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19th Annual Gill Heart Institute Cardiovascular Research Day
Lexington Convention Center
November 4, 2016

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9:00 Scientific Session I

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Yan Nie, PhD | Wakeforest University

Éva Gatineau, PhD | University of Kentucky

Samuel Slone | University of Cincinnati

Feiming Ye | University of Kentucky

Michael Petriello, PhD | University of Kentucky

Robert Helsley, PhD | Cleveland Clinic

Tara Shrout | University of Kentucky

Xiang Ye, PhD | University of Kentucky

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David Henson
Nuanced Immune Response to Apolipoprotein A-I in Cardiovascular Disease
Graduate Student
Principal Investigator - Vincent Venditto, PhD | University of Kentucky

Patrick Van Hoose, Ph.D.
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Postdoctoral Fellow
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Rahul Annabathula
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Medical Student
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Alumni Presentation
Brian Eigel, PhD
Innovative Approaches to Improving Cardiac Arrest Survival
Senior Vice President, Emergency Cardiovascular Care Programs
American Heart Association

Gill Award Lecture
Barry S. Coller, MD
From the Rivers of Babylon to the Coronary Bloodstream: The αIIbβ3 Story
Physician-in-Chief | Vice President for Medical Affairs | David Rockefeller Professor | Allen and Frances Adler Laboratory of Blood and Vascular Biology
The Rockefeller University

11:45 Lunch | Scientific Session II

Welcoming Comments
Robert DiPaola, MD, Dean, College of Medicine

Key Note Speaker
Mark Creager, MD
Vascular Disease and the Journey to Vascular Health
Immediate Past President, American Heart Association
Director, Heart and Vascular Center at Dartmouth-Hitchcock Medical Center

Schedule of the Day

1:00 Poster Session

1:00 – 2:00 – Present Odd Number Posters

2:00 – 3:00 – Present Even Number Posters

3:00 Scientific Session III

Research Blitz

Aida Javidan | University of Kentucky

Carrie Wiese | Vanderbilt University

Hisashi Sawada, MD | University of Kentucky

Sophia Yu, MD | Vanderbilt University

Jeff Chen | University of Kentucky

Sarah Anthony | University of Cincinnati

Yasir Alsiraj | University of Kentucky

Tianfei Hou | University of Kentucky

Yufeng Chu, PhD | University of Kentucky

Trainee Presentations

Keith Saum | Graduate Student
Advanced Glycation End Products Contribute to Endothelial Dysfunction through Suppression of Krüppel-like Factor 2 in Chronic Kidney Disease
Principal Investigator - Phillip Owens, PhD | University of Cincinnati

Chase Neumann | Graduate Student
Lipidomic Profiling Uncovers Land's Cycle Phospholipid Remodeling Pathways as Novel Therapeutic Targets in Clear Cell Renal Carcinoma
Principal Investigator – Mark Brown, PhD | Cleveland Clinic

Tori Stromp | Graduate Student
Differentiating Cardiac Fibrosis from Hypertrophy in Patients with End Stage Renal Disease Using Non-Contrast MRI and Imaging Guided Biomarkers
Principal Investigator – Moriel Vandsburger, PhD | University of Kentucky

Gill Award Lecture – Helen Hobbs, MD

PCSK9: From Discovery to Treatment

Investigator, Howard Hughes Medical Institute
Professor of Internal Medicine and Molecular Genetics
UT Southwestern Medical Center

5:00 Networking Reception

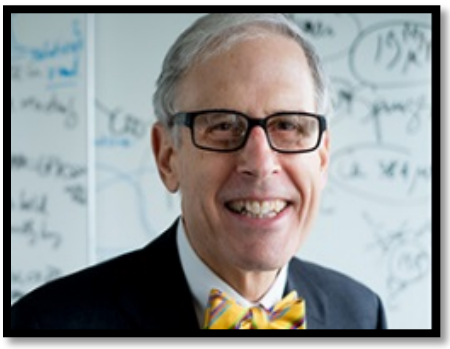
6:00 Dinner and Awards Presentations

Notes

2016 Gill Awards

The prestigious Gill Award recognizes notable and life-long achievements in research that have had a sustained impact on understanding cardiovascular biology and disease and/or that have changed the standard of cardiovascular clinical care.

2016 Gill Awardees are:



BARRY S. COLLER, MD

David Rockefeller Professor of Medicine
Head, Allen and Frances Adler Laboratory of Blood and Vascular Biology
Physician-in-Chief, Rockefeller University Hospital
Vice President for Medical Affairs
Rockefeller University

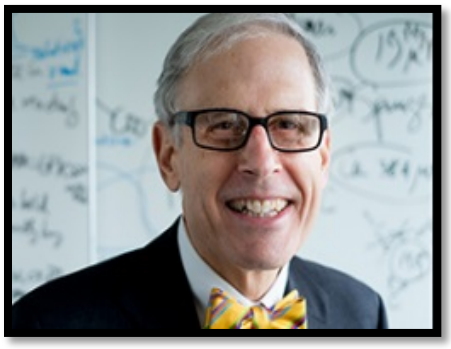
HELEN HOBBS, MD

Investigator, Howard Hughes Medical Institute
Professor of Internal Medicine and Molecular Genetics
UT Southwestern Medical Center

2016 Gill Awards

Barry Coller, MD

David Rockefeller Professor of Medicine
Head, Allen and Frances Adler Laboratory of Blood and Vascular Biology
Physician-in-Chief, Rockefeller University Hospital
Vice President for Medical Affairs
Rockefeller University



On September 1, 2001, Barry S. Coller, MD became the David Rockefeller Professor of Medicine; Head, Laboratory of Blood and Vascular Biology; Physician-in-Chief of The Rockefeller University Hospital; and Vice President for Medical Affairs at The Rockefeller University. He also serves as the founding Director of the Rockefeller University Center for Clinical and Translational Science and the Principal Investigator of the University's Clinical and Translational Science Award (CTSA) from the National Center for Advancing Translational Sciences of the National Institutes of Health. From 1993 to 2001, Dr. Coller was the Murray M. Rosenberg Professor of Medicine and Chairman of the Samuel Bronfman Department of Medicine at Mount Sinai School of Medicine in New York City, as well as Chief of the Medical Service of the Mount Sinai Hospital. Dr. Coller received his B.A. degree, magna cum laude from Columbia College in 1966, and his M.D. from New York University School of Medicine in 1970. He completed his residency in internal medicine at Bellevue Hospital in New York City and advanced training in hematology and clinical pathology at the National Institutes of Health. He joined the faculty at Stony Brook in 1976 as an Assistant Professor of Medicine in the Division of Hematology. During his years at Stony Brook he was the Clinical Director and Head of the Hematology Division, a consulting physician at the Northport VA Medical Center, Clinical Chief of the University Hospital Hematology Laboratory, a Director of the LI High Technology Incubator, a Director of the Stony Brook Foundation, and Associate Director for Biomedical Research of the Biotechnology Center for Advanced Technology. In 1982 Dr. Coller became Professor of Medicine and Pathology at Stony Brook. He was awarded the title of Distinguished Service Professor of Medicine and Pathology at Stony Brook in 1993.

Dr. Coller is a member of Phi Beta Kappa, Alpha Omega Alpha, the American Society for Clinical Investigation, the Association of American Physicians, the Institute of Medicine/National Academy of Medicine, and the National Academy of Sciences. He is a Fellow of the New York Academy of Medicine, the National Academy of Inventors, the American Association for the Advancement of Science, and the American Academy of Arts & Sciences, and a Master of the American College of Physicians.

2016 Gill Awards

Dr. Coller served as President of the American Society of Hematology in 1997-1998, the founding President of the Society for Clinical and Translational Science from 2008-2011, and as a member of the Advisory Council of the National Heart, Lung and Blood Institute from 2008-2012. In 2016 he completed service as Chair of the Steering Committee of the National Collaboratory, a trans-institute NIH Common Fund project, and he currently Chairs the Program Steering Committee of the NIH National Centers for Accelerated Innovations (NCAI). He also serves on the National Heart Lung and Blood Board of External Experts, and recently served on the NIH Advisory Committee to the Director (ACD) Intramural Research Program Working Group. Dr. Coller also serves on the National Academy of Sciences Human Gene Editing Committee.

Dr. Coller's research interests have focused on hemostasis and thrombosis, in particular platelet physiology. He developed a monoclonal antibody that inhibits platelet function and a derivative of that antibody (abciximab; ReoPro™; Centocor/Eli Lilly) was approved for human use by the FDA in 1994. It is now in clinical use throughout the United States, Europe, Scandinavia, Australia, and portions of Asia, to prevent ischemic complications of percutaneous coronary interventions such as angioplasty and stent insertion. More than 5.0 million patients have been treated with abciximab. He also developed an assay to assess platelet function, and automated derivatives of that assay to monitor therapy with abciximab, aspirin, and clopidogrel (Plavix™) have been approved for human use by the FDA (VerifyNow; Accumetrics). Dr. Coller is the recipient or a co-recipient of fourteen U.S. patents.

2016 Gill Awards

Helen Hobbs, MD

Investigator, Howard Hughes Medical Institute
Professor of Internal Medicine and Molecular Genetics
UT Southwestern Medical Center

Helen Hobbs received her undergraduate degree from Stanford University and her medical degree from Case Western Reserve University School of Medicine. After obtaining her clinical and post-doctoral training at Columbia-Presbyterian Hospital and University of Texas (UT) Southwestern Medical Center Dallas, she joined the faculty of UT Southwestern. She is currently Professor of Internal Medicine and Molecular Genetics, as well as Director of the McDermott Center for Human Growth and Development at UT Southwestern Medical Center in Dallas.

Since 2002, she has been an Investigator of the Howard Hughes Medical Institute. In partnership with Jonathan Cohen, she has identified genes and sequence variations contributing to metabolic and cardiovascular disorders with a focus on lipids and lipoproteins. Together they showed that rare genetic variations contribute to complex traits in the general population. By concentrating on alleles of low frequency and large phenotypic effect size, they have discovered new therapeutic targets for the prevention and treatment of heart disease, including PCSK9.
Most recently, they have identified genetic variants that contribute to the full spectrum of fatty liver disease, extending from hepatic steatosis to cirrhosis.

Hobbs was elected to the Institute of Medicine, American Academy of Arts and Sciences, and the National Academy of Sciences. She has been awarded prizes for her work from the American Heart Association [the Clinical Research Prize (2005) and the Distinguished Scientist Award (2007)], the Inaugural International Society of Atherosclerosis Prize (2012), the Pasarow Foundation Award in Cardiovascular Research (2013), the Pearl Meister Greengard Prize (2015), the 2016 Breakthrough Prize in Life Sciences and the Passano Award.

Notes

Cardiovascular Research Day Keynote Speaker

Mark A. Creager, MD, FAHA, FACC

Immediate Past President, American Heart Association
Director, Heart and Vascular Center
Dartmouth-Hitchcock Medical Center
Professor of Medicine and Surgery
Geisel School of Medicine at Dartmouth

Dr. Mark A. Creager is a Professor of Medicine and Surgery at the Geisel School of Medicine at Dartmouth, and Director of the Heart and Vascular Center at Dartmouth-Hitchcock Medical Center in Lebanon, NH. Dr. Creager earned his medical degree at Temple University in Philadelphia. He completed his internship, medical residency, and fellowships in Vascular Medicine and Cardiology at University Hospital in Boston.

Dr. Creager is Immediate Past President of the American Heart Association and serves on its National Board of Directors. He is an editor of the textbook, Vascular Medicine and the editor emeritus of the journal, Vascular Medicine. He is a Past President of the Vascular Disease Foundation, and a Past President and Master of the Society for Vascular Medicine. He is a Fellow of the American Heart Association and of the American College of Cardiology, and a member of the American Society of Clinical Investigation. His major research and clinical interest is in vascular medicine, specifically vascular regulatory mechanisms and the effect of treatment on patients with peripheral artery disease. He is the author of more than 350 published contributions to the medical literature, including: research papers on vascular function, book chapters, and monographs on vascular disease. Dr. Creager has been recognized with several prestigious awards and honors, including the Vascular Disease Foundation President's Award for Leadership and the American Heart Association Council on Peripheral Vascular Disease Distinguished Achievement Award, and the American Heart Association Distinguished National Leadership Award.

Cardiovascular Research Day Distinguished Alumni

Brian Eigel, PhD

Senior Vice President
Emergency Cardiovascular Care Programs
American Heart Association

Brian Eigel, PhD is Senior Vice President of Emergency Cardiovascular Care (ECC) Programs at the American Heart Association (AHA), responsible for leading the development of products and services that help people prepare and respond to first aid and cardiac emergencies.

In this role, Dr. Eigel directs the strategic vision and leadership for global science, product development, education, editorial and project management to support ECC business strategies. He oversees multiple product and service lines including education courses for healthcare providers, consumers and businesses on First Aid, CPR and AED usage. He also supports the AHA mission to fight for stronger public health policies and provide lifesaving tools and information to prevent and treat cardiac diseases.

Under Dr. Eigel's leadership, the AHA developed the 2010 and 2015 *AHA Guidelines Update for CPR and Emergency Cardiovascular Care* while collaborating with the American Red Cross on the development of the 2010 and 2015 *Guidelines Update for First Aid*.

Among the important products and services that Dr. Eigel is responsible for advancing is the AHA's Resuscitation Quality Improvement (RQI) program, a groundbreaking new approach to maintaining competence in CPR.

Dr. Eigel holds a Bachelor of Science degree from The College of William and Mary and earned his Ph.D. in Pharmacology from the University of Kentucky.

Event Supporters

Gill Foundation of Texas

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The Saha Fund for Cardiovascular Research and Education

●

The Estate and Family of Mrs. Hager Koostra

●

Mr. and Mrs. Bob Allen

Notes

Poster Judges

Thank you to everyone that participated as a judge during our poster session.

Doug Andres, PhD
University of Kentucky

Brian Bennett, PhD
University of California, Davis

Jason Brandon, PhD
University of Kentucky

Lisa Cassis, PhD
University of Kentucky

Barry Coller, MD
The Rockefeller University

Daniel Conklin, PhD
University of Louisville

Mark Creager, MD
Dartmouth-Hitchcock Medical Center

Cherry Croft, PhD
University of Kentucky

Alan Daugherty, PhD, DSc
University of Kentucky

Sean Davidson, PhD
University of Cincinnati

Frederick de Beer, MD
University of Kentucky

Marcielle de Beer, PhD
University of Kentucky

Brian Delisle, PhD
University of Kentucky

Sanda Despa, PhD
University of Kentucky

Brian Eigel, PhD
American Heart Association

Ed Fisher, MD
New York University

Ming Gong, MD, PhD
University of Kentucky

Gregory Graf, PhD
University of Kentucky

Zhenheng Guo, MD, PhD
University of Kentucky

Bernhard Hennig, PhD,
University of Kentucky

Bertha Hidalgo, PhD
University of Alabama at Birmingham

Helen Hobbs, MD
UT Southwestern Medical School

Jay Jerome, PhD
Vanderbilt University Medical Center

Sandgerk Lee, PhD
University of Kentucky

Zhenyu Li, PhD
University of Kentucky

Amelia Linnemann, PhD
Indiana University

Robert Lodder, PhD
University of Kentucky

Hong Lu, MD, PhD
University of Kentucky

Saskia Neher, PhD
University of North Carolina at Chapel Hill

Fredrick Onono, PhD
University of Kentucky

Phillip Owens, PhD
University of Cincinnati

Sabire Ozcan, PhD
University of Kentucky

Nathalie Pamir, PhD
Oregon Health & Science University

Meera Penumetcha, PhD
University of Central Missouri

David Randall, PhD
University of Kentucky

Nalini Santanam, PhD
Marshall University School of Medicine

John Stafford, MD, PhD
Vanderbilt University Medical Center

Venkateswaran Subramanian, PhD
University of Kentucky

Lisa Tannock, MD
University of Kentucky

Elizabeth Tarling, PhD
UCLA

Ryan Temel, PhD
University of Kentucky

Sean Thatcher, PhD
University of Kentucky

Michael Tranter, PhD
University of Cincinnati

Deneysvan der Westhuyzen, PhD
University of Kentucky

Vincent Venditto, PhD
University of Kentucky

Kasey Vickers, PhD
Vanderbilt University

Nancy Webb, PhD
University of Kentucky

Sidney Whiteheart, PhD
University of Kentucky

Frederique Yiannikouris, PhD
University of Kentucky

Changcheng Zhou, PhD
University of Kentucky

Xuewei Zhu, PhD
Wake Forest University School of Medicine

Poster Abstracts

Poster Index

1

Differentiating Cardiac Fibrosis from Hypertrophy in CKD Hemodialysis Patients Using Gadolinium Free Imaging and Biomarkers of ECM Turnover

Tori Stromp [1] • Tyler Spear [2] • Rebecca Kidney [2] • Kristin Andres [3] • Joshua Kaine [3] • Richard Charnigo, PhD [4] • Steve Leung, MD [5] • Vincent Sorrell, MD [5] • Moriel Vandsburger, PhD [6]

[1]Physiology, Cardiovascular Research Center, University of Kentucky • [2]Cardiovascular Research Center, University of Kentucky • [3]College of Medicine, University of Kentucky • [4]Statistics, Biostatistics, University of Kentucky • [5]Gill Heart Institute, University of Kentucky • [6]Physiology, Biomedical Engineering, Cardiovascular Research Center, University of Kentucky

Student

Purpose
Cardiac stress blood biomarkers are typically correlated to hypertrophy or diastolic dysfunction but not fibrosis in chronic kidney disease (CKD). Cardiac fibrosis can be measured without gadolinium as elevated normalized signal difference (ΔS/So) among pairs of magnetization transfer (MT) weighted magnetic resonance (MRI) images. We used MT-MRI to compare blood biomarkers to fibrosis, hypertrophy, and diastolic strain rate in CKD patients on hemodialysis (CKD5).

Hypothesis
Biomarkers of cardiac stress correlate with left ventricular (LV) hypertrophy, not fibrosis, in CKD5 patients.

Methods
MT-MRI generated maps of ΔS/So in 23 CKD5 patients and 21 controls (Fig 1A, B). Fibrotic burden was defined as divergence in ΔS/So compared to a standard distribution (Fig 1C). In a subset of 12-15 per group, serum troponin T (TnT), fibroblast growth factor 23 (FGF23), matrix metalloproteases (MMP) 2 and 9 and tissue inhibitor of MMP (TIMP) 1 and 2 were measured by ELISA and analyzed with Spearman correlations. Feature tracking measured strain.

Results
CKD5 patients had higher ΔS/So (146.2 ± 18.7%), divergence (10.0 ± 9.2%) and LV mass index (81.6 ± 28.9 g/m2) than controls (133.5 ± 11.5%, 5.4 ± 3.7%, 57.9 ± 15.5 g/m2, all $p<0.05$). Ejection fraction, diastolic strain rate and longitudinal strain were similar. While TnT, FGF23 and TIMP1/2 correlated with mass, only TIMP1 correlated with fibrotic burden (Table 1).

Conclusions
Most stress biomarkers correlate with hypertrophy but not fibrosis in CKD5 patients. Activation of cardiac fibroblasts by TIMP1 and correlation of TIMP1 with degree of fibrosis suggest TIMP1 as an alternative target to attenuate cardiac fibrosis in CKD5 patients.

2

Fine Tuning Platelet Secretion to Modulate Hemostasis

Smita Joshi, MS [1] • *Sidney. W. Whiteheart, PhD* [1]

[1]Molecular and Cellular Biochemistry, University of Kentucky

Student

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. They sense vascular damage and release 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. Our studies focus on how these VAMPs influence platelet secretion and if modulating platelet secretion can control clot formation. To address this goal, we genetically titrated the VAMPs to define their role in platelet exocytosis and in in vivo hemostasis. We gathered global VAMP-3-/- and VAMP-8-/- animals. To overcome embryonic lethality of VAMP-2-/-, we generated platelet specific VAMP-2/3-/- mice by using tissue-specific promoters and the Cre recombinase system. These animals were crossed with VAMP-8-/- mice to create platelet-specific VAMP-2/3/8-/- mice. Secretion assays were used to monitor the kinetics and the extent of release from all three platelet granules (dense, alpha and lysosomes). To understand how secretion influences hemostasis, distinct aspects of hemostasis were assessed using Tail bleeding, FeCl3 carotid injury and laser injury assays. VAMP-3-/- platelets showed no defects in secretion or tail bleeding. VAMP-2/3-/- platelets showed mild secretion defects while VAMP-2/3/8-/- platelets showed a more robust defect (about 70%), more than that observed for VAMP-8-/- platelets (about 50%). When we studied effect of secretion on hemostasis, only VAMP-2/3/8-/- mice showed significantly increased tail-bleeding times and delayed arterial thrombosis. Interestingly, VAMP-8-/- animals showed a delay in thrombus formation but no overt bleeding. This suggests that small differences in kinetics and extent of secretion from these three granules alter hemostasis and by modulating platelet secretion, we can control thrombus formation without inducing pathological bleeding. These data offer the secretory machinery as a viable target to control cardiovascular diseases.
Our work is the first comprehensive study of the differential contribution(s) of each VAMP isoform to platelet secretion and the in vivo repercussions in maintaining hemostasis. We show that 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 animal model to analyze role of platelet secretion in other vascular processes.

3

A Compromised Liver Exacerbates Organic Pollutant-induced Toxicity on the Peripheral Vasculature

Banrida Wahlang, PhD [1] • *Jordan Perkins* [1] • *Michael Petriello, PhD* [1] • *Jessie Hoffman, MS* [1] • *Bernhard Hennig, PhD* [1]

[1]UK Superfund Research Center, University of Kentucky

Fellow

Purpose: Exposure to environmental toxicants namely polychlorinated biphenyls (PCBs) is correlated with multiple health disorders including cardiovascular and liver diseases. Moreover, environmental-induced diseases can also pose as risk factors for other health complications by altering the body homeostasis. However, the responses of an injured liver to subsequent environmental insults and effects on other organs systems including the heart has not been investigated. The current study aims to evaluate the role of the liver-peripheral vasculature axis in PCB-induced toxicity, using the commercial PCB mixture Aroclor1260. Methods: Male C57Bl/6 mice were fed either an amino acid control diet (CD) or a methionine-choline deficient diet (MCD) during the 12-week study. Mice were exposed to the PCB mixture, Arcolor1260 (20 mg/kg) and analyzed for inflammatory, calorimetry and metabolic parameters. Results: Consistent with the literature, MCD diet-fed mice demonstrated steatosis, indicative of a compromised liver. Aroclor1260 exposure worsened hepatic fibrosis exhibited by the MCD groups. Interestingly, Aroclor1260 did not induce steatosis and inflammation in CD-fed mice. Notably, Aroclor1260 exposure in MCD-fed mice led to extra-hepatic toxicity such as upregulated circulating inflammatory biomarkers (ICAM-1, P-selectin, thrombomodulin), implicating endothelial cell dysfunction. Furthermore, the mice that had compromised livers and exposed to Aroclor1260 also manifested hyperglycemia and increased blood pressure which was absent in the unexposed groups. Conclusion: Taken together, these results indicate that environmental pollution can exacerbate toxicity caused by diet-induced liver injury which may be partially due to dysfunctional energy homeostasis and exacerbated inflammation. This is relevant to PCB-exposed human cohorts who suffer from alcohol or diet-induced fatty liver diseases and are at risk for cardiovascular diseases. The current study is supported by the NIEHS/NIH grant P42ES007380 and NIGMS/NIH grant 8P20GM103527-06.

4

Nuanced Immune Response to Apolipoprotein A-I in Cardiovascular Disease

David Henson [1] • *Robert Kline, MS* [1] • *Vincent Venditto, PhD* [1]

[1]Pharmaceutical Science, University of Kentucky

Student

Antibodies targeting apolipoprotein A-I (ApoA-I) have been identified in patients with cardiovascular disease (CVD), and have been shown to correlate with disease progression. ApoA-I is the major protein of high density lipoprotein (HDL). The role of IgG targeting this self-protein is unclear we hypothesize that antibodies targeting ApoA-I are both protective and pathologic and unraveling the isotype and epitope specificity of these auto-antibodies will provide a new paradigm for risk stratification in patients. To test our hypothesis, we screened human serum samples from the TRACER study to identify IgG and IgM toward a portion of ApoA-I known to undergo post-translational modification. These data highlight an inverse correlation between IgG and IgM in patients presenting with a myocardial infarction. Furthermore, we have employed an immunization strategy in mice to induce antibody responses toward specific epitopes of ApoA-I and evaluated the role of antibodies in atherosclerosis and cardiovascular disease progression. These data enable us to develop a risk stratification analysis of patients with CVD and correlate these findings into a novel mouse model of disease progression.

5

Plasma Levels of the Pro-atherogenic Nutrient Biomarker TMAO is Increased in People Highly Exposed to Dioxin-like Environmental Pollutants

Michael Petriello, PhD [1] • *Richard Charnigo, PhD* [2] • *Bernhard Hennig, PhD* [3] • *Andrew Morris, PhD* [1]

[1]Cardiovascular Research Center, Superfund Research Center, University of Kentucky • [2]Statistics, University of Kentucky • [3]Superfund Research Center, University of Kentucky

Fellow

Cardiovascular disorders are largely caused by genetic and environmental factors. Understanding how these factors intersect to determine individual disease risk is a critical challenge. Well-studied "lifestyle dependent" determinants of increased cardiovascular disease risk include smoking, physical inactivity, and poor nutrition, but emerging data now implicate exposures to persistent environmental pollutants as an important contributor to inter-individual variability in cardiovascular disease risk. Interestingly, a growing body of knowledge now implicates the importance of interactions between toxicant exposure and diet. For example, recently, we published that in preclincial models, exposure to dioxin-like environmental pollutants (polychlorinated biphenyls, PCBs) can increase circulating levels of a diet-derived biomarker of cardiovascular disease, trimethylamine-N-oxide (TMAO). Plasma TMAO levels are strongly associated with coronary artery disease and diabetes risk in humans. In our preclinical studies, dioxin-like PCBs strongly increase the enzyme responsible for TMAO production, FMO3, resulting in amplified increases in TMAO levels. We have now begun to investigate if these associations between pollutant exposure and TMAO are evident in the highly exposed Anniston, Alabama population. We have used mass spectrometry methods to quantitate TMAO in archived plasma samples, and have determined that higher body burden of dioxin-like pollutants is significantly associated with increased circulating TMAO levels in humans.

6

Inhibition of Plasma Paraoxonase 1 Activity by Isolevugladins and Its Adducts

Geetika Aggarwal, PhD [1] • *Sean S. Davies, PhD* [1]

[1]Pharmacology, Vanderbilt University

Fellow

Atherosclerosis is the leading cause of death in the United States. A polymorphism in Paraoxonase 1 (PON1), an enzyme associated with high density lipoprotein (HDL), increases risk for atherosclerosis so that individuals with PON1R isoform are more susceptible to atherosclerosis that individual with the PON1Q isoform. The PON1R isoform alters a number of PON1 activities that have been proposed for the protective effect of PON1 including its lactonase and proposed peroxidase activities. Reduced PON1 activity also occurs during exposure to lipid peroxidation associated with atherosclerosis and inflammation. Lipid peroxidation forms Isolevuglandins (IsoLG) that rapidly react with lysyl residues of proteins to alter their function. IsoLG also react with phosphatidylethanolamines (PE), which are present in HDL. We hypothesize that IsoLG and related lipid dicarbonyls inhibit plasma PON1 activity leading to increased atherosclerosis. We found that exposing plasma to IsoLG rapidly (<30 min) and potently (IC50 1.42×10-5 M) inhibits PON1 activity. This rapid inhibition is consistent IsoLG forming monoadducts with proteins or lipid molecules, rather than crosslinks which require longer timeframes. Potential mechanisms of inhibition of plasma PON1 activity by IsoLG could include competitive inhibition by IsoLG itself or IsoLG modified PE, non-competitive inhibition by covalent modification of the catalytic or by binding of IsoLG or IsoLG-modified compounds (PE or plasma proteins) to an allosteric site. Studies varying concentration of PON lactonase substrate as well as IsoLG showed that PON1 underwent non-competitive inhibition. Importantly, studies varying concentration of PON lactonase substrate and IsoLG-PE also showed non-competitive inhibition suggesting that binding of IsoLG modified compounds to an allosteric site, rather than direct IsoLG modification of PON1, most likely accounted for inhibition. Future studies will examine the ability of IsoLG to inhibit recombinant PON1 in the absence of other proteins and PE found on HDL, and to characterize the PON1 activity in the presence of dicarbonyl scavengers. The ultimate goal of these experiments is to find strategies to preserve PON1 activity under conditions where lipid peroxidation occurs, thereby decreasing risk for atherosclerosis.

7

Overexpression of SR-BI Induces Kidney Apoptosis in SR-BI Transgenic Rat

Yufeng Chu, PhD [1] • *Ling Guo* [1] • *Xiang Ye, PhD* [1] • *Jiahua Wu, PhD* [1] • *Xiang-An Li, PhD* [1]

[1]Saha Cardiovascular Research Center, University of Kentucky

Fellow

Background: Scavenger receptor class B type I (SR-BI) is a high-density lipoprotein (HDL) receptor that mediates the selective uptake of cholesteryl ester from HDL, which promotes reverse cholesterol transportation (RCT). A recent report showed that a rare loss-of-function variant in SR-BI (P376L) has impaired RCT which is associated with an increase in the risk for coronary heart disease (CHD), thus, it has been speculated that up-regulation or enhancement of SR-BI expression could be a novel therapeutic approach for reducing CHD risk. However, there are some studies in animals suggesting that overexpression of SR-BI could lead to adverse effects. SR-BI transgenic mice with very high hepatic SR-BI have an increase in atherosclerosis. In addition, an earlier in vitro study showed that overexpression of SR-BI induces apoptosis. To clarify the effect of overexpression of SR-BI, we generated SR-BI transgenic rat and assessed apoptosis and organ histology.

Methods and Results: SR-BI transgenic rat was created in a SD background using an expression vector pLncx2, which contains a constitutive CMV promoter. Western blot analysis showed high expression of SR-BI in heart, liver and kidney of SR-BI transgenic rat. Both total and free blood plasma cholesterol concentrations in SR-BI transgenic rat were significantly lower than control SD rat (total: 30.1mg/dl vs 130.8mg/dl, free: 24.1mg/dl vs 44.7mg/dl). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analysis showed profound apoptotic cells in kidney of SR-BI transgenic rat, mostly tubular epithelial cells which mainly distributed in the proximal convoluted tubules. No TUNEL positive cells were observed in liver and heart of SRBI transgenic rat. Hematoxylin and eosin (HE) staining revealed myofibrillar disarray, myocardial interstitial fibrosis and mild liver fibrosis were in SR-BI transgenic rat.

Conclusions: Our findings indicate that SR-BI overexpression induces apoptosis in renal tubular epithelial cells and causes myofibrillar disarray, myocardial interstitial fibrosis, and mild liver fibrosis. Thus, caution should be taken when considering promotion of RCT via upregulation of SR-BI.

8

Isolevuglandin, a Highly Reactive γ-ketoaldehyde Formed From the Isoprostane Pathway, Causes Structural and Functional Consequences to HDL

Linda Zhang, PhD [1] • Tiffany Pleasant [2] • Mark Borja, PhD [3] • Huan Tao, PhD [4] • Michael Oda, PhD [3] • W. Gray Jerome, PhD [5] • Patricia Yancey, PhD [4] • L. Jackson Roberts II, MD [1] • MacRae Linton, MD [4] • Sean Davies, PhD [1]

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Fellow

Background: Cardiovascular disease risk depends on levels of functional HDL particles, not HDL-cholesterol. Oxidative stress generates lipid peroxidation products, which modify proteins and lipids to render HDL dysfunctional. Isolevuglandins (isoLGs), generated in parallel to isoprostanes (a common biomarker for oxidative stress), are extremely reactive and elevated in atherosclerosis. Recently, our group observed a 42% reduction of atherosclerotic lesion size when salicylamine (SAM), a small molecule scavenger of isoLG, was administered to LDLr-/- mice. Little is known about the consequences of isoLG in modifying HDL. Aims: Effects of isoLG on apolipoprotein crosslinking, morphology and size of HDL were examined and compared to its effects on two major HDL functions, cholesterol efflux and anti-inflammation. SAM and pentapyridoxamine (PnPM) were assessed for their abilities to preserve HDL structure-function. Methods: Purified HDL from normal human plasma was incubated overnight at 37°C with isoLG at concentrations ranging from 0.1 μM to 3 μM (0.1 to 3 molar equivalence (eq) to apoA-I). Thioglycolate-induced intraperitoneal macrophages were harvested from apoE-/- mice. Results: IsoLG crosslinked major HDL apolipoproteins, apoA-I and apoA-II, starting at 0.3 eq to apoA-I. Above 1 eq, apoA-I appeared crosslinked to other proteins, demonstrating the high reactivity of isoLG. Examining particle morphology and size by transmission electron microscope showed that increasing isoLG to 3 eq shifted particle size from unmodified control HDL (6-11 nm) to two distinct sizes, 6-13 nm and 16-23 nm. In terms of function, a 72.7±0.3% decrease in 3H-cholesterol efflux from macrophages was observed at 1 eq of isoLG to apoA-I when compared to unmodified control HDL. At this concentration, HDL-ApoA-I exchange was reduced ($P<0.01$, $n=4$), from 47.4±2.8% in controls to only 24.8±5.8%, suggesting the possibility that isoLG inhibited apoA-I from disassociating from HDL to interact with ABCA1. IsoLG inhibited HDL's protection against LPS-stimulated inflammatory response in macrophages as shown by increased IL-1β and TNFα (80.7±4.2% of LPS for IL-1β; 927±309% of LPS for TNFα). The maximum inflammatory effect occurred at 0.1 eq of isoLG to apoA-I, suggesting that low modification of isoLG renders the HDL dysfunctional, independent of protein crosslinking. SAM and PnPM partially rescued apoA-I from isoLG-mediated crosslinking, with PnPM being more effective. SAM partially rescued the anti-inflammatory function of HDL. Conclusions: We report a novel pathway by which isoLG renders HDL becomes dysfunctional, by mechanisms involving isoLG-mediated alterations of HDL proteins and structure. We also demonstrate the potential of scavengers in preserving HDL and reducing atherosclerosis. Future studies will pinpoint how isoLG modifies HDL proteins (or lipids) to alter its function.

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Modulation of Gut Microbial Fermentation of Dietary Fiber by PCB126

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Exposure to environmental pollutants is associated with a greater risk for metabolic diseases including cardiovascular disease. Pollutant exposure can also alter gut microbial populations that may contribute to metabolic effects and progression of inflammatory diseases. Short-chain fatty acids (SCFAs), produced from gut microbial fermentation of dietary fibers such as inulin, exert numerous effects on host energy metabolism. SCFAs are also linked to health promoting effects, including a reduced risk of inflammatory diseases. We hypothesized that exposure to dioxin-like pollutants modulate gut microbial fermentation processes. Fecal microbes from mice were harvested and resuspended in anaerobic media containing 4 or 10g/L of inulin with or without PCB126 (0.02μM, 0.2μM, or 2μM) and incubated for 48h (37 °C). HPLC analysis revealed that PCB126 exposure differentially modulated the production of several SCFA, including succinate and propionate. Exposure to PCB126 at 0.2μM and 2μM reduced succinate production, while exposure to 2μM of PCB126 increased total fermentation acids, and in particular propionate production. It has been demonstrated that bacteria-produced succinate contributes to metabolic benefits by acting as an intestinal gluconeogenic substrate. Furthermore, there is evidence that an excess propionate and total SCFA can contribute to increased energy harvest and hepatic lipogenesis. This evidence supports the idea that pollutant exposure may contribute to alterations in host metabolism through gut microbiota-dependent mechanisms, specifically bacterial fermentation processes. (NIEHS/NIH grants P42ES007380)

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Endocytosis Mediates Platelet Responses to Circulating Viruses

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Student

Introduction- It is increasingly clear that platelet functions extend beyond hemostasis. As part of their role in innate immune responses, platelets detect bacterial and viral DNA/RNA via intracellular TLR7 and TLR9 receptors. This ability can affect the clinical courses and outcomes of both viremia and bacteremia. Chronic viral infections, e.g., HIV-1 severely increase the risk of acute myocardial infarction (MI) suggesting a dynamic platelet-virus interaction; however, the clinical importance of this are ill-defined. Since platelet hyperactivity contributes to arterial thrombosis, exacerbating MI, it seems likely that HIV-1 infections lead to some level of platelet activation. Platelets do endocytose HIV-1 virions but the molecular machinery required and its subsequent effects on platelet activation are unknown.
Methods and Results- In nucleated dendritic cells; innate immune responses to HIV-1 are mediated by pathogen phagocytosis/endocytosis, degradation to release TLR ligands, and subsequent TLR activation. We asked whether this process is recapitulated in anucleate platelets. We first examined platelets' responses to two specific nucleotide-based, TLR agonists, loxoribine (TLR7) and unmethylated CpG oligonucleotides (TLR9). Using secretion assays to measure release from all three classes of granules (alpha, dense and lysosomes), we found that both TLR agonists elicited a small (compared to hemostatic agonists, i.e., thrombin) secretory response that was sufficient to form platelet-leukocyte aggregates as measured by FACS. Additionally, both activated IKK and PI3K-Akt pathways as measured by immunoblotting. Inhibitor studies (using known inhibitors of endocytic trafficking such as Dynasore, ammonium chloride) and analysis of platelets from two different endocytosis mutants (VAMP-3-/- and Arf6-/-) demonstrated the importance of platelet endocytic trafficking in regulating responses to these intracellular TLRs. To further understand this process, we used HIV-1 pseudovirions that contain ssRNA which can be recognized by the endosomal TLR7, showing that platelets did endocytose and degrade retroviral particles to release TLR agonists, which initiate platelet activation and secretion. Fluorescence micrographs showed uptake of HIV-1 pseudovirions in both in vitro and in vivo analyses in wildtype platelets. Consistently, HIV-1 uptake and subsequent activation was abolished in endocytosis-deficient VAMP3-/- or Arf6-/- platelets.
Conclusions- Platelet endocytosis has been chiefly studied in the context of fibrinogen uptake and integrin trafficking. Our studies suggest that platelets can act at an early state of pathogen recognition during systemic viral and bacterial infections via endocytosis and are not only able to internalize pathogens but are also able to process them to initiate an immune response that lead to observed outcomes in systemic viral and bacterial infections.

11

Adipocyte-specific Deletion of HuR Reduces Diet-induced Obesity

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Staff

Obesity is classified by the World Health Organization as one of eight principle causes of preventable chronic disease, and is directly associated with decreased life expectancy due to increased risk of type II diabetes, chronic respiratory disease, stroke, peripheral vascular disease, cancer, and cardiovascular disease. As a serious threat to human health, research into novel pharmaceutical targets and therapies to combat obesity is of growing interest to the scientific community. Although the RNA-binding protein HuR (Human antigen R) is highly expressed in adipocytes, its role in adipocyte function and adipocity is largely unknown. To conclusively determine the mechanistic role of HuR in adipocyte function, our laboratory has developed an adipocyte-specific HuR knockout mouse (Adipo-HuR-/-). These mice do not differ from wild-type mice at baseline with regard to weight, glucose tolerance, or lean/fat body mass. However when placed on a chronic (20-week) high fat diet (HFD, 60% kcal/fat), Adipo-HuR-/- mice gain less weight compared to their wild-type counterparts. Ongoing efforts to identify an underlying mechanism of action have yet to identify a difference between wild-type and Adipo-HuR-/- mice following chronic HFD with regard to adaptive thermogenesis, glucose tolerance, and ratio of lean/fat body mass. In conclusion, deletion of HuR reduces diet-induced obesity, but the mechanisms remain unknown.

12

Aortic Root Surgery: A University of Kentucky Experience

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Objective
Aortic root surgery varies widely in techniques and from institution to institution. Success requires precision and superb post-operative care. The purpose of this study was to evaluate the indications and outcomes of this procedure at the University of Kentucky.

Methods
The study was carried out with IRB approval, consisting of 99 patients undergoing aortic root surgery from January 2013 to June 2015. 45 underwent procedures for aneurysm, 11 for infective native valve endocarditis, 16 for prosthetic valve endocarditis, 21 for aortic root dissection, and 14 for other reasons. The procedures were performed using homograft (10), composite (8), or biologic (81) valves. 28 of the operations included a second procedure, and 39 of the operations were redo procedures.

Results
Complications analyzed included operative death (8.9%), stroke (10%), surgical site infection (1.1%), and cardiac arrest(6.5%). For those treated for aneurysm the mortality rate was 2.2%, infective endocarditis was 7.4%, dissection was 19%, and other etiologies was 7.1%.

Conclusions
In comparing the University of Kentucky to other institutions we found that complications were on average within the range of the rates of complications of other large institutions nationally and internationally.

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Molecular MRI of Cardiac Metabolic Failure as an Early in Vivo Biomarker in Obesity Induced Heart Failure

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Student

Background: Heart failure (HF) is one of the leading causes of death in US especially among obese adults. Clinically, HF in obese patients is usually diagnosed using ventricular function measurements indicated primarily by ejection fraction. However, due to body intrinsic compensatory mechanisms that preserve cardiac output, global ventricular function is preserved until the end stages of HF. In contrast, metabolic changes occur early and gradually over time prior to functional and structural cardiac changes. Creatine (Cr) is the main metabolite of the creatine kinase (CK) system in cardiomyocytes, which plays an essential role in transferring and buffering the chemical energy in the form of ATP to ensure the supply meets the high cardiac energy demand. Numerous in vivo and ex vivo studies have attempted to map myocardial creatine content with respect to HF in both human and animal models, with consistent findings of a reduction in total myocardial creatine content. Chemical exchange saturation transfer (CEST) MRI imaging has emerged over the last decade as a novel method for in vivo molecular imaging of different endogenous substances. In our lab we optimized a new cardiac specific CEST technique which can be used for myocardial creatine mapping.

Hypothesis: Reduced cardiac creatine content can be used as an early in vivo biomarker of obesity induced heart failure.

Method: CardioCEST was used in 7T MRI to scan phantoms of different [Cr] at room temperature (RT) to detect the percent of creatine magnetization transfer asymmetry (MTRasym) at ±1.8 ppm. After further optimization on the cardioCEST sequence, another creatine phantom of 13.6mM was scanned at both RT and 37°C. In a previously published study by our lab, cardioCEST was used to probe changes in myocardial creatine in ten mice randomly assigned to either a high fat diet (HFD) or low fat diet (LFD).

Results: The phantoms used [Cr] of 0mM (saline), 5mM, 10mM, 13.6mM, and 15mM, showed that creatine MTRasym was proportional to [Cr] of 1.05%, 3.2%, 5.8%, 7.8%, and 8.8%, respectively. The optimized cardioCEST used on a 13.6mM creatine phantom increased the MTRasym at RT and 37°C to be 12.1% and 8.1%, respectively. The mice were scanned after 14 weeks on their respective diets, and results showed a significant increase in body mass in HFD mice (45 ± 1g HFD vs. 31 ± 1g LFD, $p < 0.05$). However, ventricular structural and functional measures including wall thickness (0.9 ± 0.1mm LFD vs. 0.9 ± 0.1 HFD, p = NS) and fractional shortening (34.7 ± 8.0% LFD vs. 31.1 ± 4.3%, p = NS), were similar between HFD and LFD mice. In contrast, measures of MTRasym were significantly reduced in HFD mice compared to LFD mice (7.8 ± 3.3 LFD vs. 2.7 ± 1.5 HFD, $p < 0.05$). Metabolic changes with respect to creatine may serve as an early detectable biomarker in obesity induced heart failure.

14

Atherosclerosis Development is Reduced in Mice with Blunted Biliary Cholesterol Secretion

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Staff

Excessive accumulation of cholesterol in the arteries drives atherosclerosis development. It is believed that biliary cholesterol secretion is crucial for eliminating excess cholesterol from the body via reverse cholesterol transport. In the current study, we wanted to determine the impact of reduced biliary cholesterol secretion on atherosclerosis development in mice. Decreased biliary cholesterol secretion was achieved by hepatic overexpression of human NPC1L1 (L1Tg mice) combined with knockdown of hepatic ABCG5/G8 function using an ABCG8 antisense oligonucleotide (ASO). LDLR-/- and LDLR-/-/L1Tg mice received either control or ABCG8 ASO were fed a high fat (42% Kcal)/low cholesterol (0.015% wt/wt) diet for 20 weeks. As expected, L1Tg mice and mice with hepatic ABCG8 knockdown had an >70% reduction in biliary cholesterol. The dramatic decrease in biliary cholesterol did not increase plasma cholesterol, and in fact mice with hepatic ABCG8 knockdown had reduced VLDL cholesterol. Even more surprising, aortic atherosclerosis was significantly decreased in mice with compromised biliary cholesterol secretion. LDLR-/-/L1Tg treated with ABCG8 ASO had a >90% reduction in biliary cholesterol yet had ~70% less atherosclerosis compared to LDLR-/- controls. Moreover, reducing biliary cholesterol had no impact on macrophage reverse cholesterol transport, fecal excretion of neutral sterol, and hepatic expression of genes involved in cholesterol synthesis (HMG CoA reductase/synthase) and HDL metabolism (ABCA1 and SR-BI). These results indicate that atherosclerosis development can be decreased by shunting cholesterol away from biliary secretion and potentially towards trans-intestinal cholesterol excretion or bile acid synthesis.

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A Novel Autotaxin Inhibitor Reduces Inflammation in Myocardial Infarction Pre-Clinical Model: Potential Therapeutic Targets in Ischemic Heart Disease

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Fellow

Introduction: Acute myocardial infarction (AMI) is the leading cause of death in developed countries. The bone marrow (BM) responds to AMI by the production and mobilization of inflammatory cells such as monocytes (monocytosis). While this response is essential for tissue healing after MI, pathological inflammation exacerbates tissue damage and is correlated with the development of heart failure through poorly understood pathways. Lysophosphatidic acid (LPA), produced by the ubiquitous enzyme autotaxin (ATX), is potent bioactive lipid mediator that regulates monocytosis and promotes inflammation and atherosclerosis. The role of ATX/LPA signaling nexus in cardiac inflammation has not been previously explored. We hypothesized that inhibiting ATX/LPA signaling will reduce the post-AMI inflammatory response.
Methods and Results: We generated an animal model of AMI using left anterior descending artery ligation. Following AMI, mice were randomized to receive PF-8380 (a potent ATX inhibitor) 10 mg/kg PF-8380 twice daily or placebo. Assessment of LPA in plasma samples was done by mass spectrometry. ATX activity was assessed using gene expression. Pro-inflammatory cytokines (IL-1β, IL-6, MCP-1, TNF-α) and Interferons (IFN-α, IFN-β, IFN-R) were assessed by relative quantitation of mRNA expression. Flow cytometry was done on peripheral blood, heart, spleen and bone marrow for inflammatory monocytes (CD115+, CD11b+), neutrophils (Ly-6G&C+); hematopoietic stem cells (Sca1+ c-Kit+ Lin-) and myeloid progenitors cells (Sca1+ c-Kit+ Lin-, CD16/32+, CD34+).
AMI was associated with increase in the expression of ATX activity and plasma LPA levels. This was correlated with increased PB and cardiac inflammatory monocytes and neutrophils. ATX inhibition reduced plasma LPA level which was associated with reduced numbers of circulating and cardiac inflammatory monocytes and neutrophils during peak post-AMI inflammatory phase. Moreover, expression of proinflammatory cytokines decreased at the peak of inflammatory response in BM, PB and cardiac tissues. Mechansitically, reduced inflammatory cells and inflammation was related to significant reduction in BM progenitor count and proliferation with ATX inhibition.
Conclusion: AMI leads to upregulation of ATX/LPA signaling and associated inflammatory response. ATX inhibition reduces the production and infiltration of inflammatory cells into injured myocardium. These results support the use of small molecule pharmacological inhibitors as potential therapeutic target in MI.

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Advanced Glycation End Products Contribute to Endothelial Dysfunction through Suppression of Krüppel-like Factor 2 in Chronic Kidney Disease

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Student

Background:
Patients with chronic kidney disease (CKD) have a 10-20 times higher risk of cardiovascular death than the general population and suffer from accelerated atherosclerosis. Endothelial dysfunction is thought to be a key initial event in the development of atherosclerosis, especially in CKD patients whose endothelium is constantly exposed to uremic toxins. The transcription factor Krüppel-like factor 2 (KLF2) is an important regulator of endothelial function which links changes in hemodynamics to inflammation, vasodilation, and vascular remodeling. While flow-induced changes in KLF2 expression are known to play a critical role in atherogenesis, little is known about how the uremic state of CKD effects endothelial KLF2 expression. Our objective was to determine how endothelial KLF2 is regulated in-vitro by uremic metabolites and assess the role of KLF2 in CKD related endothelial dysfunction.

Approach and Results:
Human umbilical vein endothelial cells (HUVECs) were stimulated with known uremic toxins including: P-cresol sulfate, indoxyl sulfate, carboxymethyl-lysine modified BSA (CML-BSA; an advanced glycation end product), or vehicle and analyzed for KLF2 expression. At average uremic concentrations, CML-BSA suppressed KLF2 mRNA and protein expression by 50% ($P < 0.05$) and augmented the expression of the receptor for advanced glycation end products (RAGE; 1.5 fold increase, $P = 0.005$) compared with vehicle treated cells. Transfection of RAGE siRNA attenuated the response of CML-BSA and restored KLF2 expression ($P = 0.008$). Furthermore, CML-BSA concentration and KLF2 expression are inversely correlated in end-stage renal disease, resulting in near complete suppression of KLF2 ($P < 0.0001$). To assess if these changes in KLF2 expression were linked to endothelial dysfunction, we performed monocyte adhesion assays. Our results show that endothelial monolayers treated with CML-BSA have a greater than 2-fold increase in monocyte adhesion compared to vehicle treated cells ($P = 0.002$).

Conclusions:
Our results suggest that suppression of endothelial KLF2 by uremia-derived AGEs may play an important role in the accelerated endothelial dysfunction and resulting cardiovascular disease experienced by CKD patients. Ongoing in vitro and in vivo studies utilizing endothelial specific KLF2 knockout mice will further delineate these mechanisms and assess the impact of RAGE antagonists at reducing cardiovascular disease in uremic mice.

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Insulin Receptor Signaling Plays Key Role in apoA-I Secretion from Hepatocytes

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Aim: To assess the effect of insulin receptor (IR) signaling on hepatic apoA-I metabolism.
Approach: Hepatic apoA-I expression, secretion and cellular localization were compared in IRfl/fl mice and LIRKO mice in which hepatic insulin receptors were specifically deleted by AAV delivered Cre-recombinase.
Results: IR mRNA and protein levels were markedly reduced in the livers of LIRKO mice compared to control IRfl/fl mice. As expected, LIRKO mice exhibited decreased glucose tolerance and reduced hepatic insulin signaling. Knockdown of hepatic IR decreased plasma HDL cholesterol and apoA-I levels. Whereas apoA-I mRNA levels were similar in LIRKO and control hepatocytes, apoA-I protein levels were increased in both the liver and primary hepatocytes isolated from LIRKO mice. In contrast to apoA-I, apoE and apoB protein levels in the liver and in cultured hepatocytes, as well as in the plasma, were similar in LIRKO and control mice. ApoA-I accumulation in LIRKO hepatocytes was associated with a decreased rate of apoA-I secretion, whereas rates of apoA-I synthesis were unchanged. Immunofluorescence staining demonstrated that apoA-I accumulated in LIRKO hepatocytes in membrane bound inclusions. These inclusions shared markers characteristic of early, late and recycling endosomes, and of lysosomes.
Conclusion: IR-mediated insulin signaling plays an important role in hepatic apoA-I secretion and consequent nascent HDL formation. Reduced apoA-I secretion from liver into the circulation may contribute to the lower HDL levels typically associated with insulin resistance.

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Conjugated Linoleic Acid and Nitrite Attenuate Mitochondrial Dysfunction during Myocardial Ischemia

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Fellow

Coronary artery disease and subsequent myocardial infarction (MI) are the major contributors of mortality associated with cardiovascular disease. Mitochondrial adenosine triphosphate (ATP) generation is essential for normal heart function and MI is associated with mitochondrial dysfunction. Nitrated fatty acids, arising from ischemic cardiac tissue, are cardioprotective in murine models of ischemia reperfusion injury through mitochondrial dependent mechanisms. Conjugated linoleic acid (cLA), an n-2 unsaturated fatty acid, is the preferred endogenous substrate for fatty acid nitration and previous studies have shown that co-treatment with cLA and nitrite protects against MI. It was hypothesized that co-treatment with cLA and nitrite would alter cardiac mitochondrial function and complex activity in vivo and may contribute to cardioprotection prior to and during MI. Mitochondrial respiration and complex I,II, III, and IV activity were assessed in wild-type (WT) mouse cardiac tissue following a 10 day co-treatment of cLA (20mg/kg/d) via osmotic mini-pump and nitrite (50ppm- drinking water). Mice undergoing MI were treated with cLA (10mg/kg/d) and nitrite (50ppm- drinking water) for a total of 13 days with coronary artery ligation performed on day 3 followed by mitochondrial respiration and respiratory chain activity performed on day 13. The combination treatment with cLA and nitrite resulted in a significant decrease in State 3 respiration in WT mice (141.71 vs 80.64 [Oxygen flux per V (pmoles/(s*mL], $p<0.05$). Complex I activity was increased by 32 % (3030.11 vs 4026.99 nmoles/min/mg protein, $p<0.05$), while complex II (10,058.7 vs 8565.89 nmoles/min/mg protein, $p<0.05$) and complex III (10852 vs 4570.57 nmoles/min/mg protein, $p<0.05$) activity demonstrated a 15% and 58% decrease respectively in WT mice following co-treatment with cLA and nitrite. Following MI, Complex I activity increased by 20% (3030.11 vs 3627.45 nmoles/min/mg protein, $p<0.05$) and co-treatment with cLA and nitrite further increased complex I activity after MI (3627.45 vs 7950.15 nmoles/min/mg protein, $p<0.05$). Complex II activity decreased by 52% following MI (10058.7 vs 4826.63 nmoles/min/mg protein, $p<0.05$) and remained decreased with the combination treatment of cLA and nitrite (4826.63 vs 3744.75 nmoles/min/mg protein, $p<0.05$). Co-treatment with cLA and nitrite attenuated increased State 3 respiration (84.45 vs 40.76 [Oxygen flux per V (pmoles/(s*mL], $p<0.05$), complex III activity (24728.7 vs 15591 nmol/min/mg protein, $p<0.05$) and hydrogen peroxide (9.99 vs 5.51 uM, $p<0.05$) following MI. This work reveals that co-treatment with cLA and nitrite significantly alters mitochondrial respiratory function via changes in mitochondrial complex activity. Overall, these results indicate that cLA and nitrite-mediated cardioprotection following MI may be in part due to attenuated mitochondrial respiration, complex III activity and hydrogen peroxide levels.

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Deficiency of Protease-activated Receptor 2 Attenuates Angiotensin II-induced Abdominal Aortic Aneurysm Independent of Tissue Factor

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Staff

Objective: Tissue factor (TF) is constitutively expressed in subendothelial cells, including vascular smooth muscle cells (VSMCs), and maintains hemostasis. Previously, we demonstrated C57BL/6 mice with 1% of normal TF levels (Low TF mice) had increased suprarenal abdominal aortic diameter and incidence of abdominal aortic aneurysm (AAA) compared to littermate controls. Moreover, utilizing a SM22-specific TF deficiency on a low-density lipoprotein receptor deficient (Ldlr-/-) background, this effect was VSMC dependent. TF can activate protease-activated receptor 2 (PAR-2) via a factor Xa (FXa) complex. Our objective was to determine whether the TF:FXa complex protects against aneurysm formation via PAR-2.

Methods and Results: Par-2+/+ (n = 14) and Par-2-/- (n = 13) male mice on a Ldlr-/- background were fed a fat-enriched diet and infused with angiotensin II (AngII; 1,000 ng/kg/min) for 28 days. PAR-2 deficiency attenuated abdominal aortic diameters (Par-2+/+: 1.78 ± 0.06 mm; Par-2-/-: 1.17 ± 0.03 mm; P = 0.001), the incidence of AAA (84% versus 40%; P = 0.048), and ascending aortic aneurysm (Par-2+/+: 15.26 ± 0.62 mm2; Par-2-/-: 17.43 ± 0.57 mm; P = 0.015) compared to littermate controls. To determine the effects of FXa inhibition, Ldlr-/- mice were fed a fat-enriched diet containing either the FXa inhibitor Rivaroxaban (n = 7) or a placebo (n = 8) and infused with AngII for 28 days. Rivaroxaban did not protect against AAA or ascending aortic aneurysm compared to placebo control. Total plasma cholesterol concentrations, lipoprotein cholesterol distributions, and systolic blood pressure were unchanged between any of the groups.

Conclusion: These results indicate that PAR-2 signaling enhances AAA formation and growth in a TF-independent manner.

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The Smart Heart Self-Care First Pilot Program

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Student

Purpose: The Smart Heart Self-Care First Program (SHSCF) offers a transitional care intervention focusing on the self-care of hospitalized heart failure patients who are able to be discharged home. It utilizes assessment, communication, and education components to encourage behavior modification (improved self-care) through telehealth methodology. The program also focuses on the identification of depression and anxiety symptoms in the heart failure population at the University of Kentucky as well as the impact of these psychological illnesses on outcomes including re-admissions, mortality, and quality of life.

Background and Significance: Admissions to the hospital related to heart failure (as a primary or secondary diagnosis) are among the leading cause of hospitalizations in the United States which is approximately 20%. Surprisingly, of those patients discharged with a primary diagnosis of heart failure, 20% are readmitted to the hospital within thirty days. Heart failure re-hospitalizations highlight a growing need for transitional care from the inpatient setting to the home within each community. Disease management programs focusing on self-care interventions and education are becoming increasingly popular among institutions as a means to address this problem.

Procedures: Thirty participants will be enrolled intro this randomized controlled trial. To date, twelve patients received the usual or standard of care at discharge from the University of Kentucky based on Heart Failure Core Measures. Additionally, there are 8 patients in the intervention group who received the standard of care combined with daily text messaging for thirty days after discharge from the hospital. For those patients in the intervention group, the delivery of text messages is daily through a website set up by the principal investigator. Demographic information and a text messaging comfort survey was administered to all participants prior to discharge in addition to tools to assess for depression symptoms (Patient Health Questionnaire-9), anxiety symptoms (Generalized Anxiety Disorder-7), and heart failure self-care (Self-Care Heart Failure Index Tool). Post discharge, each patient was contacted by telephone and given these tools again to assess for differences as well as a tool designed to evaluate quality of life called the Minnesota Living with Heart Failure Questionnaire. Re-admissions as well as mortality were assessed for all patients.

Results or Potential Results: Findings are pending. The study will continue to enroll participants until a sample size of 30 is obtained. Data was analyzed for the first twenty participants. Potential results include clinically significant differences in the number of re-admissions between the control and intervention groups as well as self-care scores. Also, clinically significant findings pertaining to the prevalence of co-morbid depression and anxiety may occur.

Implications for Practice: The integration of technology into heart failure education has implications to improve communication with patients after discharge by increasing engagement in self-care and boosting patient confidence. Also, the acknowledgment of psychological well-being how that impacts patient self-care is likely to occur. The diagnosis, treatment, and follow up for patients who have heart failure and also suffer from depression and anxiety may have many positive benefits in promoting holistic care for this patient population.

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Deficiency of Protease-activated Receptor 2 Attenuates the Formation and Progression of Atherosclerosis

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Objective: Atherosclerotic plaques are highly procoagulant due to the presence of tissue factor (TF). While TF reduction attenuates the formation of atherosclerosis, TF inhibition is not a feasible therapeutic option due to bleeding complications. Alternatively, protease-activated receptor 2 (PAR-2) is a putative receptor for TF and does not result in hemostatic deficiency. The objective of this study was to determine the effects of PAR-2 on the development of atherosclerosis.

Methods and Results: Relative expression of PAR-2 is increased in human coronary artery (213 fold increase) and mouse aortic arch (160 fold increase) atheroma versus control coronary and aortic arch arteries, respectively ($P = 0.001$). To determine the effect of PAR-2 deficiency on atherosclerosis, male low density lipoprotein receptor deficient (Ldlr-/-) mice (8-12 weeks old) that were Par-2+/+ or Par-2-/- were fed a fat and cholesterol-enriched diet for 12 (n = 10 each group) or 24 weeks (n = 4 each group). PAR-2 deficiency attenuated aortic root and aortic arch atherosclerosis with no effects on total plasma cholesterol concentrations or lipoprotein distributions after 12 ($P = 0.000433$) and 24 ($P = 0.097$) weeks. These reductions were attributable to non-hematopoietic-derived PAR-2 from analysis of bone marrow experiments (n = 15 for each of 4 chimeric groups; $P < 0.05$).

Conclusion: PAR-2 deficiency significantly attenuates the initiation (12 weeks) and reduces the progression (24 weeks) of atherosclerosis via non-hematopoietic derived cells.

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Role of Smooth Muscle Cell Lipid Phosphate Phosphatase 3 in Regulation of Mouse Atherosclerosis

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Background The lipid phosphate phosphatase3 (LPP3) encoded by the Ppap2B gene, is an integral membrane enzyme that degrades bioactive lysophospholipids including lysophosphatidic acid (LPA), sphingosine-1-phosphate (S1P) and related bioactive lipids, thereby terminates their signaling effects. These bioactive lipids regulate variety of vascular cell functions such as cell growth, differentiation, apoptosis and development in a wide spectrum of settings. Within the vasculature, smooth muscle cells, endothelial cells and platelets, display notable responses to LPA. We suggest that LPP3 hydrolysis of LPA is a critical step in regulating pathophysiological important responses in vascular cells.

Method and Result To investigate the possible role of LPP3 in regulation of atherosclerosis in smooth muscle cells (SMCs), we generated experimental mouse models: lacking LPP3 (SM22-Δ) mice by breeding Ppap2B fl/fl (fl/fl) mice (Previously established in our laboratory) with animals expressing Cre recombinase under control of the SM22 promoter on the low density lipoprotein receptor knocked out (LDLr-/-) background. At 4 weeks of age, littermate SM22-Δ and fl/fl mice were fed Western Diet (WD) for 12 weeks. The LPP3 mRNA expression in aortic SMCs was obviously reduced (0.139 ± 0.025 vs 1 ± 0; $P < 0.001$) and LPA content was significantly higher in the aortic arch (9.32 ± 4.66 vs 4.92 ± 1.55; $P < 0.05$) in SM22-Δ mice compared to control fl/fl mice, with no difference in plasma LPA levels in SM22-Δ and fl/fl mice (0.592 ± 0.323 vs 0.598 ± 0.246; $P = 0.9631$), indicating that LPA is probably degraded by LPP3. Atherosclerotic lesion of the aortic arch was measured by en-face analysis, and demonstrated increased atherosclerotic lesion sm22-Δ mice compared to the control fl/fl mice (18.98 ± 7.11 vs 6.06 ± 4.01; $P < 0.001$). The results support that LPP3 attenuate the development of atherosclerosis. The IL6 mRNA expression was significantly higher in the aortic arch tissues (13.6 ± 1.5 vs 1.0 ± 0; $P < 0.05$) and SMCs (1.33 ± 0.21 vs 1.0 ± 0; $P < 0.05$) in SM22-Δ vs fl/fl mice; FACS testing macrophages in aortas was significantly increased in SM22-Δ mice compared to control fl/fl mice (37173 ± 1015 vs 17638 ± 7353; $P < 0.05$); Sections of aortas at the level of the aortic root and staining with CD68 / α-SMA antibodies, indicating that the loss of LPP3, upregulated CD68 (45627 ± 4325 vs 28757 ± 1272) and α-SMA (70955 ± 21220 vs 20729 ± 3862; $P < 0.05$) in SM22-Δ versus fl/fl mice. We suggest that LPP3 deficiency increased the vascular inflammatory cell infiltration and SMCs proliferation in atherosclerotic lesion of the aorta.

Conclusions These results suggest a protective role for LPP3 in the development of atherosclerosis. Alterations in expression of LPP3 may increase vascular LPA content to promote the infiltration of inflammatory cells, including macrophages, into lesions and smooth muscle cell proliferation and thereby accelerate the development of atherosclerosis.

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Naringenin Supplementation Enhances Plaque Regression and Improves Lesion Pathology in Ldlr-/- Mice with Established Atherosclerosis.

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Student

Previous studies show that regression of atherosclerosis is achievable in mice treated by aggressive lipid-lowering. Previously, we have shown in Ldlr-/- mice that the addition the citrus flavonoid naringenin to a high-fat, high cholesterol (HFHC) diet prevents the development of metabolic dysfunction and atherosclerosis. In the present study, we hypothesized that in Ldlr-/- mice with established atherosclerosis, the addition of naringenin to a chow diet would enhance atherosclerosis regression. Ldlr-/- mice were fed a high-fat, high cholesterol diet for 12 weeks to induce metabolic dysregulation and atherosclerosis. Subsequently, the mice were switched to 1) a low-fat, isoflavone-free chow diet (IFF), 2) the IFF diet with the addition of 3% Naringenin (IFF+Nar) or 3) continuation of the HFHC diet. Compared to IFF alone, IFF+Nar enhanced weight loss (-93% vs -60%), independent of caloric intake and ambulatory activity. Metabolically, naringenin supplementation enhanced improvements in insulin sensitivity (AUC ITT -41% vs -4%), and the reversal of both hypercholesterolemia (-105% vs -96%) and hypertriglyceridemia (-79% vs -65%). IFF+Nar induced greater reductions in hepatic triglycerides, which coincided with increased expression of ATGL mRNA and protein. Echoing the metabolic improvements, intervention with IFF+Nar further reduced elevated total and Ly6Chi blood monocytes (-63% and -76%) compared to IFF alone (-37% and -41%). Relative to baseline, aortic cholesteryl ester (CE) increased with IFF alone (+40%), whereas IFF+Nar reversed aortic CE content (-19%). Compared to baseline, aortic sinus lesion size continued to increase with IFF (+47%), whereas with IFF+Nar lesion size increased only 14%, indicating almost complete attenuation of lesion growth. Both intervention diets decreased lesion apoptotic cells similarly, although IFF+Nar reduced lesion macrophages (35% vs 43%) and necrotic area (6.5% vs 7.5%, trend), compared to IFF alone. Smooth muscle content was modestly increased by the addition of naringenin (3.9% vs 2.7%, trend), but there was no significant effect on lesion collagen content (43% vs 47%). We conclude that compared to IFF alone, IFF+Nar enhances reversal of metabolic dysfunction and augments atherosclerosis regression.

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The Effects of Glucocorticoid in Lymphocyte Apoptosis and Glucocorticoid Resistance in Sepsis

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Student

Background: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection. Corticosteroid (GC) therapy is frequently employed in sepsis treatment; however, the effects of corticosteroid in sepsis treatment remain controversial. What factors affecting the effects of GC in sepsis treatment are still not completely understood. It has been reported that GC is involved in the regulation of lymphocyte apoptosis which is extensively induced during sepsis and results in immunosuppression. In addition, GC may also be associated with GC resistance that results in complicating the sepsis treatment. The roles of GC in the regulation of lymphocyte apoptosis and GC resistance in sepsis are not clear. Thus, in this study, we used RAW 264.7 cells, primary cells and C57BL/6J mice treated with LPS as a sepsis model in vitro and in vivo to clarify whether GC treatment results in lymphocyte apoptosis and GC resistance in sepsis.
Methods and Results: In this study, using 7-AAD assay and AV/PI assay, it showed that stress level of GC (145 and 1450 ng/ml) treatment significantly induced thymocyte apoptosis in vitro; however, GC supplementation (100 μg/mouse) decreased both thymocyte and splenocyte apoptosis in LPS-treated mice. This result indicated that GC supplementation decreased sepsis-induced apoptosis although thymocyte apoptosis can be induced by GC treatment in vitro. Using LPS-stimulated RAW 264.7 cells, it showed that prolonged and stress level of GC (145 and 1450 ng/ml) induced glucocorticoid resistance by mainly decreasing glucocorticoid receptor α.
Conclusions: Our data suggests that GC supplementation suppressed sepsis-induced thymocytes and splenocytes apoptosis although GC plays key roles in the lymphocyte apoptosis. In addition, the prolonged and stress level of GC treatment resulted in glucocorticoid resistance and the increase of pro-inflammatory cytokine production. Taken together, sepsis-induced lymphocyte apoptosis is not aggravated by GC treatment, but glucocorticoid resistance may be a concern for GC supplementation.

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Adipocyte Differentiation is Inhibited in Primary Brown Adipocytes Lacking CD47 Through a cMyc-dependent Mechanism

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Adipogenesis requires a precise orchestration of gene activation and suppression to regulate cellular proliferation and subsequent terminal differentiation. It has been well established that cMyc, a stem cell self-renewal gene and transcription factor, blunts preadipocyte differentiation through impaired induction of adipogenic transcription factors CCAAT/enhancer-binding protein alpha (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARγ). When activated by thrombospondin-1 (TSP1), CD47, a cell membrane receptor involved in self-recognition, has been shown to suppress cMyc expression in epithelial and endothelial cells. Previous reports have demonstrated that tissues and primary cells obtained from CD47 deficient mice exhibit elevated cMyc expression. The aim of our study was to examine whether preadipocytes isolated from brown adipose tissue of CD47 deficient mice demonstrated blunted adipogenesis due to increased cMyc expression. All in vitro experiments were triplicated. Our studies found that CD47 deficient preadipocytes exhibit enhanced proliferative capacity compared with wildtype (WT) controls. When treated with an adipogenic drug cocktail for 8 days, CD47 deficient preadipocytes accumulated significantly less lipid and blunted expression of mature brown adipocyte mRNA markers including adipocyte protein 2 (aP2), uncoupling protein 1 (UCP1), and cell death activator (Cidea), suggesting terminal differentiation is impaired with CD47 deficiency. Finally, CD47 deficiency was associated with a significant upregulation of cMyc mRNA levels and significant downregulation of C/EBPα and PPARγ mRNA levels after 8 day of differentiation. These findings imply that CD47 deficiency on preadipocytes may blunt adipogenesis in vitro by upregulating cMyc expression and subsequently blocking the transcription of adipogenic genes controlled by C/EBPα and PPARγ. In addition to regulating a number of cellular processes, this study suggests CD47 may play a unique role in regulating cellular fate. In the future, it may be necessary to adopt conditional gene knockdown models to examine the effects of CD47 deficiency in vitro.

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Clinical Signatures of Bleeding and Stroke in Patients on LVAD Support: Predictors and Outcomes

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Student

Importance: 5.7 million Americans live with heart failure (HF). Left ventricular assist device (LVAD) implantation is an increasingly common treatment for severe HF, with an estimated eligible cohort of 200,000 patients/year. Despite advancements, implantation immediately alters hemodynamics and introduces a foreign body across which blood experiences aberrant sheer stress. Clinical complications of bleeding and stroke develop secondary to a coagulopathy known as acquired von Willebrand disease. Predictors of these clinical complications remain largely unknown. This study uniquely correlates thromboinflammatory markers and LVAD types with clinical outcomes to help better understand and predict complications following LVAD implantation.

Objectives:
Primary Aim: Characterize platelet function and thromboinflammatory biomarkers alterations following LVAD implantation. Secondary Aim: Evaluate correlations between platelet function, biomarkers, laboratory trends, LVAD types, and clinical complications.
Design: Ongoing consecutive-based sample of patients undergoing LVAD implantation at the University of Kentucky. Between March 2014-October 2016, 68 enrolled patients underwent implantation of Heartmate II (n= 48), Heartmate III (n=2), Heartware (n=18). Sociodemographics: age, gender, ethnicity collected. Eligibility: First-time implant. Exclusion: Age<18, vulnerable populations. Withdraw n=1 (convenience). Follow-up between 30-180 days after implantation. Blood collections: 24-hours preoperatively and 1-, 24-, 72-, and 168-hours postoperatively. Follow-up collections: between 30-90-days postoperatively. Platelet function analyzed via impedance aggregometry and agonists. Biomarkers analysis via ELISA and MAGPIX. Clinical laboratory and complications data collected via SCM and INTERMACS.

Results: Platelet count decreased by 42% by 72-hrs post-operation (sd 208 ± 77; 120.9 ± 50)($p < 0.001$) with a corresponding 40% WBC increase (sd 8.2 ± 2.9;13.7 ± 3.6)($p<0.0001$). Platelet count recovered 168-hrs postoperatively. Platelet function via ristocetin high-concentration agonist decreased by 44% by 72-hrs post-operation, without baseline function recovery. Inflammatory profile levels via biomarkers were significantly higher than healthy levels at baseline, with further increases after implantation, without return to baseline. Average length of stay: 16.8 days. Readmission: 16.1% 3-months post-discharge, 38.7% 6-months. 1-year complications: stroke 19.3%, thrombosis 12.9%, gastrointestinal bleeding 12.9%, all-cause mortality 3%. Significant differences exist between LVAD types. Further statistical analysis will be presented.

Conclusions: LVAD implantation poses risks, but it is crucial for patients with limited cardiac function. In this study, platelet function decreases and does not return to baseline, despite return to baseline platelet counts. Inflammation markers increase and also do not return to baseline. Common complications include stroke, bleeding, and pump thrombosis. Further analysis and correlation could lead to identification of clinical signatures that predict outcomes. Further data will be presented.
Trial Registration: University of Kentucky Center for Clinical & Translational Research and Institutional Review Board.

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Reduced Mortality in Sepsis-Related Troponin Elevation with Statin and Beta Adrenergic Blockade

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Student

Background:

Type II myocardial infarction during acute sepsis has a higher mortality than compared to patients with normal cardiac troponin values. There is limited data regarding best management strategies of these patients. This study investigated the impact of aspirin, statin or beta blocker on all-cause mortality in this clinical population.

Methods:

This is a single center, non-concurrent cohort study including 899 patients who were admitted for sepsis and had a detectable troponin I (TnI) between January 2013 and December 2013. Patients were controlled for age, gender, and severity of illness (by APACHE score). Medication record was reviewed for in-hospital administration of aspirin, beta blocker, or statin. 30-day and 1-year mortality was assessed via the social security death database.

Results:

272 patients (age was 58+/- 14 years, 44% female) had full APACHE data and were included in the analysis. Using logistic regression analysis, in-hospital administration of beta blockers or statins was associated with decrease in 30-day and 1-year mortality. Aspirin was not associated with decreased mortality.

Conclusion:

In-hospital administration of beta blocker and statin (but not aspirin) therapy is associated with reduced 30-day and 1-year mortality. This suggests an anti-inflammatory and anti-catecholaminergic role of these agents and may offer new guidance for clinical management and prospective trial design.

Keywords

Type II myocardial infarctions, sepsis, elevated troponin.

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The Use of Knock-out Mice to Investigating the Role of Zinc Fingers and Homeoboxes 2 (Zhx2) During Postnatal Development and in the Control of Gene Ex

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Student

The Spear lab has had a long-standing interest in gene regulation in the liver during development and disease. Several years ago, these studies identified a novel transcriptional regulator called Zinc fingers and homeoboxes 2 (Zhx2), which is a member of a small family that includes Zhx1 and Zhx3. All Zhx proteins contain two amino-terminal C2-H2 zinc fingers and four or five carboxy-terminal homeodomains. Previous studies indicate that Zhx proteins can form homodimers and heterodimers with each other.

Zhx2 regulates numerous hepatic genes, including alpha-fetoprotein (AFP) and H19. Genes controlling lipid and cholesterol homeostasis are also regulated by Zhx2. More recently, our lab has found that a number of Cytochrome P450 (Cyp) genes and Major urinary protein (Mup) genes are also targets of Zhx2. The BALB/cJ mouse substrain contains a natural hypomorphic mutation in Zhx2, and the aforementioned targets are dysregulated in the livers of adult BALB/cJ mice. Recently, our lab developed mice that contain a floxed allele of Zhx2. By crossing these mice with transgenic mice that express the Cre recombinase in all tissues or in hepatocytes, we can knock-out Zhx2 expression in all tissues or solely in the liver, respectively.

Much of the research in the Spear lab has focused on the role of Zhx2 in liver gene expression during development and several models of liver disease. However, we have found that Zhx2 is ubiquitously expressed in all adult mouse tissues. The first part of my project has utilized whole-body Zhx2 knock-out mice to investigate the role of Zhx2 in various tissues, including kidney, brain, small intestine, liver, salivary and lacrimal glands. These studies indicate that some, but not all, previously identified Zhx2 targets are also regulated by Zhx2 in non-liver tissues. I have also carefully evaluated whether the absence of Zhx2 results in increased perinatal lethality and/or altered postnatal growth. These studies suggest that postnatal growth of male Zhx2 knock-out mice is delayed compared to wild-type Zhx2 littermates.

My second project examined subcellular localization of Zhx proteins. To accomplish this, GFP-Zhx fusion proteins were expressed in transfected cells. Zhx1 and Zhx2 localized to the nucleus whereas transfected Zhx3-GFP proteins were found in both the nucleus and cytoplasm. Moreover, when Zhx3-GFP was co-transfected with Zhx2, Zhx3-GFP was found strictly in the nucleus. This data suggests that interactions between Zhx proteins could alter protein localization. I employed bioinformatics tools to predict the 3D structures of Zhx1, Zhx2, and Zhx3 monomers, homodimers, and heterodimers.

Keywords: Zhx2, gene regulation, lipid homeostasis, bioinformatics, protein structure, knock-out mice.

Minen Al-Kafajy October 11,2016

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HDL/SRBI, Not LDL/LDLr, Pathway is Responsible for Stress Induced Steroidogenesis

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Fellow

Background: Stress induced steroidogenesis in adrenal is crucial in coping with various conditions. Glucocorticoid (GC) is synthesized using cholesterol as substrate. HDL/SRBI and LDL/LDLr are the two main pathway providing cholesterol for adrenal steroidogenesis. SRBI null mice have been found deficient in stress induced steroidogenesis, suggestting the crucial role of HDL/SRBI pathway for steroidogenesis in mice under stress conditions. However the contribution of LDL/LDLr pathway is obscure, in this study we use humanized mice (SRBI-/-|ApoBtg) to test the contribution of LDL/LDLr to stress induced steroidogenesis.

Methods and Results: To get the proposed mouse, SRBI-/- mice were bred to ApoB transgenic mice, then the F1 SRBI+/-|ApoBtg mice were backcrossed to SRBI-/- to get the F2 SRBI-/-|ApoBtg and SRBI-/-|ApoBwt mice. We first measured the lipoprotein profile in SRBI-/-|ApoBtg mice, indicating a successfully introduced LDL in the SRBI-/- mice (5.7 fold increase in the LDL fraction of FPLC profile). The cholesterol level in SRBI-/-|ApoBtg mice showed no difference (268 mg/dl vs 230 mg/dl, $p = 0.11$) compare to its littermate. And the LDL introduced in SRBI-/- mice did not restore the depleted lipid storage in the adrenal of SRBI null mice. Then we stimulated the SRBI-/-|ApoBtg mice with adrenocorticotropic hormone (ACTH). We observed that both the SRBI-/-|ApoBwt and SRBI-/-|ApoBtg showed impaired response 1 h after ACTH stimulation, while the ApoBtg control mice showed a 14.9 fold increase in response to ACTH ($p < 0.001$). To further verify this in pathological conditions, we induced steroidogenesis using cecal ligation puncture (CLP). The GC level showed no difference in SRBI-/-|ApoBwt and SRBI-/-|ApoBtg mice 3h after CLP.

Conclusions: The expression of human ApoB in SRBI null mice successfully introduced LDL in SRBI null mice. However it did not restore the impaired steroidogenesis in SRBI null mice. This demonstrates that HDL/SRBI, not LDL/LDLr, pathway is responsible for stress induced steroidogenesis.

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Regulation of PPAP2B/PLPP3 Promoter Activity by NFkappaB Family Transcription Factors

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The PPAP2B/PLPP3 gene encodes lipid phosphate phosphatase 3 (LPP3) which is an integral membrane enzyme that can dephosphorylate and inactivate bioactive lysophospholipid mediators at the cell surface and may have other roles in intracellular lipid metabolism and signaling. LPP3 expression is tightly regulated during development to control patterning and vascular development and LPP3 expression is strongly upregulated by inflammatory stimuli in blood and vascular cells. Heritable intronic variants that decrease expression LPP3 expression associate with increased coronary artery disease risk in humans while LPP3 deficiency in blood and vascular cells accelerates atherosclerosis in mice. LPP3 is biochemically indistinguishable from two related enzymes, LPP1 and LPP2 that have broad and overlapping expression patterns both during development and in adult tissues. Accordingly while these observations indicate that regulation of LPP3 expression may be a unique and important determinant of gene function essentially nothing is known about the regulatory elements and signaling pathways that control this process. We used an informatic approach to identify the PPAP2B promoter and map regulatory elements within the promoter that might be important determinants of gene expression. Using reporter constructs and mutagenesis approaches we identified functional target sequences for the Rel/NF-kappa B family of transcription factors in the PPAP2B/PLPP3 promoter. These studies provide a framework for understanding how upstream inflammatory signals increase PPAP2B/PLPP3 expression to blunt vascular inflammation and atherosclerosis and may also underlie or contribute to the unique function of this gene during development.

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Myocardial Uptake of Amylin Increases IL-1β Synthesis via Peroxidative Sarcolemmal Injury

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Fellow

Introduction: Failing hearts of patients with obesity or type-2 diabetes contain large deposits of amylin, a diabetogenic hormone co-secreted with insulin.

Hypothesis: Amylin deposition destabilizes the sarcolemma leading to peroxidative sarcolemmal damage and inflammation.

Methods: The relationship of amylin deposition with the levels of 4-hydroxy-2-nonenal (4-HNE), malondialdehyde (MDA) and cytokines (IL-1β, TNF-α, IL-6 and IL-10) were tested in myocardial tissue from patients with heart failure (HF) and obesity (BMI≥30; O-HF; N=7) or type-2 diabetes (D-HF; n=5) vs. healthy people with BMI<30 (Ctl group; N=7). To further test the hypothesis, we used diabetic rats expressing human amylin in the pancreas (HIP rats; n=25), age- and glucose-matched diabetic rats expressing only non-amyloidogenic rat amylin (UCD rats; n=12), normal mice injected with aggregated human amylin (2 μg/g body weight; n=8) or saline (n=8), and in vitro cell models.

Results: Myocyte amylin uptake is associated with increased levels of 4-HNE, MDA and IL-1β. Compared to Ctl, amylin-MDA and amylin-4-HNE adducts are elevated in both O-HF group (3.1-fold, $P<0.001$; 2.4-fold, $P<0.001$) and D-HF group (1.3-fold, $P<0.05$; 3.1-fold, $P<0.001$). IL-1β is increased by 3.4-fold ($P<0.001$) in O-HF vs. Ctl and by 1.2-fold ($P<0.05$) in D-HF vs. Ctl, respectively. These pathological changes are mirrored in HIP rats and normal mice intravenously injected with aggregated amylin, but not in diabetic UCD rats. In addition, HIP rats show elevated TNF-α, but unchanged levels of IL-6 and IL-10. In isolated cells, aggregated amylin (50 μM; 2 h) destabilizes the sarcolemma and generates reactive aldehydes leading to increased synthesis of IL-1β (2.4-fold; $P<0.001$). In contrast, incubation for the same duration with 400 mg/dl glucose had no effect on lipid peroxidation and IL-1β synthesis. Blocking either myocyte amylin uptake (by surfactants), or the lipid peroxidation chain reaction (by NAC), demonstrated that peroxidative membrane injury is upstream of IL-1β increased synthesis.

Conclusion: While further studies are necessary, these data suggest that exacerbated synthesis of IL-1β is a critical stress-activated signaling pathway in response to the interaction of aggregated amylin with myocytes.

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Concentration of Aggregated Amylin in RBCs Mediates Amylin Deposition in Cardiac Myocytes in Patients with Heart Failure and Type-2 Diabetes

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Fellow

Introduction: In patients with obesity or prediabetic insulin resistance, there is hypersecretion of both insulin and amylin. Hyperamylinemia promotes amylin aggregation, which was shown to injure the pancreas, heart, kidneys and brain.

Hypothesis: Circulating aggregated amylin serves as both a source for cytotoxicity in extrapancreatic tissues and marker of type-2 diabetes (T2D).

Methods: Red blood cells (RBCs) and cardiac myocytes from patients with heart failure (HF) and prediabetes (i.e. obesity; O-HF; n=21) or type-2 diabetes (D-HF; n=12) were tested for amylin and formation of adducts of amylin with 4-hydroxy-2-nonenal (4-HNE), a reactive aldehyde. Lean organ donors without HF or T2D served as controls (n=8). Rats expressing human amylin in the pancreas (HIP rats; n=25) and amylin knockout (Amy-KO) rats (n=8) were used for mechanistic studies.

Results: Compared to controls, amylin levels in circulating RBCs are increased 3-fold in O-HF ($P<0.001$) and 7-fold in D-HF ($P<0.001$). A significant fraction of incorporated amylin (~30%; $P<0.05$) formed amylin-4-HNE adducts suggesting peroxidative membrane damage. Paired RBCs and myocytes from the same patients showed similar size distributions of aggregated amylin. In both D-HF and O-HF, myocytes also demonstrated increased immunoreactivity for amylin-4-HNE adducts and IL-1β compared with controls. In HIP rats, analogous concentration of amylin in circulating RBCs was observed. Moreover, when Amy-KO rats were infused with RBCs from HIP rats via tail vein (300 μL daily for 7 days), both blood and heart tissue showed increased immunoreactivity for amylin, 4-HNE and IL-1β, supporting RBC amylin as a “mediator” as well as “marker” of amylin-induced stress. Treatment of HIP rats with carnosine, an aldehyde quencher (1 g/Kg; tail vein injection, daily for 7 days), blocked amylin-4-HNE adduct formation in RBCs and heart.

Conclusion: In patients with HF and T2D or obesity, concentration of secreted amylin within circulating RBCs mediates deposition of aggregated amylin in target organs, including the heart. RBC amylin, alone or in combination with other markers (e.g. A1c, amylin-4-HNE, IL-1β), may enable identification of patients at risk for heart failure or/and T2D before critical loss of β-cell mass.

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Serum Amyloid A3 Contributes to Atherosclerosis

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Staff

Cardiovascular disease (CVD) is the leading cause of death in developed nations, and is very prevalent amongst members of the United States armed forces. Serum Amyloid A (SAA) is a family of positive acute phase reactants that is used clinically as a marker of inflammation and cardiovascular risk. Several inflammatory diseases including diabetes and obesity are known to confer increased risk of developing CVD, and interestingly, are characterized by having chronically elevated SAA. Thus, we investigated the potential role of SAA in causing CVD, specifically atherosclerosis. We demonstrated that mice over-expressing human SAA1 developed more atherosclerosis in both the aortic root and on the aortic intimal surface. However, when the studies were repeated in mice deficient in both SAA1 and SAA2, homologues for the two major acute phase isoforms in humans, the incidence of atherosclerosis did not decrease. Thus, we hypothesize that SAA3, an isoform expressed in mice but not humans, acts redundantly in the absence of SAA1 and SAA2 to promote atherosclerosis. We previously demonstrated that SAA3 was produced in the liver of mice made acute phase with lipopolysaccharide. Furthermore, SAA3 has a striking sequence homology to human SAA1 which may explain its ability to affect CVD. Interestingly, SAA3 was present in aortic root atherosclerotic lesions of mice deficient in SAA1 and SAA2. To determine if SAA3 was contributing to atherosclerosis, mice were injected with an AAV to overexpress SAA3 or an ASO to inhibit SAA3's expression. Mice overexpressing SAA3 had a trend towards increased atherosclerosis while mice with SAA3 inhibition demonstrated a trend toward less atherosclerosis. Taken together, these data suggest that SAA3 is in fact a proatherogenic SAA isoform and further investigation into the mechanism of action of SAA3 is critical to combatting this endemic disease.

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Cell Encapsulation in Hydrogels – a Preliminary Step for Endothelial Progenitor Cell Isolation from Peripheral Blood

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Fellow

Myocardial infraction, also known as heart attack is one of the leading cause of death worldwide. Recent studies have shown that endothelial progenitor cells (EPC) have the capacity to regenerate vascular lining in vivo, and can restore the functions of a normal heart. Isolating these rarely present EPCs and homing them to the defect place has been a major challenge. To date, magnet activated cell sorting (MACS) and fluorescence activated cell sorting (FACS) are the two widely used techniques for isolating rare cells. Both these techniques have drawbacks with respect to time, cost, and throughput. The novel cell isolation technique currently under development, isolates antigen specific cells by encapsulating the target population in a hydrogel, which also protects the target cell from lysis in hypotonic solutions. In the current study, A549 lung cancer cell line was encapsulated in either non-degradable, PEGDA 3500 or biodegradable, gelatin methacrylate polymer solution. The viability of the polymerized cells was approximately 70% in both the groups. Approximately 20% of PEGDA encapsulated A549 cells seems to be viable even in hypotonic solution. The encapsulation was clearly seen under microscope when stained with different nanoparticles for degradable and biodegradable polymers. The main advantage of degradable polymer solution is that the cell phenotype was preserved and the cell can retain back to its activities. And as a biodegradable polymer, even its degradation products would not provoke any infectious response while degrading. The cells polymerized with gelatin methacrylate monomer have shown to attach to cell culture plate and proliferate even after polymerization. In our next experiments, we aim to encapsulate EPC cells antigen specifically in a biodegradable polymer and isolate them from peripheral blood and retain them to the defect site.

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SAA Lipoprotein Association Profile and Its Role in Cardiovascular Disease

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Staff

Despite decades of research to develop existing therapies, cardiovascular disease (CVD) is the leading cause of death in developed nations. Individuals with obesity and/or diabetes are at particularly high risk for CVD and research suggests this may be due to elevated levels of serum amyloid A (SAA). We have found that SAA induces atherosclerosis in mice. The current dogma is that SAA is exclusively an HDL associated lipoprotein. However we have found SAA on apoB-containing lipoproteins in both obese insulin resistant mice and humans and theorize that SAA can shift between lipid particles within the plasma compartment. Our preliminary data shows that SAA lipoprotein distribution differs between diabetic, MetS and normal subjects in the post-prandial period. We also have evidence that remodeling of HDL leads to the release of lipid-poor SAA that this SAA then completely associates with available lipoproteins. We also demonstrated that the presence of SAA on apoB lipoproteins increases proteoglycan binding, a key step in atherosclerosis development. For all of these reasons we hypothesize that the presence of SAA on apoB lipoproteins is atherogenic. Future studies will investigate whether intravascular HDL remodeling may lead to SAA shifting from HDL to apoB particles and how HDL remodeling and SAA shifting are affected by post-prandial dyslipidemia and insulin resistance. If this research confirms our hypotheses, then the presence of SAA on LDL or VLDL may be a new biomarker indicating humans at highest risk for cardiovascular disease.

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FGF19 Upregulates and Localizes ABCG5 and ABCG8 to the Canalicular Surface of Hepatocytes

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Background: Elevated hepatic cholesterol contributes to the development of nonalcoholic fatty liver disease (NAFLD), a condition highly associated with cardiovascular risk factors. The ABCG5 and ABCG8 (G5G8) heterodimer is responsible for up to 90% of biliary cholesterol secretion and is a potential therapeutic target for promoting cholesterol elimination. We have previously demonstrated that ursodiol (Urso), a pharmacologic bile acid, increases G5G8 protein expression and biliary cholesterol secretion. However, whole body cholesterol elimination is minimized likely due to simultaneous suppression of bile acid synthesis through upregulation of FGF15/19. The objectives of this study are to determine whether FGF15/19 regulates G5G8 and whether the effects of Urso on G5G8 requires FGF15/19-FGFR4 signaling.

Methods: Wildtype (WT) mice were injected with two doses of FGF19 or carrier (PBS) 1μg/g body weight within an 8-hour treatment window. In another experiment, WT mice were fed standard or Urso-supplemented diets in the absence or presence of FGFR4 antisense oligonucleotide (ASO) supplied by Ionis Pharmaceuticals in order to inhibit FGF15/19 signaling. In the last set of experiments, WT and G5G8 knockout (KO) mice were injected with FGF19. Body weight, liver weight, bile flow rate and plasma, hepatic and biliary lipids were measured. Immunoblotting of G5G8 and real-time PCR of genes involved in cholesterol metabolism were also conducted.

Results: As expected, mice injected with FGF19 had increased gene expression of Shp and decreased Fxr, Cyp7a1, and Cyp8b1. Abcg8 mRNA was modestly increased while both ABCG5 and ABCG8 protein were increased by more than 2-fold. Increases in G5G8 were consistent with increases in biliary total cholesterol. Both Urso and FGF19 localized G5G8 from discrete puncta dispersed in the cytosol to the canalicular surface of hepatocytes. The localization observed with Urso was impaired in the presence of FGFR4 ASO, indicating that FGF15/19-FGFR4 signaling is required. In G5G8 KO mice injected with FGF19, there was only a small increase in plasma free cholesterol and no other significant changes in cholesterol metabolism compared to wild type mice injected with FGF19.

Conclusion: FGF15/19 suppresses bile acid synthesis, post-transcriptionally upregulates G5G8, and localizes G5G8 to the canalicular surface of hepatocytes. Urso causes a similar localization of G5G8 that is dependent on FGF15/19-FGFR4. In the absence of G5G8, FGF15/19 did not acutely disrupt cholesterol metabolism.

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Serum Amyloid A: A Link Between Inflammation and Metastatic Lung Cancer

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Background: Extensive epidemiological data spanning the past three decades establish that circulating levels of serum amyloid A (SAA) predict lung cancer risk and/or poor prognosis. Whether SAA plays a direct role in cancer pathogenesis, or is merely a marker of increased risk, is not yet known. SAA is a secreted protein that is highly induced in the liver during acute infection or tissue injury. SAA exerts a variety of activities important for innate immunity, including cytokine induction in multiple cell types, leukocyte chemotaxis, and upregulation of genes involved in extracellular matrix remodeling, including matrix metalloproteinases. More recently, SAA has been shown to stimulate IL-1β secretion by activating the NLRP3 inflammasome. Notably, all of these activities attributed to SAA are characteristic of tissue microenvironments that are permissive for tumor growth and metastasis. Our studies investigate the novel hypothesis that sustained elevations of systemic SAA, such as occurs in individuals who are exposed to environmental toxins (including cigarette smoke) or have chronic obstructive pulmonary disease, leads to the development of tissue microenvironments that are conducive to lung tumor metastasis.
Methods and Results: We investigated the role of SAA in metastatic lung cancer using the Lewis lung cancer (LLC) mouse model. The LLC cells express a luciferase reporter gene, which allows for monitoring the fate of the cells in vivo. Three weeks after intravenous injection of LLC cells into syngeneic wild-type (WT) C57BL/6 mice, SAA was readily detected in lung tumor sections by immunohistochemical staining. Hepatic SAA expression and plasma SAA concentrations were both significantly increased in mice bearing LLC tumors, demonstrating a systemic inflammatory response in this lung cancer model. WT mice and SAA-deficient (“SAAKO”) mice were injected with LLC cells and euthanized after 20 days, when some of the WT mice exhibited reduced body weight. Lung tumor burden was significantly reduced in SAAKO mice compared to WT mice, as assessed by lung tumor weight, presence of lung tumor nodules, and luciferase activity in the lung. SAA gene expression was shown to vary widely (>1000-fold differences) in an initial screen of 11 immortalized human lung tumor cell lines.
Conclusions: SAA is induced both systemically and locally during the establishment of LLC tumors. SAA produced at a site distal from the cancer cells themselves is required for lung tumor development. Future studies will investigate the mechanism by which SAA exacerbates lung cancer progression and the relationship between SAA expression and tumorigenicity in human lung cancers.

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Multiple Cellular Mechanisms Underlie the Trafficking-deficient Phenotype for Kv11.1 (hERG) Mutations Linked to Long QT Syndrome

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Staff

Type 2 Long QT syndrome (LQT2) is commonly caused by missense mutations that disrupt the trafficking of Kv11.1 channels. The goal of this study is to determine the cellular mechanisms that underlie the trafficking-deficient phenotype for LQT2-linked mutations in different channel domains. We expressed the pore-domain mutation G601S and the cyclic nucleotide binding domain mutation F805C in human embryonic kidney 293 (HEK293) cells or human inducible pluripotent stem cell derived cardiomyocytes (hiPSC-CMs). HEK293 cells or iPSC-CMs expressing G601S showed a diffuse intracellular anti-Kv11.1 staining pattern that colocalized with the transitional endoplasmic reticulum (ER) marker BAP31, but not markers for the rough ER (calnexin), ER-associated degradation compartment (derlin1), ER exit sites (Sec31), or the ER-Golgi intermediate compartment (ERGIC53). Culturing cells in the drug E-4031 (10μM, 24 hrs), which corrects the trafficking-deficient phenotype for G601S, decreased its colocalization with BAP31. Unlike G601S, the trafficking-deficient phenotype for F805C is not corrected by culturing cell in E-4031. Expressing F805C in HEK293 cells or iPSC-CMs showed a very different anti-Kv11.1 staining pattern that consisted of several discrete aggregates. In iPSC-CMs, the F805C aggregates partially overlapped with derlin1 staining and co-transfected green fluorescent protein (gfp) in the cytosol. Incubating cells in the proteasome inhibitor bortezomib (10μM, 4 hrs) dispersed the F805C aggregates to generate a largely diffuse intracellular staining pattern. We conclude that G601S and F805C localize to distinct ER subcompartments: G601S is sequestered in the transitional ER subcompartment and F805C localizes to a ER associated degradation subcompartment that is sensitive to proteasome inhibition.

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Role of Machrophage - derived TSP1 in Obesity and Insulin Resistance: A Study with Tissue Specific TSP1 Deletion Models

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Staff

Background: Thrombospondin 1(TSP1) is a matricellular protein involved in a variety of diseases. Studies from our lab and others have demonstrated that TSP1 plays an important role in obesity-associated inflammation and insulin resistance. Both adipocytes and macrophages are cellular sources of obesity-induced TSP1 in adipose tissue. However, the contribution of these different cellular sources of TSP1 on obesity-associated inflammation and complications is unknown.

Methods: In this study, we determined the contribution of macrophage-derived TSP1 to the development of obesity and inflammation in a high fat diet induced obesity model. Macrophage specific TSP1 deficient mice (TSP1ΔmΦ) were generated by crossbreeding of Lyz-Cre mice with TSP1 floxed mice (TSP1fl/fl). Both male and female mice were used. Eight weeks old TSP1ΔmΦ mice and age-matched littermate (TSP1fl/fl) controls were fed with LF (10% fat) or HF diet (60% fat) for 32 weeks. Body weight was monitored weekly. GTT, ITT and other parameters were measured by the end of the study.

Results: We found that TSP1 gene expression was significantly reduced in peritoneal or bone marrow derived macrophages from TSP1ΔmΦ mice as compared to those from wild type mice (about 87% reduction). Both TSP1fl/fl and TSP1ΔmΦ mice had similar weight gain when fed with either LF or HF diet. No differences were observed in food intake, energy expenditure or physical activity between TSP1fl/fl and TSP1ΔmΦ mice under either LF or HF feeding conditions. Although TSP1fl/fl and TSP1ΔmΦ mice had similar level of obesity, TSP1ΔmΦ mice had improved glucose tolerance and insulin sensitivity. Moreover, adipose tissue macrophage infiltration as well as inflammatory cytokine production (MCP-1 and IL-1β) was significantly reduced in HF-fed TSP1ΔmΦ mice as compared to HF-fed TSP1fl/fl mice. Adipose tissue from HF-fed TSP1ΔmΦ mice also showed less Masson positive staining. Taken together, these studies suggest that macrophage-derived TSP1 stimulates macrophage accumulation in adipose tissue and promotes the development of obesity-associated inflammation and insulin resistance.

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Cloning and Expression of a Fibrinogenolytic Serine Protease from Solanum Tuberosum

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Student

Introduction: Serine proteases from plants are proposed to have a number of clinical applications, including as potential anticoagulants and antiplatelet agents. StSBTc-3, a subtilisin-like serine protease from Solanum tuberosum, was previously identified by our laboratory as a fibrinogenolytic enzyme. To further define its therapeutic value, we sought to generate recombinant StSBTc-3 for analysis. The StSBTc-3 gene was cloned from Solanum tuberosum DNA and recombinant protein was expressed in E. coli.
Methods and Results: DNA was extracted from Solanum tuberosum tubers and the gene encoding StSBTc-3 (PGSC0003DMT400027148) was amplified by Polymerase Chain Reaction using gene-specific oligonucleotide primers. The fragment corresponding to the peptidase domain (Ala-61-Thr-504) was amplified, sequenced, and cloned into pPROEX HTb and pGEX-KG expression vectors. The fragment corresponding to the hole mature protein (Thr-43-Asp-702) was also cloned into pPROEX HTb. E. coli Rosetta cells were transformed and enzyme expression was induced with IPTG. The cells were harvested and the enzyme was purified. For the clones containing the peptidase domain insert, low levels of soluble StSBTc-3 activity were obtained. However, a larger amount of insoluble enzyme was generated. Rapid refolding and slower dialysis techniques failed to generate soluble, active enzyme. The expression of the full mature enzyme increased the solubility of the protein significantly.
Conclusions: After successfully cloning the DNA encoding the peptidase domain of StSBTc-3 from Solanum tuberosum, we can express the intact full-length domain; however, the recombinant protein produced in E. coli is insoluble. The recombinant hole protein was soluble even without GST. This may suggest that the Fibronectin type III domain, missing in the first clone, helps to stabilize the protein in solution. Other expression systems are being explored to generate active StSBTc-3 for study in hemostasis assays.

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Tissue Distribution of a Col1A1-driven Inducible Cre in Rosa26 LacZ Mice

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Objective: The type 1 collage AI subtype gene (Col1A1) gene controls formation of the pro-α1(I) chain component of the large triple-stranded type I collagen. Type I collagen is composed of two pro-α1(I) chain and one pro-α2(I) chain. While extracellular matrix disruption is considered to be pivotal to development of aortic aneurysms, contribution of the pro-α1(I) chain has not been defined. The purpose of this study was to determine whether expression of the pro-α1(I) chain of collagen occurred in tissues that influence development of aortic aneurysms. This was accomplished by breeding mice that express an inducible Cre under control of the Col1A1 promoter to mice with LacZ inserted into the Rosa26 locus.
Methods and Results: Female Rosa26 mice were bred to male Col1A1-ERT-Cre+ mice to produce litter mates that were either Cre positive or negative. At approximately 8 weeks of age, mice were injected intraperitoneally with tamoxifen for 5 days. In Cre positive mice, this promotes translocation of the ERT-Cre chimera to the nuclei to remove a lox-flanked repressor of β-galactosidase expression. Whole and sectioned tissues were fixed and stained with X-Gal and eosin to observe ß-galactosidase activity and cellular cytoplasm, respectively. No staining was observed in tissues from mice not expressing Cre. The following tissues expressed ß-galactosidase activity: adrenal, brain, lung, lymph nodes, ovary, oviduct, and thymus. Staining appeared closest to the exterior membrane of the tissues. The lung expressed the highest concentration of stained tissue. No β-galactosidase activity was detected aortic tissue.
Conclusion: Multiple tissues have ß-galactosidase activity in which Cre activity was directed by the Col1A1 promoter in Rosa26-LacZ mice. However, the absence of ß-galactosidase activity in any regions of the aorta infers that the pro-α1(I) chain of collagen probably has no role in aortic aneurysm development.

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Comparison of MACS and FACS Sorting Techniques by Isolating Sca-1+ Cells from Mouse Bone Marrow

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Fellow

Cell based therapies offer a possible solution to some seemingly incurable diseases. Some of the cells with a promising future for therapeutic use are biologically very rare, such as Hematopoietic Stem Cells and Endothelial Progenitor Cells. Effective and affordable isolation of the rare cell population is necessary to be transitioned into therapeutic clinical use. Of the two widely used antigen positive isolation techniques - Magnetic Activated Cell Sorting (MACS) and Fluorescence Activated Cell Sorting (FACS), neither offers both high throughput and high purity sorting. However, a novel technique in development called Antigen Specific Lysis (ASL) is hypothesized to achieve these two criteria. This technique isolates cells by encapsulating the target population in a hydrogel protecting them from hypotonic solutions. In the current study, Sca1+ cells were isolated from mouse bone marrow and using MACS and FACS. Stem cell antigen-1 (Sca-1) is a cell marker found on Hematopoietic Stem Cells (HSCs), a type of stem cell found in peripheral blood and bone marrow. HSCs are also capable of differentiating into a multitude of blood cells. HSCs are being used therapeutically to treat leukemia, lymphoma, and blood disorders. Isolation of these cells is a key step in developing them as a new therapy. The isolated cells were compared of purity, recovery, and enrichment between the techniques. We also compared the viability and the amount of time it would take to sort a therapeutic dose of cells using each technique. Overall, the purity, recovery, and fold enrichment for FACS was higher than that of MACS. However, the time it would take to sort a therapeutic dose of Sca1+ from FACS is much longer than that for MACS. In our following study, we will be isolating these Sca1+ cells by hydrogel encapsulation using ASL and compare against MACS and FACS.

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A Novel Mechanism by Which TNF Mediates LPS-induced Vasodilatory Shock, Hypotension, and Mortality

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Staff

Objectives – Sepsis is defined by a severe systemic inflammatory response to infection, and its complications, including vasodilatory shock and hypotension, can be fatal. Vasodilatory shock has long been believed as a result of failure of the vascular smooth muscle cells (VSMCs) to respond to vasopressors to constrict and result in hypotension; however, the mechanism is largely unknown.

Approaches and Results – Here, we report that protein kinase C (PKC)-potentiated phosphatase inhibitor of 17 kD (CPI-17), a key regulator in smooth muscle contraction, was selectively downregulated by endotoxin lipopolysaccharide (LPS) in mesenteric arteries from C57BL/6 mice and by tumor necrosis factor alpha (TNF) in cultured smooth muscle tissue and cells. Downregulation of CPI-17 by LPS was associated with LPS-induced vasodilatory shock and hypotension and was largely attributed to TNF-induced CPI-17 transcriptional suppression. By various molecular approaches, including cloning, deletion, mutation, siRNA, electrophoretic mobility shift assay (EMSA), and chromatin immunoprecipitation (ChIP), we identified two GC-boxes in the proximal CPI-17 promoter as a novel TNF-response element that is critical for TNF-induced CPI-17 downregulation, and demonstrated that in the absence of TNF, SP1 bound to the CPI-17 promoter GC-boxes and positively regulated CPI-17 transcription, whereas in the presence of TNF, Krüppel-like factor 4 (KLF4) was markedly upregulated, competed with SP1 for binding to the CPI-17 promoter, and negatively regulated CPI-17 transcription through histone deacetylases (HDACs). Moreover, specific blockage of the TNF/KLF4/SP1/CPI-17 signaling by genetic deletion of TNF prevented mice from LPS-induced hypotension and mortality.

Conclusions – These data reveal a previously unrecognized but potentially important mechanism for vasodilatory shock and hypotension in sepsis, and suggest a new therapeutic strategy for treatment of patients with sepsis.

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Cardiac Ryanodine Receptors are Leaky Channels in Type 2 Diabetes and Contribute to the Development of Ventricular Arrhythmia

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Background. Type-2 diabetes (T2D) and obesity increase the risk of heart failure, arrhythmias and sudden cardiac death. However, the underlying mechanisms are poorly understood. Ryanodine Receptors (RyR) are Ca2+-release channels localized in the sarcoplasmic reticulum (SR) membrane which control both triggered and spontaneous Ca2+ release (i.e., SR Ca2+ leak), being vital for physiological myocyte contraction as well as for arrhythmogenesis. RyR activity is highly regulated by phosphorylation, oxidation and O-GlcNAcylation, post-translational modifications (PTM) with critical role in diabetes/obesity-related complications.

Hypothesis. SR Ca2+ leak is enhanced in diabetic hearts due to PTM of RyR and contributes to the triggering of ventricular arrhythmia.

Methods. To test this hypothesis we used a rat model overexpressing human amylin (HIP rat) which develops late-onset T2D and myocardial tissue from obese and non-obese patients.

Results. The frequency of spontaneous Ca2+ sparks, a measure of SR Ca2+ leak, is elevated in myocytes from HIP vs. non-diabetic rats, despite lower SR Ca2+ content. This result indicates an augmented activity of RyR in HIP myocytes, likely due to PTM of RyR. Indeed, the immunoblot analysis revealed a significant increase in RyR phosphorylation at the CaMKII site (Ser2814) and, at lesser extent, at the PKA site (Ser2808) in HIP rat hearts vs. control, without any differences in total RyR expression. Furthermore, augmented phosphorylation of PLB, the main regulator of the SERCA pump, at CaMKII site (Thr17) confirmed the activation of CaMKII in diabetic hearts, likely due to self-phosphorylation and O-GlcNAcylation of the enzyme. Although HIP hearts display oxidative stress, there was no increase in RyR oxidation vs. control hearts. We did not detect RyR O-GlcNAcylation in either HIP or control hearts. These results suggest that CaMKII-dependent RyR hyperphosphorylation is the main mechanism underlying the enhanced SR Ca2+ leak in hearts from diabetic HIP rats. Indeed, CaMKII inhibition with 1μM KN-93 dissipated the difference in Ca2+ spark frequency between HIP and control myocytes. Larger SR Ca2+ leak resulted in an increased propensity for triggering spontaneous action potentials (afterdepolarizations occurred in 5 out of 15 HIP myocytes and in 0 out of 15 control cells). In agreement with increased spontaneous activity, the SR Ca2+ load needed for generating a depolarizing transient inward current was significantly lower in HIP vs. WT myocytes. Interestingly, CaMKII-dependent RyR phosphorylation was also increased in non-failing hearts from obese vs. lean individuals, suggesting a possible early role in the development of cardiac dysfunction associated with obesity. The concomitant hyper-phosphorylation of RyR and PLB by CaMKII may also suggest a compensatory mechanism that helps maintain heart contractile function in the prediabetic state, before the onset of diabetes and its complications.

Conclusions. Our results highlight a possible association between altered cardiac RyR hyperphosphorylation, SR Ca2+ leak and ventricular arrhythmias in T2D.

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EGFR Signaling is a Key Regulator for Hepatic VLDL Secretion

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Objective – Elevation of ApoB-containing lipoproteins in blood circulation a well-established risk factor for the development of cardiovascular diseases under metabolic disease conditions like obesity, metabolic syndrome, and type 2 diabetes. Circulatory level of HB-EGF (heparin binding EGF-like growth factor), which is a ligand of EGFR (epidermal growth factor receptor), was reported to be positively correlated with cholesterol level, and with risk for coronary artery diseases in human individuals. In this study, we tested the effects of targeting of HB-EGF◊EGFR signaling on the hepatic VLDL secretion and development of hyperlipidemia and atherosclerosis in mouse models.

Methods and Results – LDLR deficient mice under high fat diet (HFD) (21% fat and 0.2% cholesterol) were treated with HB-EGF antisense oligonucleotide (ASO) for 12 weeks. At the termination step, we measured circulatory triglyceride (TG) and cholesterol levels, and size of aortic atherosclerotic lesion. To measure hepatic VLDL secretion rate, we used C57BL/6 mice, which is the genetic background of LDLR deficient mice. After 3 week ASO administration or one time intravenous injection of a water-soluble EGFR blocker BIBX1382, we intraperitoneally injected poloxamer-407 (1g/kg, lipoprotein lipase blocker) and retroorbitally collected blood samples at 0, 1, 2, 5 hour points to measure TG and apoB levels in circulation. Using same strain and treatment conditions, we also measured post-heparin plasma lipoprotein lipase (LPL) activities. First, we observed that the HB-EGF ASO administration induced a remarkable downregulation of circulatory apoB-containing lipoproteins (VLDL/LDL) and TG levels and atherosclerosis formation in the aorta. Second, HB-EGF ASO or BIBX1382 administrations significantly reduced hepatic VLDL secretion rates. However, the treatments did not induce changes for the post-heparin LPL activities.

Conclusion – Current results indicate that HB-EGF◊EGFR signaling is a key regulator for the hepatic VLDL secretion but not for VLDL clearance in circulation. This study also show a potential of use of HB-EGF◊EGFR targeting against hyperlipidemia and hyperlipidemia-associated cardiovascular diseases.

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The Role of Adipose- Derived Autotaxin on Inflammation Associated with Cardiovascular Disease

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Obesity is an established risk factor for cardiovascular disease (CVD) and stroke, and the cardiovascular complications of obesity are a leading cause of potentially preventable death. Recent evidence suggests that increased cardiovascular mortality in patients with obesity may not be fully explained by associated risk factors such as dyslipidemia, hyperglycemia, insulin resistance and hypertension. In addition to contributing to traditional CVD risk factors, obesity is also characterized by a chronic sub-acute inflammatory state termed “metainflammation” involving increased circulating inflammatory cytokines. Autotaxin (ATX), encoded by the ecto-nucleotide pyrophosphatase/phosphodiesterase 2 (ENPP2) gene, is a potent cell motility-stimulating factor that is secreted by adipose tissue. ATX hydrolyzes lysophosphatidylcholine (LPC), a lipid that is abundant in the circulation, to lysophosphatidic acid (LPA).
The two main objectives of this study were to investigate if ATX may be involved in metainflammation and to explore whether adipose ATX further contributes to physiological attributes of adipose cells and cardiovascular disease. We established two animal models with reduced ATX expression: MX1 Cre-mediated deletion of the gene encoding ATX to generate a global loss (MX1-Δ) and AdipoQ-Cre mediated loss of ATX expression in adipocytes (AdipoQ-Δ). Mice were fed a high fat diet for up to 20 weeks. RNA and proteins were extracted, qPCR and western blots were performed for ATX and inflammatory cytokines, and plasma ATX activity was determined. Results showed MX1-Δ and AdipoQ-Δ mice had a reduced level of inflammatory cytokines in adipose tissue. Furthermore, BODIPY, a fluorescent dye, was used as an indirect measurement for lipids with which we stained subcutaneous and visceral adipose tissue, in order to quantify adipose cell-size distribution. In response to quantitative adipose cell measurements, both AdipoQ-Δ and MX1-Δ mice demonstrated decreased size. The data generated from these experiments will provide important insight into potential mechanisms of how ATX and adipose tissue influence obesity induced inflammation and results from this and future projects may identify potential therapeutic targets to prevent and treat obesity induced inflammation.

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Inhibition of Myeloperoxidase-mediated HDL Oxidation by Paraoxonase 1

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Recent studies have reported that cholesterol efflux capacity, a measure of high-density lipoprotein (HDL) function in the first step of reverse cholesterol transport (RCT), is inversely associated with measures of carotid and coronary atherosclerosis, independent of HDL-C levels. Inflammation has been associated with reduced activity of HDL-associated anti-oxidative enzymes. HDL forms a functional tertiary complex with paraoxonase 1 (PON1) and myeloperoxidase (MPO). However, the impact of inhibition of MPO-mediated HDL oxidation by PON1 on HDL function is still unknown. Here, we found that MPO peroxidase activity is inhibited by PON1 as determined by an L-012 biochemical luminescence assay. In the presence of PON1, dimerization and cross-linking of apoA1 in response to oxidation of HDL by MPO was abolished, which indicates that PON1 inhibits MPO-mediated modification of ApoA1 and HDL. In the presence of PON1, malondialdehyde-HDL (MDA-HDL) adduct levels were decreased in response to MPO-mediated HDL oxidation compared to the oxidized HDL formed by MPO treatment in the absence of PON1, which indicates that formation of MDA-HDL adducts due to MPO-mediated HDL oxidation is inhibited by PON1. Using a BODIPY-cholesterol efflux assay, we also found that PON1 is able to restore the impaired cholesterol efflux from J774 macrophages caused by MPO-oxidized HDL. The amount of ApoA1 dimerization increased with increasing concentrations of MDA used to modify HDL, which indicates that there is a dose-dependent relationship between MDA and ApoA1 dimerization and cross-linking. We also found that cholesterol efflux capacity of MDA-HDL is reduced by MDA and pro-inflammatory cytokines (IL-1beta, IL-6) are increased by MDA-HDL modification in a dose-dependent manner in ApoE-/- macrophages. Therefore, PON1 inhibits MPO-mediated ApoA1 and HDL modification, decreases MDA-HDL adduct levels formed by MPO-mediated HDL oxidation, and restores the impaired cholesterol efflux capacity due to oxidized HDL by inhibition of MPO peroxidase activity.

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A Pivotal Role of Smooth Muscle Bmal1 in DOCA Plus Salt-induced Mouse Aortic Aneurysm

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Rational: Bmal1 is a basic helix loop helix (bHLH) transcription factor and is an indispensable core clock gene. Bmal1 is expressed in vascular smooth muscle and has been implicated in atherosclerosis and vascular remodeling. However, it is unknown whether vascular smooth muscle Bmal1 is involved in aortic aneurysm. We use a smooth muscle selective Bmal1 knockout (SM-Bmal1-/-) mouse and mineralocorticoid receptor agonists plus salt induced aortic aneurysm mouse model to address this specific question.
Approach and Results: Aortic Aneurysm was induced in SM-Bmal1-/- and littermate controls by subcutaneously implanting a 50mg DOCA (deoxycorticosterone acetate) pellet for 21 days or mini-pump with 200μg/Kg/day aldosterone for 28 days and drinking 0.9% saline solution. The abdominal aortic aneurysm incidence was dramatically decreased from control 30.7% (4-month-old) and 68.0% (8-month-old) to 4.0% ($p<0.05$) and 0.0% ($p<0.001$) in SM-Bmal1-/- mice respectively. This was associated with a significant decrease in the aortic inner and external diameters. While SM-Bmal1 deletion did not alter the plasma sodium concentration, it significantly decreased the elastin breakage in aorta. In situ zymography assay demonstrated a significantly suppressed gelatinase activity in SM-Bmal1-/- mouse aorta when the metalloproteinases (MMPs) were complexed with their tissue inhibitors (TIMPs). Gel zymography showed no decrease in MMP2/9 activities in the SM-Bmal1-/- mouse aorta, real-time PCR analysis showed TIMP4 mRNA and immunohistochemistry analysis showed TIMP4 protein were selectively up-regulated among the four TIMP isoforms. Mouse TIMP4 promoter contains 7 E-boxes, ChIP assay of the mouse aorta tissues showed 4 of them bound to Bmal1. Further TIMP4 promoter luciferase activity analysis demonstrated TIMP4 promoter activity was dramatically activated by the deletion of Bmal1.
Conclusions: Deletion of Bmal1 from smooth muscle cells protects mice from DOCA plus salt induced aortic aneurysm formation at least in part via up-regulating TIMP4.

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Lamb Meat Lipid Oxidation and Iron Changes during Different Routine Cooking Methods

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Student

Purpose: The main object of this study was to evaluate the influence of three cooking methods on lipid oxidation and heme iron to non-heme iron changes in raw and cooked meat treated by commercial cooking methods in Iran.
Methods: Present study was carried out in three experimental steps. Preparation of meat samples and determination of proximate composition of raw and cooked meat were conducted as the first step. Secondly, lipid oxidation was determined using three oxidation indices, including peroxide value, conjugated dienes and thiobarbitoric reactive species (TBARS). Also, effect of cooking on heme and non-heme iron content was studied. Finally, the statistical relationships between lipid oxidation indexes against total iron, heme and non-heme iron changes were investigated.
Results: Peroxide value decreased in all cooked samples. Conjugated dienes as another oxidation index increased significantly in fried and grilled samples, but not in stewed lamb meat. In contrast, stewed samples showed significant more TBARS content about 88.94% than raw meat ($p<0.05$). Total iron was reduced by 20.63 %, 15.95 % and 4.85 % after stewing, frying and grilling, respectively. Assessment of correlation between either heme or non heme iron and the investigated oxidation indexes revealed direct relationships between heme iron and conjugated dienes. Similar result was obtained for non heme iron and TBARs.
Conclusion: lamb meat lipid oxidation and non heme iron appeared in stewing fallowed by frying and grilling. Long cooking time and high water pressure in stewing could be the main reasons for accumulation of the most stable products of fat oxidation in stewed lamb meat.

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Management of Pulmonary Arteriovenous Malformations: University of Kentucky Experience

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Pulmonary arteriovenous malformations (PAVMs) are atypical vascular structures involving a direct connection between the pulmonary arterial and venous circulations. While PAVMs are a relatively uncommon disorder, unmanaged cases are at risk for the development of serious complications including embolization and infection. Since their first description in 1897, PAVMs have been identified and treated in a variety of ways. Advancements in diagnostic methods and operative techniques have allowed for more effective treatment of the disease. Most recently, the use of vascular plug embolization has been described as an effective therapeutic procedure in the management of PAVMs. A case report involving the successful treatment of a patient with PAVM using vascular plug embolization at the University of Kentucky will be presented.

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Role of Smooth Muscle Cell Specific iPLA2β in Vascular Inflammation and Neointimal Formation in a Murine Femoral Artery Wire Injury Model

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Staff

Coronary heart disease is the single leading cause of death in the United States. Coronary revascularization, including coronary artery bypass graft and percutaneous coronary intervention (PCI) is the most common modality in patients with coronary diseases. However, it is also among the most costly, and is often associated with a high incidence of restenosis. Thus, there is an urgent need to identify new therapeutic targets for coronary heart disease. Calcium independent phospholipase A2β (iPLA2β) is a member of the phospholipase A2 superfamily that acts on phospholipids to produce a free fatty acid and a lysophospholipid. iPLA2β is ubiquitously expressed and is implicated in many human diseases. To investigate whether targeting smooth muscle-specific iPLA2β is sufficient to reduce vascular inflammation and neointima formation, we established a femoral artery injury model that better mimics PCI and developed a novel tamoxifen-inducible smooth muscle-specific iPLA2β knockout mouse model (SM- iPLA2β iKO). By using genomic PCR with smooth muscle-specific Cre-mediated genomic DNA recombination, we demonstrated that iPLA2β was specifically deleted in smooth muscle tissues such as aorta, carotid artery (CA), mesenteric artery (MA), and bladder, but not that in others, including heart, kidney, lung, brain, liver, and skeletal muscle. Smooth muscle cell specific deletion of iPLA2β was verified by real-time PCR, Western blot, and iPLA2 enzymatic assay. Interestingly, SM- iPLA2β iKO mice, unlike global iPLA2β knockout mice, did not have iPLA2γ, iPLA2ζ, and cPLA2α genetic compensatory upregulation. iPLA2β protein was upregulated by wire injury and was predominantly detected in neointima area in a time dependent manner. Using a femoral artery injury model, we illustrated that wire-injury-induced neointima formation was markedly decreased in SM- iPLA2β iKO mice, which was temporally correlated with a remarkable reduction of neutrophil infiltration into neointima in SM- iPLA2β iKO mice. These data demonstrate a critical role of smooth muscle cell iPLA2β in neointima formation and inflammation in a femoral artery injury model, suggest that smooth muscle cell iPLA2β participates in the initiation and early progression of vascular inflammation and neointima formation, and indicate that iPLA2β may represent a novel therapeutic target for treatment of coronary heart disease.

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Disruption of BP Circadian Rhythm in Diabetic db/db Mice is Associated with Hyperglycemia and Desynchronization of Central and Peripheral Oscillators

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Blood pressure (BP) exhibits 24-hour rhythm. Loss of BP oscillation has been found in up to 75% diabetic patients and is associated with increased risks of target organ injuries. However, the mechanisms underlying the disruption of BP circadian rhythm in diabetes remain poorly understood. The type 2 diabetic db/db mice in C57/KsJ background provides a useful model for dissecting the mechanisms underlying the diabetes associated disruption of BP circadian rhythm as we and others have demonstrated that type 2 diabetic db/db mice in C57/KsJ background have hypertension and severe disruption of BP circadian rhythm. However, these db/db mice were severely hyperglycemic (>600 mg/dL), which only resemble a small population of the diabetic patients as the blood glucose level would not reach such high level in most diabetic patients. Moreover, deciphering the contribution of clock gene dysfunction in various systems to the disruption of BP circadian rhythm using this model requires large number of mice due to the demanded multiple time point sampling. To address these specific issues, we generated a mouse model with moderate hyperglycemia and with a luciferase reporter linked to a clock gene per2, which allows the high time-resolution monitoring of clock gene oscillation over a period of days with a single tissue. We have cross bred the leptin receptor mutated db/db mice in the C57BL/KsJ background with PERIOD2::LUCIFERASE knock in mice in C57BL/6J background. At 4-5 months of age, the blood glucose in these db/db-Per2::luc mice was higher than controls (320.3 vs 153 mg/dL) but was significantly lower than the C57/KsJ -db/db mice (608.5 mg/dL). We then determined the BP in the db/db-per2::luc mice using radiotelemetry under 12: 12 light: dark cycle. The circadian parameters of BP, including period length, amplitude and acrophase were calculated using Chronos-fit software. The results demonstrated that db/db-per2::luc mice have normal BP value but disrupted BP circadian rhythm, with decreased power of 24h oscillation, diminished amplitude and shifted acrophase. However, the extent of the disruption was significantly less than that we have reported in the C57/KsJ-db/db mice. We measured the Per2 oscillations in various isolated tissues by using LumiCycle. We found that the phase of Per2 protein oscillation is advanced in aorta, mesentery artery and liver, but delayed in SCN isolated from db/db-per2::luc mice compared to those of non-diabetic control mice . In summary, we demonstrated that the level of hyperglycemia is associated with the extent of the disruption of BP circadian rhythm in db/db mice. In addition, the phases of central and peripheral oscillators are differently affected in db/db mice.

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Tracking the Tissue Distribution of Secondary Heart Field Derived Cells

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Objective: The distribution of embryonic cells derived from the secondary heart field (SHF) has not been fully defined in mammalian systems. Determination of this lineage has been facilitated by the availability of mice that express Cre under that control of promoters of genes that are of secondary heart field origin. Monocyte-specific enhancer factor 2c (Mef2c) is considered to be specific for SHF genes. The objective of this study was to determine the tissue distribution of cells of SHF-origin. This was performed using mice expressing Cre under the control of Mef2c that also had a LacZ gene knocked into the Rosa26 locus, downstream of a lox flanked repressor.
Methods and Results: This experiment was performed using mice expressing Cre under the control of Mef2c that also had a LacZ gene knocked into the Rosa26 locus, downstream of a lox flanked repressor. The following tissues were harvested from Mef2c-Cre x Rosa26 lacZ and non Cre-expressing mice: adipose (white), adipose (brown), adrenal glands, aorta, bladder, bone, brain, diaphragm, esophagus, heart, intestine, kidney, liver, lung, ovaries, pancreas, skeletal muscle, skin, superior mesenteric artery, spleen, stomach, testis, thymus, trachea, uterus, and vas deferens. Tissues were dissected free from mice and stained with X-gal using a standardized procedure. Tissues were evaluated by comparison of endogenous β-galactosidase activity in non Cre-expressing mice, compared to those expressing Cre. β-galactosidase activity was detected in the following tissues of the Cre expressing mice: aorta, brain, heart, lung and spleen. These positive tissues were subsequently sectioned and stained to identify regions within the tissue that expressed β-galactosidase activity. This analysis demonstrated restricted localization of stained regions in each tissue. This included the right ventricle of hearts, ascending aorta, lung vascular tissue, brain outer cortex, and spleen capsule.
Conclusion: The presence of SHF-derived cells has a distinct tissue distribution as defined by this linage tracking study using a Mef2c promoter driven Cre. This information is important in interpreting ongoing studies in which mice have been developed with a functional deficiency of a gene in SHF-derived cells.

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The Role of Fatty Acid Desaturase 1 in Inflammation Initiation and Resolution in Atherosclerosis

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Student

Studies suggest that dietary enrichment of ω-3 polyunsaturated fatty acids (PUFAs) may improve cardiovascular disease (CVD) outcomes, however the mechanisms underlying these observations remain elusive. Additionally, genome-wide association studies have linked single nucleotide polymorphisms (SNPs) in genes encoding essential PUFA metabolizing enzymes, the Fatty Acid Desaturases 1, 2, and 3 (FADS1-2-3), to dyslipidemia and other metabolic risk factors known to be linked to CVD. Since enzymatic oxidation of specific PUFAs, commonly arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), generate signaling mediators that coordinate both the initiation and resolution of inflammation, understanding the interplay of PUFA metabolism and vascular inflammation has broad implications in cardiometabolic drug discovery. In an effort to change the abundances of distinct PUFAs and associated lipid mediators, we have used antisense oligonucleotides (ASO) to inhibit Fads1, the only known mammalian Δ5 desaturase enzyme that can generate endogenous AA or EPA, in Ldlr-/- mice fed diets enriched in ω-3 Fads1 substrate PUFAs. Fads1 knockdown alters the circulating PUFA pool, specifically resulting in accumulation of its substrate fatty acids (dihomo-γ-linolenic acid and eicosatetraenoic acid) and marked reduction of its enzymatic products (AA and EPA). Metabololipidomic profiling reveals that ω-3 PUFA feeding increases the production of EPA- and DHA-derived pro-resolving lipid mediators compared to control diet. Interestingly, Fads1 knockdown diminishes levels of these same pro-resolving mediators, and concomitantly reduces the observed protective effects of ω-3 PUFA feeding on diet-induced atherosclerosis. Fads1 inhibition also results in striking reorganization of circulating monocytes subsets in a highly diet-specific manner. Collectively, our studies provide new information regarding the relative contribution of endogenous (FADS1-derived) and exogenous (diet-derived) EPA and DHA in the production of specialized pro-resolving lipid mediators, and how lipid mediators affect cardiometabolic phenotypes. Furthermore, our studies provide the first mechanistic understanding behind the genetic link between FADS1-2-3 SNPs and atherosclerosis.

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Lineage Tracking of Fibroblasts in the Aorta during Angiotensin II Infusion

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Student

Objective: S100A4, also known as fibroblast specific protein 1, governs epithelial to mesenchymal transition and is thought to be specifically expressed in fibroblasts. The S100A4 promoter is used to drive Cre recombination in fibroblast specific gene expression. However, the S100A4 promoter is potentially active in cell types in addition to fibroblasts. Our previous studies have demonstrated angiotensin II (AngII) infusion increases aortic medial cells expressing S100A4 promoter driven Cre in mice ubiquitously expressing a conditional LacZ gene. The purpose of this study was to determine whether cells tracked with a S100A4 driven Cre retain markers for fibroblasts or expressed characteristics of smooth muscle cells.

Approach and Results: Mice expressing Cre under the control of the S100A4 promoter were bred into transgenic mice with a repressed lacZ gene at the Rosa26 locus. At 8-10 weeks of age, mice were infused subcutaneously with either saline or AngII (1,000 ng/kg/min) for 28 days. Following infusion, aortas were dissected free and sections were obtained from the ascending, descending, and abdominal aortic regions. Expression of Cre was identified by the presence of β-galactosidase and was colocalized with immunostaining for smooth muscle cells (α-smooth muscle cell actin) or reticular fibroblasts (ERTR7). Colocalization was assessed by confocal microscopy. As noted previously, AngII infusion increased β-galactosidase tissue staining in the ascending and abdominal aortic regions, but not the descending region. β-galactosidase immunostaining was more closely colocalized with α-smooth muscle cell actin immunostaining than with ERTR7 immunostaining in all aortic regions.

Conclusions: AngII infusion drives an increased expression of S100A4 in medial cells tracked with a S100A4 promoter driven Cre. Despite S100A4 being defined as a fibroblast specific gene, lineage tracked cells primarily had expression of a smooth muscle cell marker. It remains to be determined whether this reflects a transformation of fibroblasts into smooth muscle cells, dedifferentiation of smooth muscle cells, or promiscuous expression of the promoter.

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Lipidomic Profiling Uncovers Land's Cycle Phospholipid Remodeling Pathways as Novel Therapeutic Targets in Clear Cell Renal Carcinoma

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Student

Recent epidemiological studies have shown obesity to be a major risk factor for the development of clear cell renal cell carcinoma (ccRCC), and obese patients have strikingly elevated mortality. The mechanisms by which obesity alters ccRCC progression are unknown, but there is mounting evidence that early disease pathogenesis is associated with aberrant lipid accumulation in proximal tubule epithelial cells. For instance, the name "clear cell" originates from the characteristic clear cytoplasmic inclusions that closely resemble unilocular lipid droplets. Furthermore, urinary biomarker studies have found that ccRCC patients have elevated urinary levels of the lipid droplet-associated perlipin-ADRP-Tip47 (PAT) family of proteins, which are intracellular regulators of lipid storage. To better understand lipid metabolic alterations in human ccRCC, we performed a Triple-TOF mass spectroscopy liipidomic analysis of matched-pair stage IV ccRCC tumors and adjacent non-tumor tissue. Our Lipidomic analysis showed a specific lipid signature within the ccRCC tumor, with a particularly striking elevation in cholesterol esters, which likely makes up the neutral lipid core of the large lipid droplets seen in ccRCC tumor cells. In addition to alterations in neutral lipids, ccRCC was characterized by marked reorganization of the Land's cycle, which dictates the saturation/unsaturation of cellular membrane phospholipids. In particular, phosphatidylethanolamine (PE) species showed clear acyl chain switching characterized by loss of shorter saturated species and increases in longer polyunsaturated fatty acid (PUFA)-containing species. At the same time several species of phosphatidylcholine (PC) (32:1/32:0) were elevated in the tumor as opposed to the adjacent normal tissue. The corresponding lysophophatidylcholine (LPC) precursors were reciprocally reduced in tumor vs. non-tumor tissue. LPCAT1, a key regulator of this LPC to PC conversion, exhibited a 3-fold increased expression in tumor tissue. Using The Cancer Genome Atlas ccRCC cohort (n=403), LPCAT1 high expression (2 SD > μ) significantly decreases patient survival from 116 month median survival (n=359) to 52 month median survival (n=54). Collectively, our results indicate that targeting ccRCC-specific lipid metabolic alterations could represent a novel therapeutic strategy for patients suffering ccRCC. In addition to these human data, we will discuss mouse models of obesity-driven ccRCC that we are developing to test such preclinical leads.

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The Gut Microbe-Derived, Trimethylamine (TMA), Contributes to the Etiology of Ethanol-Induced Liver Disease

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Fellow

Objectives:
Sustained alcohol abuse initiates a progressive disease pathogenesis involving simple steatosis, steatohepatitis, fibrosis, cirrhosis, and in some cases hepatocellular carcinoma. There is mounting evidence that microbes resident in the human intestine represent a key transmissible factor promoting inflammatory diseases including ALD. However, mechanisms by which gut microbiota synergize with excessive alcohol intake to promote ALD are poorly understood. We have recently revealed a metaorganismal pathway where nutrients in high fat foods can be metabolized by gut microbes to generate trimethylamine (TMA), which is subsequently metabolized by the host enzyme flavin-containing monooxygenase 3 (FMO3) to produce the pro-atherogenic metabolite trimethylamine-N-oxide (TMAO). Hepatic FMO3 mRNA levels are suppressed in alcoholics and breath levels of TMA correlate with the severity of alcoholic hepatitis. Thus, this study investigates the role of the gut microbe-derived metabolite TMA in ethanol-induced liver injury.

Methods and Results:
We first explored a novel approach to block TMA production by using a small molecule inhibitor targeting the TMA-producing enzyme, CutC/D. This inhibitor, known as CC08, was used during chronic pair- and ethanol-feeding (25 days Lieber DiCarli feeding). After 25 days of feeding, ethanol treatment increased both plasma TMA and TMAO levels. In mice with CC08 supplementation, TMA and TMAO production was significantly suppressed. Further, CC08 treatment abrogated ethanol-induced hepatic steatosis, while reducing hepatocellular inflammation. We then used antisense oligonucleotides (ASOs) targeting FMO3 to inhibit host enzymatic conversion of TMA to TMAO in mice challenged with the chronic ethanol-feeding paradigm. FMO3 ASO treatment reduced hepatic FMO3 mRNA and protein levels greater than 90%. ASO-mediated knockdown of FMO3 resulted in a large increase in substrate (TMA) to product (TMAO) ratio in pair-fed mice, and this was exacerbated in ethanol-fed mice. Additionally, FMO3 knockdown in ethanol-fed animals completely prevented ethanol-induced lipid droplet accumulation, yet promoted immune cell infiltration and hepatocyte apoptosis. Collectively, these results suggest that elevation of TMA (driven by ethanol and FMO3 knockdown) exacerbates ethanol-induced liver injury.

Conclusions:
Our results establish TMA as an important regulator of ethanol-induced liver injury. Inhibition of TMA production by utilizing small molecule inhibitors targeting the TMA lyase, such as CC08, may represent a novel therapeutic approach to combat ethanol-induced hepatic steatosis and hepatocellular inflammation.

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PI(4,5)P2 is Translocated by ABCA1 to the Cell Surface where it Mediates Apolipoprotein A1 Binding and Nascent HDL Assembly, and it is Carried on HDL

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Fellow

Rationale: The molecular mechanism by which ABCA1 mediates cellular binding of apolipoprotein A-I (apoA1) and nascent HDL assembly is not well understood.
Objective: To determine the cell surface lipid that mediates apoA1 binding to ABCA1 expressing cells and the role it plays in nascent HDL assembly.

Methods and Results: Using multiple biochemical and biophysical methods, we found that apoA1 binds specifically to phosphatidylinositol (4,5) bis-phosphate (PIP2). Flow cytometry and PIP2 reporter binding assays demonstrated that ABCA1 led to PIP2 redistribution from the inner to the outer leaflet of the plasma membrane. Enzymatic cleavage of cell surface PIP2 or decreased cellular PIP2 by knockdown of phosphatidylinositol-5-phosphate 4-kinase impaired apoA1 binding and cholesterol efflux to apoA1. PIP2 also increased the spontaneous solubilization of phospholipid liposomes by apoA1. Using site directed mutagenesis; we found that ABCA1's PIP2 and phosphatidylserine translocase activities are independent from each other. Furthermore, we discovered that PIP2 is effluxed from cells to apoA1, where it is associated with HDL in plasma, and that PIP2 on HDL is taken up by target cells in a scavenger receptor-BI (SR-BI) dependent manner. Mouse plasma PIP2 levels are apoA1 gene dosage dependent and are > 1 uM in apoA1 transgenic mice.

Conclusions: ABCA1 has a PIP2 floppase activity, which increases cell surface PIP2 levels that mediate apoA1 binding and lipid efflux during nascent HDL assembly. We found that PIP2 itself is effluxed to apoA1 and it circulates on plasma HDL, where it can be taken up via the HDL receptor SR-BI.

Current Studies: We are extending these studies by engineering a novel recombinant Grip-apoA1 protein. Grip binds to PIP2 specifically and addition of Grip to cholesterol loaded macrophages robustly increased apoA1-ABCA1 mediated cholesterol efflux. We are studying effect of Grip injections in mouse model and characterizing recombinant Grip-apoA1 protein cholesterol efflux activity. We believe that recombinant Grip-apoA1 protein can be used as novel therapeutic to treat Coronary Artery Disease (CAD).

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Angiotensin II Infusion Does Not Influence the Distribution of Cardiac Neural Crest-Derived Smooth Muscle Cells in the Ascending Aorta

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Fellow

Objective: Angiotensin II (Ang II) causes vascular pathology including hypertrophy and aneurysms. Smooth muscle cells (SMCs) exert a pivotal role in vascular remodeling. The localization of these vascular pathologies may be associated with distinct embryonic origins of SMCs. While there is evidence that most SMCs derive from the cardiac neural crest in the ascending aorta, other data has inferred a greater complexity of origins for this cell type. The aim of this study was to determine whether heterogeneity of the embryonic origin of SMCs in the ascending aorta is influenced by AngII.

Methods and Results: ROSA26 lacZ mice were bred to mice expressing Cre under control of the Wnt1 promoter to track SMCs of cardiac neural crest origin. Gross examination of beta-galactosidase stained aortas from adult mice demonstrated cells of cardiac neural crest present from the sinotubular ascending aorta to just distal to the subclavian branch. For further examination of cell type distribution, both sagittal and cross sections were obtained. These stained sections demonstrated there was a distinct distribution on the anterior aspect of the ascending aorta, with SMCs of cardiac neural crest origin only being present in the inner laminal layers. On the proximal area of this region, there was transmural staining for beta-galactosidase. Much of the aortic arch to the subclavian branch also had transmural beta-galactosidase staining. Previous studies have demonstrated that infusion of AngII promotes pathology that varies along the length of the ascending aorta. Also, the medial pathology is most pronounced on the adventitial aspect. Therefore, we examined whether the distribution of cardiac neural crest-derived SMCs was influenced during AngII infusion. However, after 28 days of pressor infusion rates of AngII, there was no overt change in the distribution of cardiac neural crest-derived SMCs in the ascending aorta.

Conclusion: SMCs from the outer aspect of the media are derived from a different embryonic origin. Cardiac neural crest derived-SMCs retain their unique distribution in the inner media of the ascending aorta during AngII infusion in adult mice.

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Inducible Depletion of Calpain-2 Attenuates Angiotensin II-induced Cytoskeletal Protein Destruction During Aortic Aneurysm Development in Mice

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Student

Background and Objective: Chronic infusion of angiotensin II (AngII) induces abdominal aortic aneurysms (AAAs) in normal and hypercholesterolemic mice which lead to disruption of structural integrity of aortic wall due to the dissociation of cytoskeletal structural proteins that bridge SMC contractile filaments with extracellular matrix by proteases. By using AngII-induced AAA mouse model, we demonstrated that AngII infusion significantly increased fragmentation of cytoskeletal structural protein, Filamin A, in AAAs. Further, using pharmacological inhibitor and genetic deficient mice, we identified that calpain-2 (a class of calcium-activated, neutral cysteine proteases) play a critical role in AngII-induced AAA formation in mice. The purpose of this study was to determine the functional contribution of calpain-2 in AngII-induced cytoskeletal structural protein destruction during AAA development.

Methods and Results: Calpain-2 floxed mice that were hemizygous for Cre-ERT2 in an LDLr -/- background were produced by breeding male Cre-ERT2 to female calpain-2 floxed mice. At 8 weeks of age, male Calp-2 x Cre-ERT2 (Cre+) and non-Cre littermates (Cre-) mice were injected with tamoxifen (25 mg/kg, i.p.) daily for 5 consecutive days. After 2 weeks, Western blot analyses showed a complete depletion of calpain-2 protein in the aorta from Cre+ mice compared to Cre- littermates. Male Cre+ and Cre- (N=12 each) mice were fed a Western diet and infused with saline or AngII (1,000 ng/kg/min) by osmotic minipumps for 2 weeks. AngII infusion significantly ($P<0.05$ vs saline) increased c-terminal fragmentation of cytoskeletal structural protein, talin and kinases such as integrin linked kinase-1 (ILK-1), and focal adhesion kinase in addition to filamin A. Inducible depletion of calpain-2 significantly ($P<0.05$; Cre+ vs Cre-) blunted AngII-induced fragmentation of talin, filamin A and ILK-1. Further siRNA mediated silencing of calpain-2 in aortic SMCs and fibroblasts significantly reduced AngII-induced fragmentation of filamin A and talin.

Conclusion: These findings suggest that calpain-2 plays a critical role in AngII-induced cytoskeletal structural protein fragmentation during AAA development in mice.

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MicroRNA-146a Deficiency Prevents PCSK9 Gain-of-Function Mutation-induced Hypercholesterolemia in Mice

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Student

Background and Objective: Mimetic mediated activation of microRNA 146a (miR-146a) reduces atherosclerosis via suppression of nuclear factor-κB-driven inflammation in mice. The purpose of this study was to determine whether miR-146a influences plasma cholesterol in hypercholesterolemic mice.

Methods and results:
To induce hypercholesterolemia, female C57BL/6 miR-146a WT (n=8) and miR-146a KO (n=8) mice were injected intraperitoneally with an adeno-associated viral vector (AAV) expressing the proprotein convertase subtilisin/kexin type 9 (PSCK9 D377Y) gain-of-function mutant at a dose of 3 x 10 10 genomic copies/mouse. After infection, mice were fed a Western diet (42% calories from saturated fat) for sixteen weeks, and plasma PCSK9 and total cholesterol concentrations were monitored monthly using an enzymatic assay. Plasma PCSK9 concentrations were profoundly increased 4 weeks post injection (Baseline: WT – 179 ± 12; KO – 207 ± 12; Week 4: WT - 1700 ± 148; KO - 2689 ± 305 ng/ml) and remained significantly high during 16 weeks (WT - 882 ± 142; KO - 718 ± 109 ng/ml) of Western diet feeding. Consistent with increased plasma PCSK9 concentrations, plasma cholesterol concentrations were increased in both groups of mice. Interestingly, miR-146a KO group mice showed less significant increase in plasma cholesterol compared to WT group (Baseline: WT – 88 ± 3; KO – 83 ± 3; Week 4: WT - 328 ± 25; KO - 195 ± 18 mg/dl) irrespective of the comparable plasma PCSK9 levels. Also, lipoprotein distribution analysis with size exclusion gel chromatography revealed that miR-146a KO mice showed a strong reduction in high density lipoprotein (HDL) particles while very low density lipoprotein (VLDL) and low density lipoprotein (LDL) particles were not affected.

Conclusion: Our findings suggests that miR146a plays a critical role in the regulation of HDL particles in PCSK9 gain-of-function mutant-induced hypercholesterolemia in mice. Future studies will identify gene targets influenced by miR-146a in regulating HDL-cholesterol in hypercholesterolemic mice.

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An XX Sex Chromosome Complement Promotes the Development of Obesity, Hypercholesterolemia and Atherosclerosis in Male and Female Ldlr-/- Mice Fed a We

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Student

Background: Underlying mechanisms contributing to sexual dimorphism of cardiovascular diseases are not well understood. Sex hormones are primary contributors to sexual dimorphism of cardiovascular diseases. By comparison, little is known regarding the contribution of genes on sex chromosomes (XX and XY) to sexual dimorphism of cardiovascular diseases, even though the X chromosome contains around 5% of the human genome. In this study, we hypothesized that genes on sex chromosomes influence the development of obesity, hypercholesterolemia and atherosclerosis.

Methods and Results: Transgenic male mice with deletion of Sry from the Y-chromosome expressing Sry on autosomes (8-12 weeks of age) were bred to female Ldlr-/- mice to generate male and female mice with an XX or an XY sex chromosome complement (FXX, FXY, MXX, MXY). Mice were fed a Western diet (Teklad TD88137) for 3 months. XX mice exhibited increased body weight compared to mice with an XY sex chromosome complement, regardless of gonadal sex (FXX, 41.2 ± 2.4; FXY, 31.7 ± 2.5 g; $P<0.05$; MXX, 51.5 ± 1.2; MXY, 41.7 ± 1.8 g; $P<0.05$). Moreover, XX mice had significantly increased serum cholesterol concentrations, regardless of gonadal sex (FXX, 2501 ± 192; FXY, 890 ± 141 mg/dl; $P<0.05$; MXX, 3814 ± 344; MXY, 1297 ± 385 mg/dl; $P<0.05$). Elevations in serum lipids were manifest as increased VLDL and LDL-cholesterol. The extent of atherosclerosis in aortic arch was significantly increased in XX compared to XY females (XXF, 26.3 ± 1.6; XYF, 17.4 ± 3 % lesion surface area; $P<0.05$). However, most likely due to the profound extent of atherosclerosis, there was no significant difference in aortic arch atherosclerosis between XX and XY males (XYM, 35.4 ±1.4; XXM, 33 ± 2% lesion surface area). In the aortic sinus, atherosclerotic lesion surface area was significantly increased in XX mice, regardless of gonadal sex (FXX, 60.4 x 104 ± 3.6 x 104; FXY, 32.4 x 104 ± 3.8 x 104 µm2; $P<0.05$; MXX, 67.1 x 104 ± 9.6 x 104; MXY, 36.2 x 104 ± 3.7 x 104 µm2; $P<0.05$).

Conclusion: Results demonstrate that an XX sex chromosome complement promotes diet-induced obesity, hypercholesterolemia and atherosclerosis regardless of gonadal sex. Future studies will identify whether genes on the X or Y chromosome contribute to these effects.

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Pyocyanin, a Pathogen-associated Ligand of the Aryl Hydrocarbon Receptor, Reduces Differentiation of 3T3-L1 Adipocytes

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Objectives
The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor known for regulation of genes involved in xenobiotic metabolism of environmental toxins, such as polychlorinated biphenyls (PCBs). Previously, we demonstrated PCB-induced activation of AhR regulated adipocyte differentiation in vitro and promoted adipose inflammation in mice with diet-induced obesity. Recent studies demonstrated that AhR senses bacterial pigments to regulate immune and inflammatory responses, suggesting a link between the microbiome, AhR activation, and immune response. The purpose of this study was to determine if pathogen-associated AhR ligands regulate differentiation of 3T3-L1 adipocytes.

Methods/Results
Preadipocytes were incubated with vehicle (VEH) or increasing concentrations of pyocyanin throughout differentiation. Pyocyanin (100 μM) treatment significantly reduced 3T3-L1 differentiation as quantified by neutral lipid staining with Oil Red O (VEH: 0.88 ± 0.09; pyocyanin: 0.42 ± 0.05; absorbance of Oil Red O; $P<0.0001$) corresponding with reduced mRNA abundance of aP2 (VEH: 1.3 ± 1.3; pyocyanin: 0.25 ± 0.1; $P<0.05$) and PPARγ (VEH: 1.1 ± 0.6; pyocyanin: 0.64 ± 0.2; $P<0.05$). Moreover, pyocyanin robustly increased mRNA abundance of both AhR (VEH: 1.0 ± 0.3; pyocyanin: 13.1 ± 2.7; $P<0.0001$) and CYP1A1 (VEH: 1.2 ± 0.6; pyocyanin: 3.4 ± 1.1; $P<0.0001$). The effect of pyocyanin to reduced Oil Red O staining was not attenuated by pretreatment with 6, 2', 4'-trimethoxyglavone (TMF), a known AhR antagonist (VEH: 1.00 ± 0.09; pyocyanin: 0.57 ± 0.04; TMF+pyocyanin: 0.50 ± 0.06; absorbance of Oil Red O; $P<0.0001$). Similarly, in fully differentiated adipocytes, pyocyanin (100 μM) treatment reduced neutral lipid staining (VEH: 0.17 ± 0.012; pyocyanin: 0.08 ± 0.008; absorbance of Oil Red O; $P<0.0001$) and significantly increased AhR mRNA abundance (VEH: 1.0 ± 0.3; pyocyanin: 3.3 ± 0.8; $P<0.0001$).

Conclusions
Results demonstrate that pathogen-associated AhR ligands regulate adipocyte differentiation. Future studies will focus on AhR-mediated effects of these ligands to regulate adipocyte function in vitro and in vivo.

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miR-182 and miR-183 Mediate the Glucose-lowering Effects of the Bile-Acid Resin, Colesevelam

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Colesevelam is a bile acid resin clinically used to treat Type 2 diabetic (T2D) patients. Although its hypoglycemic mechanism is not completely known, clinical trials suggest increased secretion of gastric incretins, i.e. glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP), may underlie its glucose-lowering properties. Nevertheless, our recent work suggests that an incretin-independent mechanism contributes to colesevelam's glycemic effect. Using a rodent model, we treated Zucker Diabetic Fatty (ZDF) rats with colesevelam (2% in diet) for 8-weeks. Despite no apparent change in GLP-1 secretion nor GIP levels, improvements in glucose tolerance and insulin secretion were detected 1-week post treatment, suggesting other non-incretin mechanisms are likely involved. microRNAs (miR) are powerful regulators of glucose metabolism and contribute to the pathogenesis of T2D. Hence, we performed small RNA sequencing on total RNA isolated from livers of ZDF rats treated with colesevelam for 4-weeks to determine whether miRNAs may underlie the hypoglycemic effects of Colesevelam. Strikingly, we found that the miR-182/183/96 cluster, previously shown to be down regulated in T2D models, were elevated in the livers of colesevelam treated rats ($p<0.05$; $n= 11$). miR-182 and miR-183 expression have previously been shown to be regulated by cellular cholesterol and sterol response element binding protein 2 (SREBP-2). In addition, we found miR-182 and miR-183 target genes, Tcf7l2 and Foxo1, to be decreased in the livers of colesevelam treated rats ($p<0.05$, $p=0.06$, respectively, $n=8$). To test the hypothesis that miR-182 is required for colesevelam's effect, we conducted an 8-week study where we treated db/db mice with 2% colesevelam or vehicle chow (ad lib access), in addition to a locked-nucleic acid (LNA) inhibitor of miR-182 or vehicle every 2-weeks. We found that in the first 4-weeks of the study, weekly fasting glucose levels and glucose tolerance (GTT, performed at week 4)) showed improvements in mice treated with Colesevelam, but not those given Colesevelam + LNA-182. However, these effects were lost as the diabetic phenotype progressed later in the study. Despite the worsening diabetic phenotype in mice treated with both colesevelam and LNA-182 inhibition, we did not observe differences in fasting insulin, C-peptide, or plasma lipid levels. Based on our results, we propose that the hepatic increase in miR-182 and miR-183 in response to colesevelam precede the improvements in gastric incretins and beta-cell function, and likely contribute to the glucose-lowering effects of colesevelam.

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TGFβ Attenuation via Platelet Inhibition Increased Abdominal Aortic Aneurysm Rupture in Mice

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Objective: Platelets are critical in both maintaining hemostasis and propagating thrombosis. While platelets and platelet-specific cytokines are elevated in patients with abdominal aortic aneurysm (AAA), their role in the pathogenesis of aneurysm disease remains unclear. The purpose of this study was to determine the effects of platelet inhibition or depletion in a mouse model of angiotensin II (AngII)-induced AAA.

Methods and Results: To determine temporal accumulation of platelets, low-density lipoprotein deficient (Ldlr-/-) mice were fed a high fat (21% milk fat) and cholesterol (0.2%) diet (HFD) and infused with AngII (1,000 ng/kg/min) for 1, 2, 3, 5, 7, and 28 days. Longitudinal immunohistochemical assays demonstrated platelet accumulation in the suprarenal abdominal aorta at day 1 prior to macrophage accumulation on day 3. The platelet-derived cytokine platelet factor 4 (PF4) was highly correlated to increasing abdominal diameter ($R2 = 0.914$, $P < 0.001$). Genetic deficiency of platelet receptors (protease-activated receptor 4, thrombin receptor on platelets; P2Y12, ADP receptor on platelets) in Ldlr-/- mice fed a HFD and infused with AngII for 28 days resulted in no difference in abdominal aortic diameter but augmented rupture-induced death versus proficient mice ($P < 0.001$). Administration of anti-platelet therapies (P2Y12 inhibitor clopidogrel, direct thrombin inhibitor dabigatran etexilate, and thromboxane receptor inhibitor aspirin) also significantly augmented rupture-induced death versus placebo ($P < 0.05$). To determine if platelet depletion recapitulated this effect, Ldlr-/- male mice were depleted of circulating platelets utilizing an anti-CD42b antibody, compared to an irrelevant IgG control, injected intravenously every three days concurrent with diet and AngII infusion. All platelet depleted mice diet of abdominal rupture-induced death by day 12 of infusion ($P < 0.001$). This effect is highly pervasive, as it was confirmed successfully in apolipoprotein E deficient (apoE-/-) and normolipidemic C57BL/6J mice infused with AngII, as well as in a second model of aneurysm utilizing deoxycorticosterone acetate (DOCA) salt. Interestingly, platelet deficiency resulted in almost complete ablation of transforming growth factor β1 (TGFβ1) serum levels after 1 day and throughout the remainder of the study.

Conclusion: Platelets are the first cell to accumulate in the abdominal aorta during AAA formation. Platelet depletion and subsequent TGFβ attenuation is associated with pervasive AAA rupture. Inhibition of platelet function may be detrimental in an expanding aortic lumen.

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The Role of HuR in Modulating Cardiac Hypertrophic Signaling

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HuR is a widely expressed RNA binding protein that interacts with specific AU-rich domains in target mRNAs and regulates their expression by modulating stability and/or translation. Our results show that HuR undergoes cytoplasmic translocation, indicative of its activation, in hypertrophic cardiac myocytes. Specifically, HuR translocation is significantly increased in: a) isolated cardiomyocytes (NRVMs) following treatment with phenylephrine (PE) or angiotensin II; and b) myocytes from 8 weeks post-transverse aortic constriction (TAC) hearts. In NRVMs, HuR activation is dependent on p38 MAPK and is necessary for hypertrophic signaling. RNAi-mediated knockdown of HuR inhibited the PE-induced transcriptional activation of NFAT and the subsequent increase in cell size and expression of hypertrophic marker genes ANF and RCAN. In addition, the overexpression of HuR is sufficient to induce hypertrophic signaling in NRVMs. To determine if HuR plays a similar role in hypertrophic signaling in vivo, we created an inducible cardiomyocyte-specific HuR deletion mouse (iCM-HuR-/-) and assessed its cardiac structure and function following TAC. While indistinguishable from wild-type (WT) mice at
baseline, the iCM-HuR-/- mice had significantly less hypertrophy than WT at 8 weeks post-TAC (as measured by the heart weight to body
weight ratio). In addition, the iCM-HuR-/- mice exhibited significantly preserved ejection fraction (38.2% EF in KO vs. 28.4% EF in WT; $P < 0.01$) and reduced chamber dilation compared to WT (64.3 µl ESV and 102.9 µl EDV and in KO vs. 97.1 µl ESV and 135.4 µl EDV in WT; $P <$
0.01) at 8 weeks post-TAC. In vivo hemodynamic measurements also showed that the iCM-HuR-/- mice maintained an improved average rate of developed pressure (8909 mmHg/s +dP/dT and -8593 mmHg/s -dP/dT in KO vs. 7741 mmHg/s +dP/dT and -7859 mmHg/s -dP/dT in WT; $P < 0.05$). Thus, cardiac-specific deletion of HuR reduces the development of hypertrophy in response to pressure overload and significantly preserves left-ventricular chamber structure and function. In conclusion, our results demonstrate for the first time that HuR is necessary and sufficient to induce hypertrophic signaling and plays a central role in the development of pathological hypertrophy and cardiac remodeling.

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Cholesteryl Ester Transfer Protein Raises Plasma Triglycerides and Impairs Triglyceride Clearance via Gonadal Hormones in Male Mice

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Elevated plasma triglycerides are a major risk factor for cardiovascular disease in men and women but the degree of risk is sex-specific, suggesting a role of sex hormones in triglyceride (TG) metabolism. Cholestryl ester transfer protein (CETP) shuttles TG and cholesterol esters between lipoproteins and contributes to decreased HDL with obesity. We have recently shown that CETP contributes to estrogen-regulated TG metabolism. In this study, we aimed to define whether CETP also modulates androgen regulation of TG metabolism.
As mice naturally lack CETP, we used male CETP transgenic mice as a model to define how CETP impacts androgen-regulated TG metabolism. Male CETP transgenic mice and nontransgenic litter mates (WT) were used to evaluate TG clearance, chylomicron TG production, and VLDL liver production. Males were orchiectomized to remove contribution of endogenous gonadal hormones, and allowed to recover prior to studies. Plasma TG and cholesterol were measured from plasma, and lipoproteins separated using FPLC. All data are summarized using mean and standard error, one-way ANOVA, or two-way ANOVA as appropriate. P-values <0.05 were considered statistically significant.
CETP expression in males did not alter body weight, but increased plasma triglycerides by almost two-fold. As TG concentration is a balance of intestinal production of TGs as chylomicrons, packaging of TG into VLDL by the liver, and TG clearance from plasma, we measured TG production in WT and CETP male mice. CETP expression did not alter chylomicron TG production nor VLDL production but increased postprandial TG excursion, suggesting that CETP contributes to impaired TG clearance in males. Orchiectomy reverted the lipid profile and postprandial TG excursion back to WT, suggesting CETP requires gonadal hormones to raise plasma triglycerides and impair triglyceride clearance.
CETP has a role in sex hormone-mediated TG metabolism. In males, CETP drives androgen-mediated TG elevation via impaired clearance. CETP may be an important variable in sex-differences in cardiovascular disease risk.

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The Inducible Deletion of Adipocyte PRR Reduces Body Weight and Fat Mass in Obese Male Mice

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Objectives:
Obesity contributes to approximatively 2.5 million deaths every year. It is associated with life threatening conditions including type 2 diabetes mellitus, liver and cardiovascular diseases. Recently, we found that constitutive adipocyte (pro)renin-receptor (PRR) deficiency induces lipodystrophy, liver steatosis and increases blood pressure in male mice. However, constitutive adipocyte PRR deficiency prevented high-fat diet-induced obesity and drastically decreased fat mass and suggesting that PRR might be a new therapeutic target for obesity. The purpose of this study was to investigate whether temporally-controlled induction of PRR deletion in adipocyte, used as a model to mimic a therapeutic intervention, decreases obesity and fat mass in male mice.

Methods and results:
Male mice expressing an inducible adipocyte-specific Cre under the control of the adiponectin promotor were bred to female PRRfl/fl mice to generate control male (PRRfl/Y) and inducible adipocyte-PRR deficient male mice (PRR-ERT). At 7 weeks of age, PRR-ERT and control mice were injected intraperitoneally on 5 consecutive days with tamoxifen (TMX, 1 mg in 100 µl of sunflower oil, 80 mg/kg) (n=3-4 mice/ group). The induction of adipocyte PRR deletion led to a significant reduction of PRR mRNA levels and PRR protein levels confirming the effective deletion of PRR in adipose tissue. To further investigate the effect of inducible adipocyte PRR deletion on body weight and fat mass in obese mice, PRR-ERT and control mice were fed a high fat diet for 23 weeks. After 23 weeks of high fat feeding, PRR-ERT and control mice were injected intraperitoneally on 5 consecutive days with tamoxifen (TMX) or with vehicle (Veh, sunflower oil) (n=3-4 mice/ group). Preliminary data suggested that the induction of adipocyte PRR deletion decreased significantly body weights (Veh-PRR-ERT, 54.8 ± 2.5g; TMX- PRR-ERT, 38.1 ± 6.4g, $P<0.05$) and reduced significantly the subcutaneous fat mass (Veh-PRR-ERT, 2.90 ± 0.61g; TMX- PRR-ERT, 0.38 ± 0.09 g, $P<0.05$) and the epidydimal fat mass (Veh-PRR-ERT, 1.83 ± 0.59g; TMX- PRR-ERT, 0.67 ± 0.03g, $P<0.05$). Consistent with our previous study showing that adipocyte PRR regulated genes involved in adipogenesis, when challenged with a HF diet, the inducible deletion of adipocyte PRR led to a downregulation of genes involved in late stage of adipocyte differentiation and genes involved in lipid metabolism. PPARγ and FAS mRNA abundance was decreased by 68% in adipose tissue of PRR-ERT mice compared to control mice. Interestingly, temporally-controlled induction of PRR deletion in adipocyte did not change liver weights. Taken together, our data confirmed that PRR is a master regulator of body weight, fat mass and adipocyte differentiation. Blood pressure analysis using radiotelemetry are currently under investigation.

Conclusions:
These results have identified a new signaling pathway leading to the regulation of adipogenesis, lipogenesis and lipolysis through the activation of adipocyte PRR signaling. Further investigations will determine the mechanism by which adipocyte PRR regulates adipogenesis and lipogenesis and whether PRR can be used as a therapeutic target to treat obesity and its associated comorbidities.

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Megalin Inhibition Regulates Angiotensinogen and Angiotensin II in Mice

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Student

Objective: Angiotensinogen (AGT) is the unique precursor for generation of angiotensin II (AngII) product. Megalin interacts with both AGT and AngII in kidneys. The purpose of this study was to determine whether inhibition of megalin influences systemic and local AGT and AngII regulation.

Methods and Results: Male C57BL/6J mice were fed a normal laboratory diet and injected intraperitoneally with PBS or megalin antisense oligonucleotides (ASO; 120 mg/kg/week) for 5 weeks. Urine was collected using metabolic cages 2 days prior to termination. At termination, plasma was collected with either EDTA alone or protease inhibitor cocktails to measure plasma AGT and renin, and angiotensin peptides, respectively. Megalin ASO administration reduced megalin mRNA by 80% in kidneys, and led to striking increases concentrations of AGT and renin in urine (PBS versus megalin ASO for AGT: 12 ± 1 versus 3876 ± 509 ng/ml; $P < 0.001$; for renin: 30 ± 24 versus 1758 ± 509 ng AngI/ml/hr; $P < 0.001$). Surprisingly, megalin inhibition also modestly increased plasma AGT concentrations (~17%), accompanied by increased mRNA expression of AGT in liver and kidneys. Plasma renin and AngII concentrations did not differ between PBS and megalin ASO injected mice. Renal AngII, but not AngI, was reduced by megalin inhibition. These findings demonstrate that megalin regulates liver and renal AGT expression as well as renal AngII production. Our bioinformatic analysis identified several well conserved sequences of AGT including the loop formed by residues 291-301 containing strictly conserved solvent accessible hydrophobic residues W292, V299 and S298. Surface plasmin resonance demonstrated that mutation of residue 292 from W to A diminished its binding to megalin.

Conclusions: Megalin regulates systemic and renal AGT in addition to renal AngII production. A conserved residual W292 may contribute to AGT interaction with megalin.

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Macrophage SR-BI Regulates Autophagy in Atherosclerosis via TFEB/SREBP2 Pathway

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Background: Autophagy is a cellular self-eating, life-sustaining process that plays crucial roles in cell lipid homeostasis, inflammatory responses, ER stress and cell death. Previous studies have found that loss of scavenger receptor class B type I (SR-BI) is associated with cellular cholesterol accumulation and lysosome dysfunction in macrophages and erythrocytes.
Methods and Results: GFP-LC3 transgenic and bone marrow transplantation studies were applied in ApoE and LDLR deficient mice to examine the effects of hematopoietic SR-BI deletion on atherosclerotic lesion autophagy. Analysis by electron and fluorescence microscopy and Western Blot revealed that autophagy was stimulated under the condition of cholesterol induced stress in primary macrophages, and SR-BI deficiency resulted in significantly decreased autophagy, as evidenced by ~80% fewer autophagosomes and 31.5 to 67.1% reduction of critical autophagy markers including Beclin-1, VPS34, p62 and LC3II. Pharmacologic activation of autophagy reduced the levels of lipid droplets, foam cell formation, cellular cholesterol, cell apoptosis and inflammatory responses in WT macrophages, but failed to do so in SR-BI-deficient cells. In atherosclerotic plaques of ApoE-deficient mice, hematopoietic SR-BI deletion caused 45.9 and 56.2% ($P < 0.05$ and 0.01) decreases in expression of p62 and LC3II, respectively, compared with WT hematopoietic cells, indicating defective autophagy in advanced atherosclerotic lesions. Furthermore, we found that SR-BI deficiency in atherosclerotic aorta tissue led to 37.8% to 84.6% ($P<0.05$ to 0.01) reductions in a wide range of autophagy and lysosome mRNA markers including ATG5, ATG6/Beclin-1, ATG7, LC3, Rab5, RAB7, Rab9 and TFEB. RNA-Seq and real time PCR analysis showed that the expression of transcription factors TFEB and SREBP2 was significantly attenuated in SR-BI deficient vs WT macrophages under conditions of cholesterol stress. SREBP2 transcriptionally up-regulated TFEB expression and activity in macrophages. In addition, TFEB nuclear translation and gene transcriptional activity were ~50% and 67.2% reduced in SR-BI-deficient vs WT macrophages. Overexpression of TFEB dramatically rescued defective autophagy activity in SR-BI-deficient macrophages.
Conclusions: SR-BI deficiency causes defective autophagy in macrophages and advanced atherosclerotic lesions, which accelerates the formation of more complex atherosclerotic lesions with features of unstable plaques in ApoE-deficient mice. TFEB represents a novel target gene of SREBP2 in macrophages. Macrophage SR-BI modulates expression and function of critical autophagy players via the TFEB pathway, identifying this pathway as a new target for modulating autophagy and the treatment of atherosclerosis.

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Chronic Kidney Disease-Associated Atherosclerosis is Attenuated by Dual Inhibition of microRNA-92a and microRNA-489

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Chronic kidney disease (CKD) subjects have an increased risk of developing cardiovascular disease, namely atherosclerosis. Endothelial dysfunction and inflammation are linked to the development of these diseases and recent work has identified a number of microRNAs (miRNAs) involved in these pathologies. As such, endothelial miRNAs are potential novel therapeutic targets to prevent and treat atherosclerosis. Here, results support that aortic endothelial miR-92a-3p and miR-489-3p levels are increased in a mouse model of CKD-associated atherosclerosis. A combinatorial miRNA inhibition strategy resulted in the loss of both miR-92a-3p and miR-489-3p in the endothelium and significantly reduced the atherosclerotic burden by 40% (lesion area). Total RNA sequencing (mRNA) identified many endothelial genes, pathways and processes to be significantly altered in response to in vivo miRNA loss-of-function, including immune responses and inflammation. Results suggest that the reduction in atherosclerosis levels were not likely to be linked to changes in cholesterol levels, adhesion molecule expression, or kruppel-like factor 4 (Klf4) expression. Nevertheless, the observed phenotype correlates to altered signal transducer and activator of transcription 3 (STAT3). Fam220a, a negative regulator of STAT3 phosphorylation and target of miR-92a-3p, mRNA levels was significantly reduced in CKD-atherogenic mice compared to controls, but miRNA inhibition in vivo blocked the Fam220a repression. Moreover, gene reporter (luciferase) assays with site-directed mutagenesis confirmed FAM220A as a direct target of miR-92a-3p. Furthermore, FAM220A mRNA levels were repressed in human coronary artery endothelial cells (HCAEC) with miR-92a-3p over-expression and resulted in increased phosphorylation of STAT3. Collectively, these results suggest that endothelial miR-92a-3p and miR-489-3p contribute to CKD-linked atherosclerosis.

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HDL-mediated tRNA-Derived Small RNA Communication in Systemic Lupus Erythematosus

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Systemic Lupus Erythematosus (SLE) is a debilitating disease primarily in women involving complex T and B cell dysregulation and production of autoantibodies against a myriad of self-antigens. SLE presents with dysfunctional HDL and we have previously found that HDL-microRNAs (miRNA) are significantly altered in SLE; however, miRNAs are just one of many types of sRNAs. As such we hypothesized that HDL-sRNA cargo and cell-to-cell communication in SLE extend beyond miRNAs. Indeed, we found via high-throughput small RNA sequencing (sRNAseq) tRNA-derived sRNAs (tDRs) were the most abundant class of sRNAs on HDL and were significantly altered in SLE subjects compared to controls (n=6-8, $P<0.05$). In addition, circulating levels of angiogenin, an RNaseIII enzyme responsible for tDR cleavage from parent tRNAs, was also found to be significantly increased in plasma ($P<0.05$) from SLE subjects compared to controls. To determine if tDRs are altered in CD4+ T cells in SLE subjects, real-time PCR was used to quantify candidate tDRs, and we found that tDR-GlyGCC levels were significantly increased 4.2-fold in SLE ($P<0.01$) and readily exported to HDL. Excitingly, using total RNAseq in silico predicition, and gene reporter (luciferase) assays we identified ROCK2, a critical regulator of CD4+ T cell differentiation, is a direct tDR-GlyGCC target gene. Moreover, activated human CD4+ T cells transfected with tDR-GlyGCC mimetics demonstrated reduced ROCK2 and STAT3 phosphorylation and consequently reduced inflammatory cytokine secretion (IL-17 and IL-21; $P<0.05$). To determine if T cell exported tDR-GlyGCC is transferred to other cells by HDL, ex vivo studies were completed using Trans-PhotoActivatable-Ribonucleoside-CrossLinking-ImmunoPrecipitation high-throughput Sequencing (Trans-PAR-CLIPseq). Using this approach, we found a cassette of CD4+ T cell-originating sRNAs, including tDR-GlyGCC, that were transferred by HDL to recipient immune cells. Here, we demonstrate that HDL facilitates intercellular transfer of sRNAs between immune cells and we have shown for the first time a role for tDR-GlyGCC in regulating T cell signalling.

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Absence of Liver Estrogen Alpha Signaling Predisposes Mice to Atherosclerosis During Western-type Diet Feeding

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Absence of liver estrogen alpha signaling (ERα) caused insulin resistance in high-fat diet fed mice. Since insulin resistance is a risk factor for cardiovascular disease, we hypothesize that liver ERα knock-out mice may be predisposed to atherosclerosis during Western-type diet feeding. Liver ERα knock-out (LKO) mice and their wild-type (WT) littermates were fed a Western-type diet for 7 months (210±12 days), and atherosclerosis were evaluated. We saw that deletion of liver ERα signaling increased atherosclerosis by ~7.9-fold for female LKO mice in comparison to the WT controls. Deletion of liver ERα did not increase atherosclerosis for male mice, while atherosclerosis increased ~5.5-fold for male WT mice in comparison to female WT mice. Deletion of liver ERα increased serum cholesterol levels significantly only for female mice, and Western-type diet increased cholesterol levels by 22% for male mice in comparison to female mice. In vivo cholesterol reverse transport assay showed that liver ERα deletion significantly decreased cholesterol efflux from peripheral tissue to liver for females but not for males, while cholesterol efflux decreased by 34% in male mice compared to female mice. Consistently, liver ERα deletion decreased cholesterol secretion in feces by 38% for females but not for males, while secretion of radiolabeled cholesterol in feces decreased by 36% in male mice compared to female mice. Immunoblotting showed that liver SR-B1 and LDLr levels decreased in LKO female mice compared to their littermates, and liver LDLr levels were significantly lower in male mice compared to female mice. Our results suggest that liver ERα signaling plays important roles for cholesterol reverse transport and is protective for atherosclerosis in female mice during Western-type diet feeding.

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XANTHIPPE: Examining the effect of Ticagrelor on Platelet Activation, Platelet-Leukocyte Aggregates, and Acute Lung Injury in Pneumonia

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Introduction: Despite advances in supportive treatment and antibiotic therapy for pneumonia, significant improvement in the early and late mortality have yet to be realized and increased rates of adverse cardiovascular events remain an issue. Recently, a role for platelets in inflammatory and immune responses has been identified in addition to their established contribution to hemostasis and thrombosis. Data generated from preclinical animal models and retrospective analysis of clinical interventions suggests that anti-platelet therapy may improve outcomes in patients hospitalized with pneumonia. The XANTHIPPE study was undertaken to establish the effect of ticagrelor on markers of inflammation and thrombosis in patients with pneumonia and to explore its safety in this patient population.

Methods: Patients (n = 60) admitted to our institution for pneumonia were randomized within 1 day of hospitalization (or diagnosis) to receive placebo or ticagrelor (180 mg loading dose followed by 90 mg twice daily) for up to 7 days. The primary endpoint was change in platelet-leukocyte aggregates between baseline and 24 hours. Secondary endpoints included change in platelet function, biomarkers of inflammation and thrombosis, lung function, and adverse events within the first 30 days of hospitalization.

Results and Conclusions: Subjects enrolled in XANTHIPPE ranged in age from 18 – 92 of which 53% were female. A significance difference in the percent of leukocytes with attached platelets over the first 24 hours was observed between groups (-16.62 ± 6.58 vs 2.56 ± 4.18, P = 0.0176). Effects on biomarkers of platelet function, inflammation, and lung function will be presented. Our results should provide valuable mechanistic insight into platelet function in pneumonia and may demonstrate beneficial effects of anti-platelet therapy in this setting.

Notes

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