchitecture, and mechanical integrity of the bone, and their impact on causing the pathological diseases in the bone. Sesamoid bones are analyzed using a micro computed tomography scanner in order to map the microarchitecture within the bone itself. From the scans, we also isolate the micro-vasculature tree or interconnected canals inside the bone. We hypothesize that If these canals are damaged, even slightly, then a resulting pathological complex could arise in the bone. With depleted nutrients to the bone leading to possible degradation of bone mechanical integrity, it is foreseeable that a change in the mechanical properties of the bone such as Young's modulus, fracture toughness, and yield strength will be affected negatively; and with continued loading to the joints, a risk of fracture, breakage, or fragmentation of the bone significantly increases. This, in the racing industry, often leads to not only the end the horse's career but also its life.

Once the vasculature of the bone is depicted, mechanical testing will be done to slices of the bone sample. The vasculature will then be reexamined to evaluate the integrity of the canals. If a quantifiable relationship from mechanical loading and the health of the canals, ultimately the health of the overall bone sample, can be made, measurements can be considered to reduce stress sustained to the joints.

From this point, further investigation could relate these findings to human gait. Bone injuries are a very common injury to humans, especially athletes and the elderly. By understanding the natures of these injuries in relation to mechanical loading and vascular health, we can continue to improve healthcare for those at-risk patients by preventative measures and possible repair of central bone vasculature.

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Graduate Student

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An In-situ and Real-time Means to Detect Biomarkers Crucial to Cardiovascular Diseases

Atherosclerosis is a disease in which plaque builds up within arteries, leading to the blockage of blood flow. High cholesterol is one cause of arterial plaque buildup. Currently, there is a large need in the research field for a device capable of monitoring and analyzing concentration of various biomarkers including cholesterol in-situ and in real-time, as the current method only provides detection through a blood test with results taking days to receive. Implementation of such a device would provide relatively instant results and would advance the science by providing in-situ and real-time monitoring of cholesterol concentrations, with the potential for use with other measured parameters.

Two amperometric biosensors in a three-electrode configuration were developed to detect cholesterol by the immobilization of a cholesterol oxidase at the working electrode. Two cholesterol oxidase (ChOx) immobilization methods were utilized: self-assembled monolayers (SAM) of octadecanethiol (ODT) or thioglycolic acid (TGA) and electropolymerization of pyrrole. One sensor using a SAM of ODT was created by dissolving ODT in ethanol to create a solution. Another sensor using a SAM of TGA was created by dissolving TGA in ethanol to create a solution. Each sensor was then submerged in its corresponding solution for 6 hours. When removed, a cyclic voltammetry experiment was used to ensure the working electrode was functionalized. Each SAM electrode was then functionalized with the cholesterol enzyme by submerging the three-electrode system in a solution of pyrrole and cholesterol oxidase in Phosphate Buffer Solution (PBS). The sensor utilizing the method of electropolymerization of pyrrole was created by submerging the three-electrode system in a solution of pyrrole and cholesterol oxidase in PBS. Each sensor was attached to the potentiostat and a galvanostatic experiment was run. The functionality of each sensor was determined by performing amperometric experiments with differing concentrations of cholesterol stock solution.

Sensitivity, or the ability to detect concentration changes, is used to determine the effectiveness of the sensors. All three sensors presented a linear relationship between cholesterol concentration and current produced. However, the sensor that showed the best sensitivity was the pyrrole electropolymerization sensor.

In continuation, optimization of the pyrrole sensor should be performed. This should include improved sensor storage for increased longevity and continued experiments to create an improved calibration curve for cholesterol detection. Optimization of the SAM method could also be performed by determining an improved method of cholesterol enzyme attachment. Furthermore, the inclusion of cholesterol esterase as an additional cholesterol enzyme is another potential improvement that should be explored for cholesterol sensors.

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Graduate Student

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3D ultrasound evaluation for acute thymus involution in septic mice

Objectives
Sepsis induces acute thymus involution, which is reversible depending on the recovery from sepsis. Therefore, monitoring thymus involution during sepsis will broaden the understanding of sepsis. Previously, several studies applied a two dimensional ultrasound (2D-US) method to assess the thymus volume. However, a 2D-US can only assess one section in the thymus, which may not precisely evaluate the total thymus volume. To address this issue, we developed a three dimensional ultrasound (3D-US) method to assess thymus volume in septic mice.
Methods
We induced sepsis with cecum ligation and puncture (CLP) in 6-weeks-old C57BL/6J male mice. We measured the thymus volume at day 0, day 3 and day 10 with a 3D-US that is reconstructed by computer-aided analysis. The thymus weight was measured ex vivo. Then, we assessed the correlation between thymus volume and thymus weight.
Results
We induced sepsis with 25G and half ligation. The 10 days-survival percentage was 71.4 %. The mean thymus weight was 63.4 ± 3.4 mg, 7.5 ± 0.6 mg, and 19.9 ± 2.4 mg at day 0, day 3 and day 10 respectively. The mean thymus volume was 34.4 ± 1.8 mm3, 2.9 ± 0.4 mm3, 7.9 ± 2.0 mm3 at day 0, day 3 and day 10 respectively. Both of the thymus volume and the thymus weight were significantly reduced at day 3 after CLP (p<0.001). The thymus volume was well correlated with the thymus weight at day 0, day 3 and day 10 respectively (day0, r = 0.787, p = 0.004; day3, r = 0.719, p = 0.044; day10, r = 0.787, p = 0.02).
Conclusion
We developed a 3D-US method to monitor sepsis-induced thymus involution. The thymus volume assessed by 3D-US significantly correlated with the thymus weight at every time point. This 3D-US method is non-invasive and can be translated to investigate the clinical importance of thymus involution in septic patients.

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Graduate Student

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Quantifying the effects of HCM using MRI

Hypertrophic cardiomyopathy (HCM) is a cardiac disease that mainly results from genetic mutations, which in turn cause the heart myocardium to become significantly thick and sometimes contributes to myofiber disarray. The thickened myocardium can interrupt the functionality of the heart in different ways. It can either stiffen the left ventricle, which reduces the capacity of blood that the LV can hold and the amount of blood pumped out to the body, or it can restrict the blood flow out of the heart due to an enlarged septal wall. HCM has affected a large number of Americans (nearly 700,000) and is considered as the most common cause of cardiac death in young people.

This study was designed to track several influences of HCM, including dimensional (e.g. thickened wall, reduced LV cavity volume, etc.) and structural (e.g. stress, strain, stiffness, etc.) changes in the heart. Therefore, a large group of healthy and sick mice having HCM caused by mutations in sarcomeric proteins were bred at the University of Kentucky. This disease progresses in such a way that its effects on the left ventricle's myocardium increases as the mice get older. For this purpose, based on a pre-scheduled timetable, the healthy and sick mice have been scanned several times starting at the age of 6 months old until the age of 14 months old via the 7T MRI scanner in the University of Kentucky MRISC. The high resolution MRI images acquired thus far have shown an increase in the thickness of left ventricular wall and decrease in the cavity diameter in mid-ventricular end-diastolic slice. Furthermore, strain analysis has been obtained on a number of healthy and sick mice via software developed in MATLAB named DENSAnalysis in order to detect any functional changes (e.g. principal and shear strains) in the LV during a heartbeat in comparison with the healthy mice.

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Faculty

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Cost-effectiveness of Humanitarian Pediatric Cardiac Surgery Programs in Low- and Middle-Income Countries

Abstract Importance: Endorsement of global humanitarian interventions is based either on proven cost-effectiveness or perceived public health benefits. The cost-effectiveness and long term benefits of global humanitarian pediatric cardiac surgery are unknown and its funding is insufficient. Objective: To determine the cost effectiveness of the intervention (multiple 2-week long humanitarian pediatric cardiac surgery program development trips to various low and middle income countries). The secondary objective was to produce a measure of the long lasting effects of global humanitarian programs. Design: International, multicenter cost effectiveness analysis of a cohort of patients undergoing surgical treatment in low and middle income countries for congenital heart disease by a global charity organization (The William Novick Global Cardiac Alliance) during 2015. Secondary objective, estimated improvement in the United Nations Human Development Indicators (life expectancy, years of schooling and gross national income) for each individual survivor, as proxy for long term benefits of the intervention. Setting: Low and middle income countries (LMIC) where the intervention took place, including: China; Macedonia; Honduras; Iran; Iraq; Libya; Nigeria; Pakistan; Russia and Ukraine. Participants Costs calculated for every patient operated in the countries included. Primary and secondary outcomes were calculated for survivors under 16 years of age treated for a diagnosis of congenital heart disease. Main Outcomes Primary outcome: Cost-Effectiveness of the intervention. Secondary outcome: Potential gains in life expectancy, years of schooling and gross national income per capita for each survivor. Results: During 2015, 446 patients were served in 10 countries at an overall cost of $3,210,873. The cost-effectiveness of the intervention was $166 per Disability Adjusted Life Year averted. Each survivor on the cohort (n=390) potentially gained 43.4 DALYs averted, 3.8 extra years of schooling, and $315,409 of extra Gross National Income per capita during their extended lifetime (at purchasing power parity and 3% discounting). Conclusions and Relevance: Humanitarian pediatric cardiac surgery in LMIC is highly cost-effective. It also leaves behind a lasting humanitarian footprint by potentially improving individual development indices.

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Graduate Student

Podium Presentation – No Poster

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Recruitment From Myosin OFF State Steepens ESPVR in Finite Element Model of Left Ventricle

Finite element (FE) modeling is becoming increasingly prevalent in the world of cardiac mechanics, however many existing FE models are phenomenological and thus do not capture cellular level mechanics. This work implements a cellular level contraction scheme into existing nonlinear FE code to model ventricular contraction. Specifically, this contraction model incorporates three myosin states: OFF, ON, and an attached force-generating state. It has been speculated that force dependent transitions from the OFF to ON state may contribute to length-dependent activation at the cellular level (PMID: 30054031). This work investigates the contribution of this OFF state to ventricular level function, specifically the Frank-Starling relationship, as seen through the end systolic pressure-volume relationship (ESPVR).

Five FE models were constructed using geometries of rat left ventricles obtained via cardiac MRI. FE simulations were conducted to optimize parameters such that the FE predicted ventricular pressures for the models were within 5% of experimentally measured LV pressures. The models were validated by comparing FE predicted strain to experimentally determined strain. Simulations mimicking vena cava occlusion generated descending pressure volume loops from which ESPVRs were calculated. In simulations with the inclusion of the OFF state using force-dependent transitions to the ON state, the ESPVR calculated was steeper than in simulations excluding the OFF state. This suggests that force-dependent recruitment of thick filaments from the OFF state at the cellular level contributes to the Frank-Starling relationship observed at the organ level.

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Postdoc

Podium Presentation – No Poster

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Single-cell transcriptome analysis of human ascending thoracic aortic aneurysms reveals a cell atlas of the aortic wall

Ascending thoracic aortic aneurysm (ATAA) can lead to aortic rupture and other life-threatening complications. To date, no medications are clinically available that reliably prevent ATAA development and progression. Therefore, it is critical to understand the pathogenesis of ATAA and identify molecules that can be targeted to prevent disease formation and progression. The aortic wall consists of multiple cell types with various functions. Determining the makeup of cell populations in the aortic wall of ATAA patients and characterizing the genome-wide gene expression of each cell type may provide insight into the cellular and molecular basis of ATAA. Here, we performed single-cell RNA sequencing analysis of aortic tissues from patients with sporadic ATAA (women, n=4; men, n=3) and from heart donors (controls; women, n=1; men, n=1). We identified 7 smooth muscle cell (SMC) (or SMC-like) clusters, 4 fibroblast clusters, and 2 endothelial cell clusters, as well as immune cells including T cells, monocytes/macrophages, natural killer cells, and B cells. Of the SMC (or SMC-like) clusters, 1 displayed a contractile phenotype, and other 6 exhibited a unique molecular signature in addition to contraction. Gene ontology analysis of the conserved genes from those 6 SMC (or SMC-like) clusters revealed functions in the extracellular matrix (n=1; synthetic SMCs), angiogenesis and SMAD signaling (pericytes; n=1), the endoplasmic reticulum stress response (n=1), the inflammatory response (n=1), and migration (n=2). Within the SMC (or SMC-like) clusters, the proportion of synthetic SMCs and pericytes was slightly higher in ATAA tissues than in control tissues. In addition, synthetic SMCs of ATAA tissues showed the downregulated expression of genes associated with copper and zinc ion binding, which are critical to maintaining vascular SMC function. In pericytes of ATAA tissues, we observed the downregulated expression of genes associated with the bone morphogenetic protein signaling pathway, proliferation, migration, and the response to cytokines. Overall, our data illustrate a comprehensive cell atlas of the aortic wall in ATAA tissues and provide molecular insight into the function of each cell type in the aortic wall.

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Graduate Student

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HuR dependent regulation of inflammatory remodeling following myocardial ischemia/reperfusion injury

Myocardial infarction, resulting from ischemia/reperfusion (I/R) injury due to the obstruction of coronary blood flow, affects an estimated 800,000 Americans annually resulting in cost of nearly 12 billion dollars to the health care system. Recent medical advances have led to improved survival following acute myocardial infarction (MI), but unfortunately, the post-MI inflammatory and fibrotic remodeling of the heart is driving an increase in the prevalence of heart failure. Thus, increasing our understanding of the molecular mechanisms that promote inflammatory remodeling of infarcted myocardium following I/R injury remains an unmet need. The objective of this work is to determine the mechanistic role of HuR on the regulation of cardiac cytokine/chemokine expression and the functional effect of HuR on macrophage infiltration and polarization post-I/R.

To address this work, we subjected wild-type mice to 30 minutes of LAD (left anterior descending) coronary artery ligation followed by reperfusion at the appropriate time-points (2hrs, 24hrs, 3 days, 7 days, and 14 days). Hearts were then collected and processed for qRT-PCR, Western Blotting, IHC, and Picrosirius Red Staining. Preliminary data from our lab shows that HuR is activated at 2 hours and 7 days post-I/R, pharmacological inhibition of HuR reduces acute mRNA expression of IL-6, TNF-alpha, and ICAM-1 independent of acute (24 hour) infarct size, and a single dose of HuR inhibitor administered at reperfusion is sufficient to reduce macrophage infiltration at 7 days post-I/R and fibrosis at 2 weeks post-I/R.

In conclusion, we have shown HuR inhibition results in a significant blunting of pro-inflammatory cytokine and chemokine gene expressions two hours post-reperfusion *in vivo*, suggesting that HuR activity is necessary for the early induction of inflammatory gene expression networks. In addition, it appears that HuR inhibition attenuates macrophage infiltration and fibrosis formation following ischemia/reperfusion injury at 7 and 14 days post-I/R, respectively. Thus, indicates a possible intricate role for HuR in post-MI inflammatory and fibrotic remodeling.