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September 6-8, 2023

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Barbara Kahn, MD

George Richards Minot Professor of Medicine
Harvard University

Adipose Triglyceride Lipase (ATGL) and Fatty Acid Esters of Hydroxy Fatty Acids (FAHFAs) in Diabetes

Fredrickson Keynote Address

Alan Remaley, PhD

Senior Investigator
National Heart, Lung, and Blood Institute

Increasing Phosphatidylethanolamine Content in the Endoplasmic Reticulum

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Presentations from Abstract Submissions

Larry Rudel Award Lectures

Nour Mouannes | Cleveland Clinic
The Ability of Dietary Polyunsaturated Fatty Acids to Protect Against Hepatic Inflammation and Non-Alcoholic Steatohepatitis is Dependent on Gut Microbes

Mikala Zelows | University of Kentucky
Carnitine Palmitoyltransferase 1a Modulates Hepatic Lipid and Lipoprotein Metabolism

Anh Cao | Harvard University
Long Acyl Chains as a Primal Molecular Cue for Identifying Potential Threats

Selected Abstract Presentations

Wenceslao Martinez-Navarrete | University of Chicago
Acute and Long-Term Changes in Bone Marrow Immune Populations in Response to Weight-Loss and Regain

Carol Beatty | Augusta University
Multiplex Analysis of Inflammatory Proteins Associated with Risk of Coronary Artery Disease in Type 1 Diabetes Patients

Kara Timinski | Cleveland State University
Disulfiram Reduces Atherosclerosis and Enhances Efferocytosis, Autophagy, and Atheroprotective Gut Microbiota in Hyperlipidemic Mice

Ayoung Kim | Washington University at St Louis
Amelioration of Liver Failure in Short Bowel Resection by Intestinal LXR Agonism

Prasun Kumar Dev | University of South Carolina
Association of HDL Lipid Classes and HDL Traits Before and After Exercise Training

Emerging Career Scientist Presentations

Sumita Dutta PhD | Cleveland Clinic
Commensal Gut Bacteria Derived N-Acyl Serinols Can Regulate Host's Postprandial Metabolic Homeostasis

Robert Nate Helsley PhD | University of Kentucky
Loss of Carnitine Palmitoyltransferase 1a Reduces Polyunsaturated Fatty Acid Levels and Drives Microvesicular Steatosis in Livers of Female Mice

Xiangbo Ruan PhD | Johns Hopkins University
A Novel Non-Coding Genetic Variant Affects Blood Lipids by Regulating a Human-Specific Long Non-Coding RNA

Invited Speakers

J Mark Brown PhD | Cleveland Clinic

Sergio Fazio MD | Regeneron

Jane Ferguson PhD | Vanderbilt University

Scott Gordon PhD | University of Kentucky

Gwendalyn Randolph PhD | Washington University in St. Louis

Gissette Reyes-Soffer MD | Columbia University

Ze Zheng PhD | Medical College of Wisconsin

Norbert Leitinger PhD | University of Virginia

Ada Weinstock PhD | University of Chicago

Lin Zhu MD PhD | Vanderbilt University

Brandon Davies PhD | University of Iowa

Amanda Doran MD PhD | Vanderbilt University

DON FREDRICKSON LIPID RESEARCH CONFERENCE

Lipid Metabolism, Lipoproteins, and Atherosclerosis

September 6-8, 2023

Vanderbilt University
Homewood Suites Vanderbilt
Nashville, TN

Wednesday, September 6

4:30 pm Welcome

John Stafford, Kasey Vickers, Sean Davies | Vanderbilt University

4:45 pm Session 1

Modulators of Triglyceride Metabolism and Lipoprotein Lipase

Chair: Kasey Vickers PhD | Vanderbilt University

4:45 pm **Sergio Fazio MD PhD** | Regeneron
ODYSSEY Outcome Trial: Analysis of Familial Hypercholesterolemia and Type III Hyperlipidemia

5:15 pm **Adam Mullick PhD** | Ionis Pharmaceuticals
ApoC-III Inhibition for the Treatment of Severe Hypertriglyceridemia

5:45 pm Break

6:00 pm **Keynote Address**
Alan Remaley MD PhD | National Heart, Lung, and Blood Institute
Hypertriglyceridemia: Update from Diagnostics to New Therapies

7:00 pm Welcome Reception

DON FREDRICKSON LIPID RESEARCH CONFERENCE

Thursday, September 7

8:30 am Session 2

Intestinal and Gut Microbiome Regulation of Lipid Metabolism

Chair: Sean Davies PhD | Vanderbilt University

8:30 am **Scott Gordon PhD** | University of Kentucky
Insight into the Chylomicron Secretion Pathway from a Model of Impaired Dietary Lipid Absorption

9:00 am **Gwendalyn Randolph PhD** | Washington University in St. Louis
HDL Trafficking Through Lymphatics or Not – Implications in Gut-Liver Crosstalk and Atherosclerosis

9:30 am **Selected Trainee Presentations**

Wenceslao Martinez-Navarrete | University of Chicago
Acute and Long-Term Changes in Bone Marrow Immune Populations in Response to Weight-Loss and Regain

Carol Beatty | Augusta University
Multiplex Analysis of Inflammatory Proteins Associated with Risk of Coronary Artery Disease in Type 1 Diabetes Patients

10:00 am Break

Session 2 continued

Chair: Kasey Vickers PhD | Vanderbilt University

10:15 am **Jane Ferguson PhD** | Vanderbilt University
Microbiome and Cardiovascular Risk

10:45 am **J Mark Brown PhD** | Cleveland Clinic
Diet-Gene Interactions and Fatty Liver, Microbe Metabolites

11:15 am **Selected Trainee Presentation**

Kara Timinski | Cleveland State University
Disulfiram Reduces Atherosclerosis and Enhances Efferocytosis, Autophagy, and Atheroprotective Gut Microbiota in Hyperlipidemic Mice

Ayoung Kim | Washington University in St Louis
Amelioration of Liver Failure in Short Bowel Resection by Intestinal LXR Agonism

Prasun Kumar Dev | University of South Carolina
Association of HDL Lipid Classes and HDL Traits Before and After Exercise Training

12:00 pm Lunch

Faculty and Staff **– Lunch on Your Own**

Trainees **– Career Development Session: Panel Discussion with Investigators Across the Spectrum of Careers in Research**
(Prior Registration Required)

Moderator:
Brittney Poole, Graduate Research Assistant, Division of Pulmonary, Critical Care and Sleep Medicine, University of Florida

Panelist:
Jane Ferguson PhD, Assistant Professor of Medicine | Vanderbilt University
Adam Mullick, PhD, Vice President, Cardiovascular and Renal Drug Discovery | Ionis Pharmaceutical
Michael Orr PhD, Senior Scientific Project Manager | Vanderbilt University
Alan Remaley MD PhD, Senior Investigator, Lipoprotein Metabolism | National Heart Lung and Blood Institute
Gissette Reyes-Soffer, MD, Assistant Professor of Medicine | Columbia University
Ada Weinstock PhD, Assistant Professor of Medicine | University of Chicago

1:45 pm Session 3

Emerging Career Scientists Session

Chair: John Stafford MD PhD | Vanderbilt University

1:45 pm **Sumita Dutta PhD |** Cleveland Clinic
Commensal Gut Bacteria Derived N-Acyl Serinols Can Regulate Host's Postprandial Metabolic Homeostasis

2:15 pm **Robert Nate Helsley PhD |** University of Kentucky
Loss of Carnitine Palmitoyltransferase 1a Reduces Polyunsaturated Fatty Acid Levels and Drives Microvesicular Steatosis in Livers of Female Mice

2:45 pm **Xiangbo Ruan PhD** | Johns Hopkins University
A Novel Non-Coding Genetic Variant Affects Blood Lipids by Regulating a Human-Specific Long Non-Coding RNA

3:15 pm Break

3:30 pm Session 4

Oxidized Phospholipids, Lipoprotein (a)

Chair: Sean Davies PhD | Vanderbilt University

3:30 pm **Gissette Reyes-Soffer MD |** Columbia University
Advances in LP(a) Physiology and LPA Gene Variants

4:00 pm **Ze Zheng PhD** | Medical College of Wisconsin
The Reciprocal Regulation of Lipoproteins and Blood Clotting System

4:30 pm **Norbert Leitinger PhD** | University of Virginia
Targeting Oxidized Phosphatidylcholines in Cardiometabolic Disease

5:00 pm Networking Reception

5:30 pm Poster Session

5:30 pm **Odd Posters**

6:30 pm **Even Posters**

DON FREDRICKSON LIPID RESEARCH CONFERENCE

Friday, September 8

8:30 am Session 5
Larry Rudel Award Lectures
Chair: Ryan Temel PhD | University of Kentucky

8:30 am **Nour Mouannes** | Cleveland Clinic
The Ability of Dietary Polyunsaturated Fatty Acids to Protect Against Hepatic Inflammation and Non-Alcoholic Steatohepatitis is Dependent on Gut Microbes

8:45 am **Mikala Zelows** | University of Kentucky
Carnitine Palmitoyltransferase 1a Modulates Hepatic Lipid and Lipoprotein Metabolism

9:00 am **Anh Cao** | Harvard University
Long Acyl Chains as a Primal Molecular Cue for Identifying Potential Threats

9:15am Virgil Brown Lecture
Barbara Kahn MD | Harvard Medical School
Adipose Triglyceride Lipase (ATGL) and Fatty Acid Esters of Hydroxy Fatty Acids (FAHFAs) in Diabetes

10:15 am Break

10:30 am Session 6
Atherosclerosis Regression and Efferocytosis
Chair: Lin Zhu MD PhD | Vanderbilt University

10:30 am **Ada Weinstock PhD** | University of Chicago
Weight Loss, Weight Regain and Atherosclerosis

11:00 am **Lin Zhu MD PhD** | Vanderbilt University
Reducing Atherosclerosis in A Model of Menopause

11:30 am **Brandon Davies PhD** | University of Iowa
Everything ANGPTL3 All at Once

12:00 pm **Amanda Doran MD PhD** | Vanderbilt University
Immune Cells and Atherosclerosis

12:30pm Closing Remarks

Poster Presentations

Name	Poster Board
Garrett Anspach	13
Sarah Anthony	31
Alexander Bashore	17
Carol Beatty	24*
Nilam Bhandari	38
Anh Cao	11*
Mark Castleberry	2
Isha Chauhan	22
Sivaprakasam Chinnarasu	3
Prasun Kumar Dev	9*
Presley Dowker-Key	29
Sumita Dutta	36*
Reza Fadaei	15
Nicholas Gill	32
Kailash Gulshan	37
Robert Helsley	12*
Rachel Hohe	6
Anant Jaiswal	20
Ayoung Kim	5*
Mindy Kim	27
Kala Mahen	33
Wenceslao Martinez-Navarrete	7*
Nour Mouannes	14*
Khaga Raj Neupane	19
Brittney Poole	16
Ashutosh Prince	1
Maya Rodriguez	23
Xiangbo Ruan	39*
Siarhei Salamevich	26
Erika Savage	30
Fubiao Shi	4
Huan Tao	28
Ryan Temel	8
Kara Timinski	18*
Clint Upchurch	10
Mark Vander Roest	35
Mikala Zelows	34*
Xueheng Zhao	25
Lin Zhu	21

*Podium Presentation

Abstract

Title

Progressive familial intrahepatic cholestasis modulates membrane elasticity, dipole potential and cell membrane protrusions.

Ashutosh Prince
Cleveland State University

Introduction

Integrity of hepatic canalicular membrane is essential and excessive cholesterol extraction by bile salts can be detrimental. Human mutations in ATP8B1, a P-type ATPase, cause progressive familial intrahepatic cholestasis type (PFIC1), a disease characterized with enhanced biliary cholesterol excretion and liver damage. The underlying mechanisms for how loss of ATP8b1 compromise canalicular membrane integrity are not clear.

Objective

To investigate the biophysical properties of canalicular membrane in WT vs. PFIC1 cells.

Methods and Results:

Using Crispr-Cas9 strategy, we generated a variety of human cell lines (macrophages, hepatocytes, and kidney) carrying a homozygous knockout of ATP8b1 and subjected them to atomic force microscopy and dipole potential measurement assays. Our findings revealed a significant increase in elasticity in THP-1 ATP8b1$^{-/-}$ and HEK ATP8b1$^{-/-}$ cells vs. WT cells. The Young's modulus for WT THP-1 and HEK293 cells was ~ 680 ± 25 Pa and 1479 ± 48 Pa, while the THP-1 ATP8b1$^{-/-}$ and HEK293 ATP8b1$^{-/-}$ cells exhibited values of ~ 296±10 Pa and 697±17 Pa, respectively. The dipole potential of HEK293 ATP8b1$^{-/-}$ and HepG2 ATP8b1$^{-/-}$ cells vs. corresponding WT cells was reduced by ~ 38% and ~ 45%, respectively. Formation of membrane protrusions was significantly impaired in ATP8b1$^{-/-}$ cells, showing physiological impact of altered membrane environment.

Conclusion

ATP8b1 modulates membrane elasticity and membrane potential to maintain appropriate canalicular membrane environment for controlled lipid extraction by bile salts. ATP81 regulates membrane properties in several tissues.

Low-density lipoprotein delivers extracellular RNAs to platelets

Mark Castleberry[1], Wen Dai[1], Ziyu Zhang[1], and Ze Zheng[1,2,3,4]

[1] Versiti Blood Research Institute; Milwaukee, 53226, USA.

[2] Department of Medicine, Medical College of Wisconsin; Milwaukee, 53226, USA.

[3] Cardiovascular Center, Medical College of Wisconsin; Milwaukee, 53226, USA.

[4] Department of Physiology, Medical College of Wisconsin, Milwaukee, 53226, USA.

Platelet activation and aggregation are central to many processes that underly hemostasis and atherothrombotic disease progression. These anucleate cells are derived from their megakaryocyte precursors and circulate at high concentrations in the blood (150,000-400,000 platelet/ µl). Platelets circulate in the blood in a resting state and rapidly become activated in response to the prothrombotic stimuli. Activation triggers the release numerous hemostatic proteins that are stored within the granules of the platelet, including plasminogen activator inhibitor-1 (PAI-1) which inhibits fibrinolysis by binding to tissue plasminogen activator and inhibiting its ability to activate plasminogen. Despite lacking a nucleus, platelets maintain the ability of *de novo* protein synthesis and secretion for up to 10 days following activation. Both very-low-density lipoprotein (VLDL) and oxidized low-density lipoprotein (LDL) have been shown to stimulate, or promote, platelet activation through their interactions with their membrane receptors (e.g. CD36). Although it is known that LDL can deliver its lipid cargos to platelets, the ability of LDL to deliver its RNA cargos to platelets has yet to be characterized. I have recently published on the ability of LDL to serve as a mediator of a highly dynamic intercellular RNA transport system between disparate cell types. To evaluate the potential for LDL-mediated RNA delivery to platelets, we independently labeled the protein and RNA cargos of these particles, using fluorescence labeling technologies, and utilized both live imaging and fluorimetry to characterize the delivery of LDL-associated RNAs and the pathways that mediate this phenomenon. Our new data indicate that LDL is internalized by platelets, likely through an endocytic pathway involving dynamin 2, and delivers its RNA cargos to *ex vivo* resting platelets. Preliminary data suggest that apoB-lipoproteins drive platelet PAI-1 synthesis; possibly through their ability to deliver hepatocyte-specific RNA cargos to platelets. Ongoing *ex vivo* and *in vivo* studies are aimed at defining the functional consequences of apoB-lipoprotein RNA delivery to platelets and to define the pathways that regulate this process.

Sex-specific effects of Cholesteryl Ester Transfer Protein on dyslipidemia and Metabolic Associated Fatty Liver Disease

Sivaprakasam Chinnarasu [1,3]; Sophia Yu[1]; Uche Anozie[1,3]; Lin Zhu[1,3]; John M. Stafford[1,2,3]

[1]Tennessee Valley Health System, Veterans Affairs, Nashville, TN, USA
[2]Department of Molecular Physiology & Biophysics, Vanderbilt University, Nashville, TN, USA
[3]Division of Diabetes, Endocrinology and Metabolism, VUMC, Nashville, TN, USA

Abstract:

Significance: Cholesteryl Ester Transfer Protein (CETP) is a lipid transfer protein expressed in the liver and adipose in humans which shuttles triglycerides and cholesteryl esters between the lipoproteins. Metabolic Associated fatty liver disease (MAFLD) is a wide spectrum of disease from simple steatosis to steatohepatitis and hepatocellular cancer, which remains without effective therapies and so far, no FDA approval drugs available. There are male-female sex differences in the pathophysiology of MAFLD and the associated dyslipidemia that gives rise to cardiovascular risk. Understanding mechanisms of sex differences in MAFLD and lipoprotein metabolism may yield novel therapeutic targets. However, the role of CETP in sex associated risks of MAFLD has not been well defined.

Approach: Mice naturally lack CETP, and thus transgenic approaches can be used as a model to define the contribution of CETP toward dyslipidemia and MAFLD. We used both mice transgenic for the human CETP gene regulated by its natural flanking sequences (huCETP) and a liver-targeted CETP-expressing adeno-associated virus (AAV8-TBG-(GFP/CETP), here termed hAAV-CETP) injected mice models to achieve our goals.

Results: We identified that in both models CETP has sex-specific effects on dyslipidemia and MAFLD. High-fat diet (HFD) fed mice increased body weight and adiposity in both female and male mice regardless of CETP expression. In male hAAV-CETP mice liver weight increased, and glucose tolerance worsened compared to hAAV-GFP control. In female hAAV-CETP mice, glucose tolerance was improved consistent with our prior clamp results in huCETP mice. In male mice hAAV-CETP expression resulted increased oil-red-O staining and more lipid droplet and accumulation compared to both hAAV-GFP and female hAAV-CETP groups. We found in male mice hAAV-CETP worsened steps in hepatic lipid metabolism, while CETP improved steps in hepatic lipid metabolism in females (Acc, Ldlr, Mttp and Dgat1). Similarly, glucose metabolism genes (Pepck and G6pc) were upregulated by CETP male mice and downregulated by CETP female mice. Liver fibrosis marker genes mRNA levels upregulated on male CETP but no changes on female mice. We performed liver mRNA sequencing from huCETP mice that underwent orchiectomy and testosterone treatment. We found that approximately 1500 genes differentially expressed by the presence of CETP in the male liver. Most of these were androgen-dependent.

Conclusion: Our studies support a role of CETP expression with regard to sex-specific risk of MAFLD and dyslipidemia. This may suggest that CETP could be target to improve MAFLD in males and may facilitate sex-specific precision medicine.

Key words: MAFLD, Cholesteryl ester transfer protein, lipid metabolism, testosterone.

PKA phosphorylation of RAPTOR potentiates mTORC1 and controls adipose tissue homeostasis

Fubiao Shi[1#], Dianxin Liu[1#], Wei Zhang[1], Jian-Liang Li[3], Sheila Collins[1,2]*

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[3]National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA
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Norepinephrine stimulates the adipose tissue thermogenic program through a β-adrenergic receptor (βAR) - protein kinase A (PKA) signaling cascade. We have demonstrated that a noncanonical activation of the mechanistic target of rapamycin complex 1 (mTORC1) by PKA is required for the βAR-stimulation of adipose tissue browning. Here we report that PKA phosphorylation of RAPTOR at Ser791 (S791) is required for mTORC1 activation and essential for adipose tissue homeostasis. To establish the *in vivo* function of S791 phosphorylation, we generated a mouse model with adipocyte-specific knock-in of a phosphorylation-resistant Ser to Ala mutation in *Raptor* (*Raptor*^{AdS791A}). On the chow diet, *Raptor*^{AdS791A} mice had normal body weight and glucose tolerance but showed compromised thermogenic response when treated with β_3-AR selective agonist CL316,243 (CL). When fed with a high-fat diet (HFD), male *Raptor*^{AdS791A} mice gained similar body weight but had smaller epididymal white adipose tissue and enlarged liver with aggravated hepatic steatosis. Euglycemia-hyperinsulinemic clamp studies revealed that *Raptor*^{AdS791A} mice were more insulin resistant, showing reduced glucose infusion rate, higher hepatic glucose production, and declined skeletal muscle glucose uptake. Mechanistically, in primary adipocytes that express phosphorylation-resistant RAPTOR S791A protein, CL-stimulated mTORC1 activity was impaired and the protein level of peroxisome proliferator-activated receptor gamma (PPARγ) was significantly reduced. In contrast, in HIB-1B brown adipocytes that stably express phosphorylation-mimetic RAPTOR S791D protein, the PPAR signaling pathway is up-regulated, and adipocyte thermogenic and mitochondrial gene programs are significantly enhanced. Furthermore, PPARγ protein levels are also reduced in adipose tissues of *Raptor*^{AdS791A} mice, suggesting that RAPTOR S791 phosphorylation might act through a PPARγ-dependent mechanism to control adipose function. Taken together, our study revealed the *in vivo* function and mechanistic insight of PKA phosphorylation of RAPTOR and demonstrated that PKA phosphorylation of RAPTOR at S791 potentiates mTORC1 activity and controls adipose tissue homeostasis through the PPARγ pathway.

Amelioration of liver failure in short bowel resection by intestinal LXR agonism

Ayoung Kim[1], Hannah Phelps[2], Deanna Davis[1], Lingaiah Maram[3], Michael Cameron[4], Bahaa Elgendy[3], Brad Warner[2], Gwendalyn Randolph[1]

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[4]Department of Molecular Medicine, UF Scripps Biomedical Research, Jupiter, FL, 33458, United States

Short gut syndrome (SGS) is a form of intestinal failure that arises as a consequence of surgical removal of a substantial portion of the small intestine. Due to the loss of absorptive capacity, SGS patients require specialized additional interventions to acquire nutrition and sustain their health. The morbidity associated with SGS is notably high and encompasses complications like intestinal failure-associated liver disease (IFALD). IFALD manifests with cholestasis, steatosis, hepatitis, fibrosis, or cirrhosis, with variations in these features depending on the patient. However, effective therapies specifically targeting IFALD after SGS have yet to be established. Our previous studies revealed that ablation of enterocyte ABCA1 led to a significant decrease in portal vein HDL levels. This, in turn, contributed to increased LPS activity reaching the liver, exacerbating liver fibrosis following small bowel resection. Increasing intestinal HDL levels via the oral administration of a low dose of whole-body liver X receptor (LXR) agonist GW3965, which activates intestinal ABCA1, demonstrated protection against liver fibrosis. In this study, we conducted further research to enhance our understanding and develop evidence-based treatments for SGS-associated IFALD. We investigated the use of a gut-restricted LXR agonist WUSTL0717 wherein an amide group replaces a carboxyl group in GW3965, increasing its lipophilicity. Detailed pharmacokinetic analysis in mice revealed that the compound is largely intestinally retained and cleared within 1 hour after oral administration. This compound induced LXR target genes including ABCA1 in all regions of the small intestine, but not in the liver. Transcript levels of apoA1, the core protein for HDL, showed a slight increase selectively at the ileum. The compound treatment resulted in a reduction of IFALD, demonstrated by decreased fibrosis observed through assessments using second harmonic signal and Sirius Red staining, as well as decreased blood biochemical analysis of ALT and AST enzymes. Additionally, the compound showed an effect on restoring body weight, which could be attributed to its potential to enhance the ability to acquire nutrition and sustain health in SGS. Our data suggest the possibility of activating LXR in the intestinal epithelium to provide therapeutic benefits for treating IFALD after SGS.

Macrophage Cholesterol Homeostasis and Inflammation is Shaped by ASTER-C

Rachel C. Hohe[1,2,3], Iyappan Ramachandiran[1,2], Venkateshwari Varadharajan[1,2], Rakhee Banerjee[1,2], Kevin Fung[1,2], William J. Massey[1,2], , Amy C. Burrows[1,2], Amanda L. Brown[1,2], Anthony J. Horak 3rd [1,2], Kala K. Mahen[1,2,3] , Sumita Dutta[1,2], and J. Mark Brown[1,2,3]

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[3]Molecular Medicine Doctoral Program, School of Medicine, Case Western University, Cleveland, OH 44106, USA

In mammalian cells, cholesterol is predominantly found in the plasma membrane (PM). However, regulation of cholesterol synthesis and metabolism primarily occurs at intracellular membranes like the endoplasmic reticulum (ER), mitochondria, and nucleus. Cholesterol trafficking from the PM to these membranes is tightly controlled to maintain lipid homeostasis and cellular signaling balance. A new family of ER-resident proteins, known as ASTERs (ASTER-A, -B, and -C), plays a crucial role in facilitating nonvesicular influx of free cholesterol by forming membrane-membrane contact sites at cholesterol-rich lipid raft domains. ASTER-B functions as a liver X receptor (LXR)-stimulated cholesterol transporter, promoting PM-to-ER sterol transfer and subsequent cholesterol ester (CE) storage in the adrenal gland. Our research focuses on investigating the contribution of ASTER-C in macrophage cholesterol homeostasis and inflammation. We utilize gain- and loss-of-function approaches in murine macrophages. While ASTER-C is not directly stimulated by LXR agonism, it enhances LXR agonist-stimulated reorganization of macrophage cholesterol homeostasis. Knockout of ASTER-C in bone marrow-derived macrophages (BMDMs) results in reduced expression of lipid metabolic genes, including ABCA1 and LPCAT3, which are normally induced by LXR activation. On the other hand, overexpression of ASTER-C in Raw 264.7 macrophages enhances LXR-stimulated expression of ABCA1. Moreover, ASTER-C-deficient BMDMs display impaired LXR-driven anti-inflammatory responses. Notably, ASTER-C deletion also affects macrophage cholesterol homeostasis and function. ASTER-C null macrophages exhibit reduced cholesterol esterification rates and impaired apoA1-stimulated cholesterol efflux. Furthermore, the ability of lipopolysaccharide (LPS) to suppress cellular cholesterol efflux is lost in ASTER-C null macrophages. Together, our findings highlight ASTER-C as a novel regulator of macrophage cholesterol homeostasis and inflammatory pathways. This research has significant implications for understanding diseases associated with abnormal cholesterol homeostasis and unresolved inflammation, such as atherosclerosis, advanced liver disease, and various cancers.

Acute and long-term changes in bone marrow immune populations in response to weight-loss and regain

Wenceslao Martinez-Navarrete, Aleepta Guha Ray, Destini Wiseman, Rotem Kalev-Altman, Oluwatomilayo Ojo, Ada Weinstock

Chronic low-grade inflammation is a hallmark of obesity. Weight loss (WL) improves obesity-related comorbidities, however, subsequent weight-regain (RG) is linked to worsened diseases compared to never losing weight. Therefore, understanding how weight loss and regain impact immunity is critical. We hypothesize that WL promotes inflammation resolution, associated with a reduction in myeloid cells, while RG enhances inflammation by increasing myeloid cell abundances, compared to obese conditions. To test this, we characterized changes in immune populations in response to WL and RG using flow-cytometry in obese male C57Bl/6J mice. Bone marrow and peripheral blood were analyzed after 5,17,28, and 42 days of WL (induced by caloric restriction) or after 17 days of WL followed by RG (ad-libitum access to food) for 7,14,21,28, and 35 days. We found that WL reduces total circulating monocytes, while pro-inflammatory (Ly6Chi) monocytes are retained in the bone marrow. Despite reduced circulating lymphocytes, bone marrow B- and T-cells, and their progenitors, increased. Conversely, RG increases total bone marrow monocytes and shifts towards greater pro-inflammatory and fewer patrolling (Ly6Clo) phenotype. Concordantly, higher circulating monocyte and lymphocyte numbers were observed in RG, together with increased numbers of their progenitors. In summary, we show that WL promotes monocyte and lymphocyte retention in the bone marrow resulting in decreased circulating numbers, suggesting WL regulates immune trafficking rather than production. In contrast, increased circulating monocytes in RG are associated with an expansion of pro-inflammatory monocytes and their progenitors in the bone marrow, indicating heightened production of inflammatory cells. Further understanding these dynamic processes will direct strategies to hinder obesity-related inflammation.

Approaches to create a diet-induced, nonhuman primate model of non-alcoholic steatohepatitis

Lei Cai[1], Peter I. Hecker[1], Mogens Vyberg[2], Ryan E. Temel[1]

[1]Saha Cardiovascular Research Center and Department of Physiology, University of Kentucky, Lexington, KY; Department of Pathology, Hvidovre Hospital, DK-2650 Hvidovre, Denmark

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease in the United States. It is estimated that ~25% of the population has NAFLD with the vast majority of individuals having benign hepatic steatosis. However, as many as 6.5% of U.S. adults have nonalcoholic steatohepatitis (NASH), which is a more severe form of NAFLD characterized by steatosis, hepatocyte death, inflammation, and fibrosis. The development of preventions and treatments for NASH has been hampered due the lack of large animal models that develop human-like pathology. A recent study reported that NASH was induced in 20 male cynomolgus monkeys that had been screened for metabolic abnormalities from over 1000 animals. Since most nonhuman primate (NHP) researchers do not have the ability to prescreen large numbers of animals, our group attempted to make a diet-induced, NHP NASH model. The first diet we designed was designated the “fast food diet” (FFD) because of its high lard/high fructose content and the inclusion of a high fructose drink with a composition similar to soda. The FFD was fed to 12 young adult, male cynomolgus monkeys for 24 months, and liver samples were collected via laparoscopy at 12, 18, and 24 months on FFD. We found that compared to monkeys fed standard NHP diet, animals fed FFD had significant elevations in hepatic lipid levels based upon biochemical and histological analysis. However, the level of hepatic steatosis was highly variable and none of the animals fed FFD showed signs of significant hepatic inflammation, cell death, or fibrosis. In addition, it was found that the level of hepatic steatosis was largely unchanged between 12 and 24 months of FFD feeding. We then made a second diet that was based upon the Gubra Amylin NASH (GAN) diet, which is used to induce NASH in mice. Similar to the mouse diet, the NHP GAN diet contained palm oil as its major fat source and high levels of fructose and cholesterol. The GAN diet was fed to 6 young adult, male cynomolgus monkeys for 18 months, and liver samples were collected via laparoscopy at 6, 12, and 18 months on GAN diet. While causing high-grade hepatic steatosis to develop in all animals, the GAN diet, like the FFD, did not produce pathology consistent with human NASH. In conclusion, we were unable to produce an NHP NASH model using two different dietary approaches.

Association of HDL lipid classes and HDL traits before and after exercise training

Prasun K. Dev[1], Eric C. Leszczynski[1], Charles S. Schwartz[1], Jacob L. Barber[2], Sujoy Ghosh[3,4], Robert E. Gerszten[2], Michael Olivier[6], Anand Rohatgi[7], Clary B. Clish[5], Claude Bouchard[4], Mark A. Sarzynski[1]

[1]University of South Carolina, Columbia, SC
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Introduction: The composition of HDL particles greatly influences their function, and studies have revealed numerous HDL-associated lipid species (HDL lipidome). However, the association between the abundance of HDL lipid species with HDL-related traits is understudied. Moreover, the effects of exercise training on the HDL lipidome and its relationship with HDL structure and function are largely unknown.
Methods: Plasma based measurements were taken in 154 sedentary but apparently healthy individuals before and after 20 weeks of exercise training in the HERITAGE Family Study. The HDL-sized plasma fraction was obtained through FPLC-SEC using three gel filtration columns and concentrated with Amicon filters. Untargeted lipidomic analysis was performed on HDL-sized plasma fraction using the C8-pos LC-MS method by the Broad Institute. Data from 144 known lipid species were used to create nine lipid classes (Figure 1) by summing the concentration of all lipid species in the same class. Measured HDL-related traits included HDL-C, HDL-TG, NMR subclasses, hepatic lipase activity, cholesterol efflux, and HDL lipid peroxidation (HDLox). Spearman correlation was used to test the associations between HDL lipid classes and HDL traits at baseline and in response to exercise training while adjusting for age, sex, and race. Significance was set to FDR<0.05.
Results: HDL surface lipids (PC, PE, SM, Ceramides) and cholesterol esters were positively associated ($r = 0.38$ to 0.62, FDR < 0.05) with the concentration of HDL-C, apoA-I, and large HDL-P and HDL size at baseline (Figure 1A) and in ($r = 0.26$ to 0.38, FDR < 0.05) response to exercise training (Figure 1B). HDL-TG and HDL-DG were negatively associated ($r = -0.28$ to -0.17, FDR < 0.05) with hepatic lipase activity at baseline only. The HDL-LPC, -PC, and -PE classes were positively associated with cholesterol efflux capacity and negatively associated with HDLox at baseline (Figure 1A) and in response to exercise training (Figure 1B).
Conclusions: Our findings reveal several associations between HDL lipid classes and HDL traits that are independent of exercise training. We found that higher abundance of HDL phospholipid and sphingolipid species was related to higher global cholesterol efflux and anti-oxidative capacities of HDL before and after exercise training.

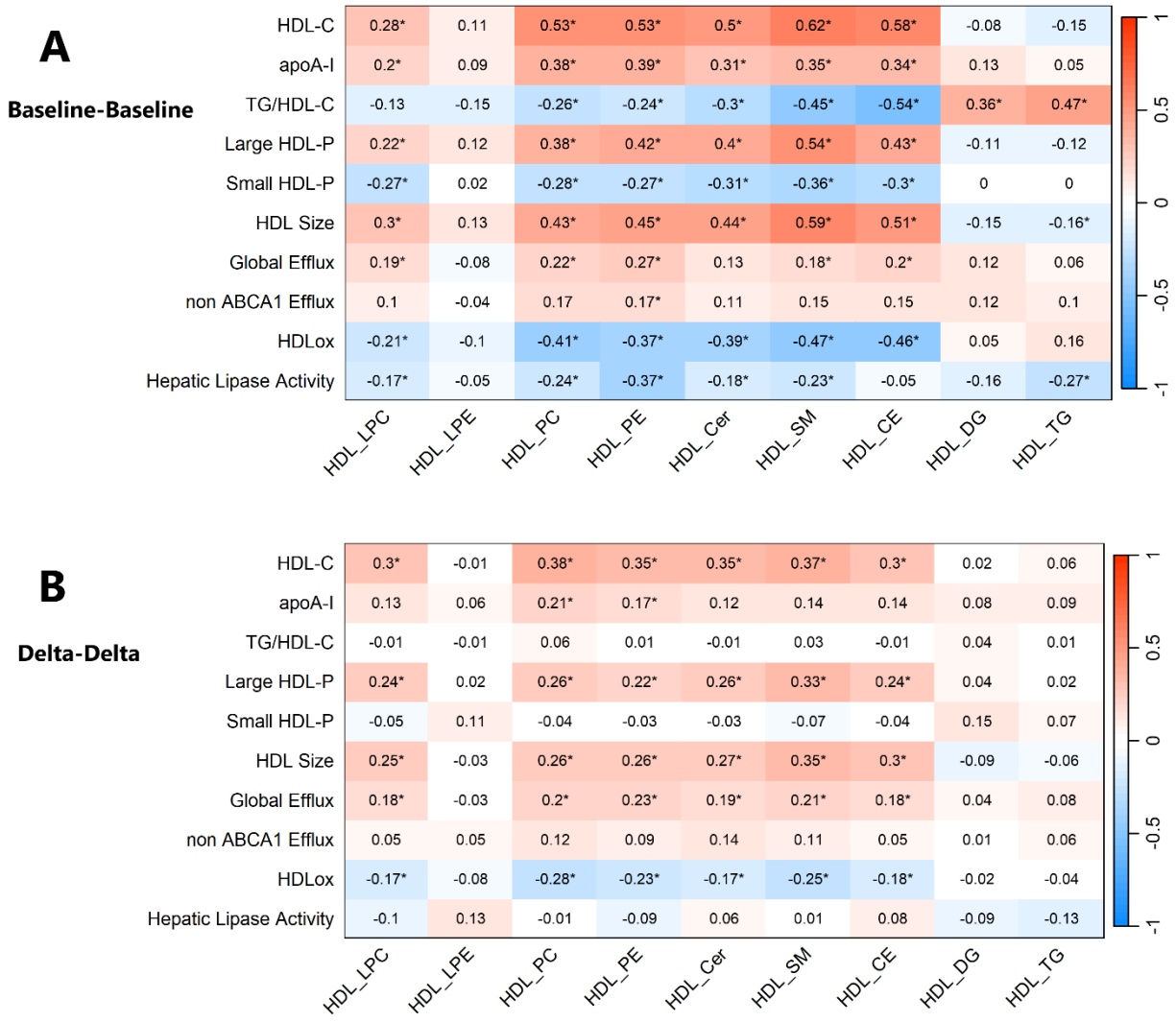

Figure 1. Heatmap of Spearman correlation values between HDL lipid classes (x-axis) and HDL traits (y-axis) while adjusting for age sex, and race. (A) Correlation between baseline HDL lipid classes and baseline HDL traits. (B) Correlation between exercise training induced change in abundance of HDL lipid classes and change in HDL traits. *FDR< 0.05. Abbreviations- LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine, PC: phosphatidylcholine, PE: phosphatidylethanolamine, Cer: ceramide, SM: sphingomyelin, CE: cholesterol ester, DG: diglyceride, TG: triglyceride, HDLox: HDL lipid peroxidation.

Plasma oxidized phospholipidome as an indicator of comorbidities in critically ill patients

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Critical illness is hallmarked by increased oxidative stress; however, antioxidant therapies developed to combat elevated oxidative stress have yielded mixed results. Therefore, understanding the composition of prooxidant lipid species present during settings of critical illness is essential for efficacious treatment of disease. To identify and quantify oxidized phospholipids produced during critical illness, we developed a liquid-chromatography mass spectrometry-based method to assess the composition and quantity of oxidized phosphatidyl choline (OxPCs) species present in a cohort of critically ill patients admitted to the medical intensive care unit at the University of Virginia. We identified and quantified 19 OxPC species present in a 91-patient cohort ranging from mid-nanomolar to high micromolar concentrations. Hierarchical clustering analysis revealed that structurally similar oxidized phospholipids correlated strongly with each other. Using this agnostic clustering analysis as well as groups of OxPCs based on chemical similarity, we identified clinical parameters, including creatinine, blood urea nitrogen, alanine aminotransferase, aspartate aminotransferase, and body mass index, that strongly associated with one or more groups establishing unique fingerprint of associations with different pathological indications. This work demonstrates that OxPCs are present in the plasma at biologically relevant concentrations in numerous pathologies that manifest in critically ill patients, elucidates a novel coregulation between structurally similar OxPCs in disease settings, and identifies new pathologies in which to further study the mechanisms through which OxPCs regulate physiological and cellular functions.

Title: Long Acyl Chains as a Primal Molecular Cue for Identifying Potential Threats

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Abstract

Caspase-11 functions as a pattern-recognition receptor (PRR) that detects bacterial lipopolysaccharides (LPS) in the cytosol of human and murine cells. The specific molecular pattern recognized by Caspase-11 has been challenging to identify due to the complexity and heterogeneity of LPS. Our biochemical investigations have revealed that the caspase activation and recruitment domains (CARD) of Caspase-11 binds to highly soluble lipids with 14-carbon or longer acyl chains, resulting in increased structural stability that can resist trypsin digestion. The Alphafold model of Caspase-11 CARD predicts a hydrophobic cleft exposed to solvent, which appears capable of accommodating the acyl chain during docking analysis. Through mutagenesis experiments, we identified a hydrophobic residue in the predicted model responsible for binding the acyl chain, which is also crucial in LPS-induced inflammatory activities in immune cells. This hypothetical hydrophobic cleft is positioned adjacent to positively charged residues known to play a vital role in LPS binding, together forming what we term long acyl-chain recognition motif (LARM). Using this motif as a guide, we identified previously undefined lipid receptors in in primitive animals, such as *Hydra vulgaris* and *Caenorhabditis elegans*. Some of these LARM-containing caspases bind to mono-acylated lipids with 14-carbon or longer acyl chains but not LPS, suggesting that caspases capable of binding acyl chains predated the evolution of LPS-binding caspases. We propose that highly soluble long-chain lipids represent an ancient molecular pattern which underlies the inflammatory activities of host and microbial lipids with inflammatory activities.

Loss of Carnitine Palmitoyltransferase 1a Reduces Polyunsaturated Fatty Acid Levels and Drives Microvesicular Steatosis in Livers of Female Mice

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Background: Loss-of-function variants in carnitine palmitoyltransferase 1a (CPT1a) associate with reductions in circulating polyunsaturated fatty acids (PUFAs). Loss of hepatic PUFAs contribute to the progression from simple steatosis to more severe metabolic dysfunction-associated steatohepatitis (MASH). Therefore, the goal of this study was to determine the impact of liver-specific CPT1a deletion (LKO) on PUFA metabolism and MASH across male and female mice.

Methods: Eight-week old male and female LKO (*Cpt1a*$^{\Delta Alb}$) and littermate controls (*Cpt1a*$^{F/F}$) were placed on a low-fat or high-fat diet (HFD; 60% kcal fat) for 15 weeks. Glucose and insulin tolerance tests were completed after 10 and 12 weeks on the diet, respectively. Mice were necropsied after a 16 hour fast to induce hepatic fatty acid oxidation, and tissues and serum were collected and utilized for shotgun lipidomics, matrix-assisted laser desorption ionization for mass spectrometry imaging (MALDI-MSI), kinase profiling, bulk RNA sequencing, transmission electron microscopy, and protein expression by immunoblotting.

Results: Male LKO mice displayed improved insulin sensitivity, had lower circulating alanine aminotransferase (ALT) levels, but did not exhibit changes in hepatic triglycerides or cholesterol levels as compared to male control mice. Female LKO mice, however, displayed significant increases in serum ALT levels which associated with greater deposition of hepatic triglycerides and cholesterol, as compared to female control mice. Histologically, female LKO mice displayed diffuse, panlobular microvesicular steatosis, while male LKO mice exhibited only slight periportal steatosis. Mice with CPT1a deletion

had increased monounsaturated fatty acid (MUFA)-containing phospholipids at the expense of DHA-containing phospholipids in both whole liver and in lipid droplet fractions. Utilizing MALDI-MSI, we observed spatial heterogeneity of DHA-containing PE species (38:6 and 40:6 PE) in control mice, which was largely absent with *Cpt1a* deficiency. Male and female mice responded to *Cpt1a*-deficiency by increasing the expression of genes involved in lipid droplet turnover (*Plin2, Cidec, G0S2*) and in PUFA metabolism (*Elovl5, Fads1, Elovl2*), while only female LKO mice increased genes involved in inflammation (*Ly6d, Mmp12, Cxcl2*). Kinase profiling showed decreased protein kinase A (PKA) activity, which coincided with increased PLIN2, PLIN5, and G0S2 protein levels and decreased triglyceride hydrolysis in LKO mice.

Conclusions: Liver-specific deletion of CPT1a promotes sexually dimorphic MASH in mice, thereby identifying a new mechanism by which females protect themselves from diet-induced liver injury.

Title: Exploring Altered Fatty Acid Metabolism in Human Hepatocellular Carcinoma

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

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

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

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

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

The Ability of Dietary Polyunsaturated Fatty Acids to Protect Against Non-Alcoholic Steatohepatitis (NASH) is Dependent on Gut Microbes.

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*Authors equally contributed to this work

Non-alcoholic steatohepatitis (NASH) is a rapidly expanding form of liver disease with limited treatment options. Several clinical trials have shown that dietary ω-3 polyunsaturated fatty acid (PUFA) supplementation, in particular eicosapentaenoic acid (EPA, 20:5,ω3) and docosahexaenoic acid (DHA, 22:6,ω3), improve health outcomes in NASH patients by suppressing hepatic lipogenesis and resolving hepatic inflammation. In parallel, there is emerging evidence that the gut microbiome can powerfully impact NASH via similar mechanisms. Although dietary PUFA supplementation can strongly impact host lipid metabolic pathways in the liver, it is often overlooked that gut microbiota can also synthesize and degrade diverse lipids. In fact many microbe-associated molecular patterns (MAMPs) such as lipopolysaccharide (LPS) and triacylated lipopeptides signal to host toll-like receptors via their lipid moieties. Although there is a wealth of knowledge regarding diet-microbe-host interactions on carbohydrate and protein co-metabolism, comparatively little is known in regard to metaorganismal metabolism of dietary fatty acids. Here, we hypothesized that the ability of dietary PUFAs to suppress NASH is dependent on microbe and host co-metabolism of lipids. To test this, we fed either conventionally-raised or germ-free C57BL6/N mice six sterile diets with well-defined levels of either saturated monounsaturated, ω6 PUFAs, or ω3 PUFAs and comprehensively examined the diet-microbe-host interactions as they relate to NAFLD progression. Compared to a SFA control diet (palm oil with lard), which effectively promoted obesity and NASH, both ω6 (borage oil) and ω3 PUFAs (fish oil) reduced body weight and liver weight in conventional, but not germ-free mice. Furthermore, the ability of dietary SFA, MUFA, and PUFAs to uniquely alter the hepatic lipidome was clearly altered in germ-free versus conventional mice. Of particular interest, the ability of dietary ω3 PUFAs to increase certain species of pro-resolving lipid mediators in the liver was prevented in germ-free mice. Collectively, this study provides a comprehensive lipidomic analysis defining unique dietary fatty acid-microbe-host interactions, and have uncovered new insights into how metaorganismal metabolism impacts liver disease.

NAPE-PLD protects against the inflammatory effects of pyrrole-modified phosphatidylethanolamines

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Introduction: Oxidative stress and subsequent lipid peroxidation contributes to various chronic pathological conditions, such as atherosclerosis, insulin resistance, endothelial dysfunction, and inflammation. Lipid peroxidation constitutes a major source of reactive lipid aldehydes. Recently, Uchida et al demonstrated that lipid peroxidation leads to the formation of pyrrole adducts on the lysyl residues of protein and that both succinaldehyde (SUC) or the combination of glyoxal and glycoaldehyde are reactive lipid aldehydes that can produce such pyrrole adducts. We had previously found that another family of reactive lipid aldehydes, the isolevuglandins (IsoLGs), form oxidized pyrrole adducts on the lysyl residues of proteins (IsoLG-Lys) and on the amine headgroups of phosphatidylethanolamines (IsoLG-PEs). Because IsoLG-PEs induced inflammatory responses in macrophages, we therefore tested whether SUC would react with PE to form pyrrole-PE and whether this pyrrole-PE would also induce the inflammatory response of macrophages. We had also previously found that IsoLG-PE was inactivated through hydrolysis by the enzyme NAPE-PLD. We therefore tested whether pyrrole-PE was also a substrate for NAPE-PLD.

Method: SUC was reacted with PEs of varying O-acyl chain lengths, and the formation of pyrrole-PE monitored by LC/MS/MS. The inflammatory activity of the resulting product was monitored using RAW64.7 Murine Macrophages stably transfected with an NFkB-luciferase reporter plasmid. The ability of recombinant mouse Nape-pld to hydrolyze Pyrrole-PE was determined via LC/MS/MS.

Results: Reaction of one molar equivalent SUC with dihexanoyl-PE (dHPE), dilauroylPE (dLauPE), or dipalmitoyl-PE (dPPE), all resulted in the formation of products with a mass of +55.6 amu greater than the starting PE mass, consistent with the formation of a pyrrole-PE product (Pyr-PE). Both Pyr-dLauPE and Pyr-dPPE induced NFkB activation in the RAW264.7 NFkB-Luc reporter cell line in a concentration-dependent manner, while Pyr-dHPE did not. 2 h incubation with recombinant Nape-pld resulted in extensive hydrolysis of Pyr-dPPE. Treatment of RAW264.7 NFkB-Luc reporter cells with the small molecule NAPE-PLD activator VU534 resulted in reduced NFkB activation.

Conclusion: Our results suggest that lipid peroxidation is likely to result in the formation of Pyr-PE, and that this Pyr-PE may contribute to the pro-inflammatory effects of lipid peroxidation, and that NAPE-PLD may act to suppress inflammation in part by hydrolyzing Pyr-PE, along with hydrolyzing IsoLG-PE. Future studies will examine the extent to which Pyr-PE forms in vivo under conditions of oxidative stress and whether Nape-pld$^{-/-}$ mice show higher levels of Pyr-PE and inflammatory responses.

Sirtuin3 Rescues Hepatic Phenotype of Alpha-1 Antitrypsin Deficiency Through Activation of Lipophagy

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Alpha-1 Antitrypsin Deficiency (AATD) is a rare genetic disease characterized by misfolding and accumulation of mutant alpha-1 antitrypsin (ZAAT) in the endoplasmic reticulum of hepatocytes. Accumulation and aggregation of misfolded hepatic ZAAT causes a toxic gain of function which, is theorized to be the main cause of AATD-mediated liver disease. Liver disease associated with AATD presents variably, ranging from transient cholestasis, steatosis, and fibrosis to cirrhosis and liver cancer. AATD individuals with liver disease stay asymptomatic until late-stage cirrhosis, making it difficult to diagnose in early stages. Currently, there is no treatment option for AATD-mediated liver disease except for liver transplantation. Sirtuin3 (SIRT3), a mitochondrial NAD^+-dependent deacetylase is a positive regulator of autophagy and lipophagy in the liver. We hypothesize that overexpression of SIRT3 mediates hepatic ZAAT degradation and decreases hepatic lipid toxicity through lipophagy. Here, miRNA sequencing from plasma extracellular vesicles showed that SIRT3 activity is dysregulated in the liver of AATD individuals compared to healthy controls. Therefore, utilizing RNA analysis, protein analysis, immunohistochemistry and immunofluorescence microscopy, we investigated the role of SIRT3 in the pathophysiology of AATD-mediated liver disease. Additionally, we have developed a novel humanized AATD mouse model that have been utilized to confirm our *in vitro* findings. In this study, we showed SIRT3 overexpression reduces ZAAT aggregates and lipid content of ZAAT expressing hepatocytes. We found that SIRT3 enhances lipophagy in ZAAT expressing hepatocytes, thereby mediating removal of misfolded ZAAT aggregates assisted by lipid droplets. In this context, SIRT3 activation eliminates the hepatic toxic gain-of-function associated with both lipid accumulation and

polymerized ZAAT. The discovery of SIRT3 as a modifier of both lipophagy and autophagy overcomes a major challenge for the development of new therapies for AATD-mediated liver disease and provides an alternative treatment approach for AATD individuals with liver disease.

Single-cell multimodal profiling of atherosclerosis identifies CD200 as a cell surface lineage marker of vascular smooth muscle cells and their derived cells

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Vascular smooth muscle cells (VSMCs) play a central role in the development of atherosclerosis owing to their capability to phenotypically transition into either a protective or harmful state. However, the ability to identify and trace VSMCs and their progeny is limited due to the lack of well-defined cell surface markers. Therefore, investigations into VSMC fate must utilize lineage-tracing mouse models, which can be time-consuming and challenging to generate and are not feasible in humans. Here, we employed CITE-seq to phenotypically characterize the expression of 119 cell surface proteins in mouse atherosclerosis. We revealed that CD200 is a highly expressed and specific marker of VSMCs, which persists even with phenotypic modulation. We validated our findings using a combination of flow cytometry, qPCR, and immunohistochemistry, all agreeing that CD200 can identify and mark VSMCs and their derived cells in early to advanced mouse atherosclerotic lesions. Additionally, we describe a similar expression patern of CD200 in human coronary and carotid atherosclerosis. Thus, our data supports the use of CD200 as a lineage marker for VSMCs and VSMC-derived cells in mouse and human atherosclerosis.

Disulfiram reduces atherosclerosis and enhances efferocytosis, autophagy, and atheroprotective gut microbiota in hyperlipidemic mice.

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Cleveland State University

Abstract

Aims: Pyroptosis executor Gasdermin (GsdmD) promotes atherosclerosis in mice and humans. FDA-approved anti-alcoholism drug Disulfiram (DSF) was shown to potently inhibit GsdmD pore-forming activity, but in-vivo efficacy of DSF in reducing atherosclerosis is not yet explored. We used human/mouse macrophages and hyperlipidemic mouse model of atherosclerosis to determine efficacy and mechanism of atheroprotective activities of DSF.

Methods and results: DSF-fed hyperlipidemic apoE$^{-/-}$ mice showed significantly reduced IL-1β release (~80% reduction) upon in-vivo Nlrp3 inflammasome assembly. Atherosclerotic lesions were reduced in DSF-fed mice (~27% and 29% in males and females, respectively) DSF-fed hyperlipidemic mice also showed reduced necrotic cores (~44% and 49% in males and females, respectively). Mechanistically, DSF modulated several atheroprotective pathways such as autophagy, efferocytosis, phagocytosis, and gut microbiota profile. DSF induced autophagy in macrophages and HepG2 cells, in liver tissue, and in atherosclerotic plaques of hyperlipidemic mice. DSF induced efferocytic, and phagocytic activities of human THP-1 macrophages. Cell-surface expression of MerTK (efferocytosis receptor) was markedly increased in DSF-treated macrophages. The 16sRNA sequencing of DSF-fed hyperlipidemic mice showed a highly significant enrichment in atheroprotective gut microbiota Akkermansia and reduction in atherogenic Romboutsia species.

Conclusions: Taken together, our data shows that the atheroprotective mechanism of action of DSF entails modulation of multiple atheroprotective pathways.

Impact: We provide pre-clinical data showing that DSF can serve as a stand-alone or adjuvant therapeutic (along with LDL lowering drugs such as statins) for treating cardiovascular disease.

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

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

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

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

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

This research was funded by NIH grant R01DK133184.

Trained Immunity & SETDB2: A Crosstalk between Epigenetics and Metabolism

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Several pro-inflammatory signals such as microbial cell derived β-glucan, or western diet induced sterile inflammation have been identified as initial “triggers” that train macrophages to respond to a later secondary challenge. This memory response is mediated at least in part through epigenomic changes to the chromatin landscape. SETDB2 is a putative epigenomic modifying enzyme and is a member of the KMT1 family of lysine methyltransferases. Our lab has previously shown that SETDB2 participates in glucocorticoid receptor (GR) dependent chromatin changes in hepatic gene expression during fasting where SETDB2 works together with GR to activate genes through a chromatin looping mechanism that is independent of the s-adenosyl methionine (SAM) binding function of its SET domain. We also showed a role for SETDB2 in atherosclerosis, where bone marrow transfer from global $SETDB2^{GT}$ deficient mice into LDLRKO mice resulted in more severe atherosclerotic plaques. In macrophages SETDB2 is induced by IFN signaling, where it is associated with the repression of proinflammatory gene expression during the later stages of the inflammatory response to prevent runaway inflammation.

To more fully characterize the role of SETDB2 in innate immune responses, at first, we compared bone marrow-derived macrophages (BMDMs) that are pre-trained by β-glucan followed by a secondary stimulation with LPS after 5 days. This results in a super-induction of SETDB2 expression along with a super-induction of proinflammatory cytokines and lactate production. This super-induction was blunted in BMDMs from a myeloid-specific SETDB2 knockout mouse (SETDB2mKO). RNA-sequencing analysis showed ~2200 genes regulated by β-glucan training and differentially expressed between WT vs. SETDB2mKO BMDMs. Further k-means clustering revealed a dynamic profile of transcripts that are clustered into five unique patterns of expression. Among them, cluster-1 contained genes that are super-induced by SETDB2 in WT but the response was blunted in the SETDB2mKO macrophages. In contrast, cluster-2 genes correlated negatively with SETDB2 and β-glucan; the LPS dependent activation observed in WT was suppressed by β-glucan pre-training and this suppression was blocked in the Setdb2mKO. The genes from both clusters were enriched for several metabolic and inflammatory pathways. Cluster-1 enriched genes were involved in glycolysis, hypoxia, and CCL pathways, whereas cluster-2 uniquely contained genes from Interferon and CXCL signaling pathways. To evaluate the potential role of SETDB2 enzyme activity in cluster-1 vs. cluster-2 gene regulation, we developed a SETDB2 knock-in mouse (SETDB2KI) that mutated two amino acids that would cripple its enzymatic function. Interestingly, unlike to SETDB2mKO, β-glucan associated super-induction of cluster-1 genes in SETDB2KI BMDMs was indistinguishable from WT. However, β-glucan mediated repression of the LPS induced cluster 2 genes associated with Interferon gamma and alpha pathways were lost in SETDB2KI BMDMs, which is similar to SETDB2mKO.

Taken together, our preliminary data suggest that SETDB2 regulates different immune response pathways by two different molecular mechanisms; one associated with gene repression and may require its enzymatic activity while the other is involved in gene induction and does not require SETDB2 enzyme activity but instead may be mediated through chromatin looping similar to the role for SETDB2 we uncovered in liver GR signaling during fasting.

The Transition to NASH Could Be Sex and Context Dependent

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About one-third of non-alcoholic fatty liver disease will progress to irreversible non-alcoholic steatosis hepatitis (NASH), however, no drugs specific for NASH have been proven by FDA. Small heterodimer partner (SHP, Nr0b2 gene) is an orphan member of the nuclear receptor superfamily and heterodimerizes with a variety of nuclear receptors to modify the expression of genes whose products regulate metabolism and sex-differences biology. NASH is closely associated with liver inflammation, and hepatic expression of Nr0b2 is negatively associated with liver inflammation.

To better understand the transition from steatosis to NASH, hepatic lipid metabolic and inflammation pathways were studied in male and female hepatic Nr0b2 knockout mice (Δhep) and their wild-type (WT) littermates. The hepatic deletion of Nr0b2 in mice fed a fructose diet resulted in increased hepatic fatty acid oxidation and decreased liver TG, which was associated with increased liver lipid uptake receptor SRBI and decreased blood cholesterol in both sexes. Despite the improved blood and liver cholesterol and TG profile, liver myeloid cell infiltration and mRNA levels of inflammatory markers including interferon γ (Ifnγ) were increased in Δhep mice. Interestingly, the increases in fatty acid oxidation and inflammation induced by the fructose diet in Δhep mice were more pronounced in females than males. Furthermore, lower liver TG content that was associated with better glucose tolerance was observed in male mice compared to female mice, which was not affected by the hepatic deletion of Nr0b2.

To further study the role of inflammation in the transition to NASH, Δhep mice were reconstituted with Ifnγ$^{-/-}$ bone marrow to suppress the inflammation while fed a fructose diet. We observed that liver inflammation and collagen content were decreased by the reconstitution of Ifnγ$^{-/-}$ bone marrow, and liver lipid uptake receptors and proteins in fatty acid oxidation pathways were also partially reversed by the ablation of Ifnγ in myeloid cells in Δhep mice. Glucose tolerance was improved by the ablation of Ifnγ only in female Δhep mice. RNAseq results showed that networks of genes involved in NASH development and PPARα-mediated fatty acid oxidation pathways were upregulated by the hepatic deletion of Nr0b2, and networks of genes in inflammation and fibrosis were suppressed by the ablation of Ifnγ in myeloid cells in the liver in Δhep mice. RNAseq data also showed that genes involved in bile acid (BA) metabolism pathways were upregulated in female mice compared to male mice, indicating that the more severe inflammation and lipotoxicity in female mice may be caused by the activated FXR-mediated pathways by BA. Our studies showed that deletion of Nr0b2 can contribute to NASH by increasing the expression of genes in inflammatory pathways. This biology is compounded by lipotoxicity from high-fructose feeding. Both drivers of NASH can be ameliorated by the ablation of inflammation pathways in myeloid cells.

Title: Classification of Sitosterolemia-Associated Mutations in ABCG5 and ABCG8

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Background: Sitosterolemia is a rare, recessive form of familial hypercholesterolemia and is caused by mutations in ABCG5 or ABCG8. ABCG5 and ABCG8 form an obligate heterodimer that promotes biliary secretion and opposes intestinal absorption of cholesterol and phytosterols. Whole genome and exome sequencing has revealed over 2000 pathogenic or likely pathogenic loss of function mutations of ABCG5 and ABCG8. Missense mutants in clinically confirmed Sitosterolemia are of particular interest for their potential to reveal structure-function relationships in ABCG5/ABCG8. Two classes of compounds enhance the activity (potentiators) or folding (correctors) of disease-causing mutants of ABCC7, the gene defective in cystic fibrosis. These compounds have shown promise in restoring the activity of other ABC transporters, suggesting they may be useful in the treatment of sitosterolemia. Therefore, we classified missense mutants of ABCG5 and ABCG8 to examine the underlying molecular defect causing the disease with the goal of examining their rescue by these compounds.

Methods: We mapped 57 missense mutants of ABCG5 and ABCG8 onto the primary protein structure of each half transporter. Mutants were generated through site-directed mutagenesis and confirmed with Sanger sequencing. Plasmids encoding normal and mutant proteins were co-transfected into human Huh-7 hepatocytes and analyzed for maturation by the appearance of the high molecular weight, mature forms of each glycoprotein in SDS-PAGE.

Results: Mutations in ABCG5 and ABCG8 cluster in the nucleotide-binding domain and transmembrane spanning domain of each half-transporter. Of the mutants analyzed, 57% were maturation-incompetent and potentially capable for rescue by correctors. Most maturation-competent mutants were located on the cell surface suggesting they may be amenable to enhanced activity by potentiators. However, several maturation-competent mutants displayed intracellular staining patterns and are unlikely to benefit from either class of compounds.

Conclusion: Formation and trafficking of the ABCG5 ABCG8 heterodimer provides insight into ABC transporters and diseases associated with loss of function alleles. Partial rescue of disease-causing mutants within ABCG5 and ABCG8 may be feasible with regulators of proteostasis and small molecule chaperones.

Higher plasma apoB and LDL-cholesterol in germ-free mice

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Background: SARS-Cov-2 or rhinovirus infection is associated with dyslipidemia. Our previous studies found that hypertriglyceridemia developed during hospitalization is associated with 2x higher mortality, after adjusting for age, gender, obesity, diabetes, and steroid treatment in severe COVID-19 patients. RNA viruses, such as SARS-CoV-2, often hijack the lipid metabolism system to sustain their infection and amplification. Low density lipoproteins (LDL) are particles that carry lipids and RNAs, which interact with immune cells through receptor-mediated pathways. However, the mechanism between lipoprotein and immune response is unclear.

Aims: We are using germ-free mice as a model lacking immunological stimulus to investigate changes in lipoproteins.

Methods: Germ-free mice are born, housed, bred, and euthanized in an axenic containment. This environment reduces immunological stimuli from infection. Germ-free mice were age, weight, gender, and genetic background matched to the control mice raised in a non-germ-free environment.

Results: Low density lipoprotein receptor (LDLR) is the major receptor that clears LDL particles from the circulation through hepatocytes. Compared to the control mice, germ-free mice have lower liver LDLR levels in both mRNA and protein levels than their controls. Apolipoprotein B is the scaffold protein that holds the structure of LDL particles. Germ-free mice have higher plasma apoB and total cholesterol levels compared to their controls. Separating lipoprotein fractions by size-exclusion fast protein liquid chromatography (FPLC) revealed the LDL fractions in germ-free have higher cholesterol.

Conclusion: Germ-free mice have higher apoB and LDL-cholesterol in circulation and lower LDLR in the livers, indicating a slower LDL clearance rate.

Multiplex analysis of inflammatory proteins associated with risk of Coronary Artery Disease in type 1 diabetes patients

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Objective: Chronic uncontrolled hyperglycemia, a precursor to chronic low-grade inflammation, is a leading cause of coronary artery disease (CAD) due to plaque buildup in type-1 diabetes (T1D) patients. We evaluated levels of 22 inflammatory markers in cross-sectional serum samples from 1222 subjects, to evaluate their potential as risk factors for CAD in T1D patients.
Methods: The T1D subjects were divided into two groups, those without CAD (n=1107) and with CAD (n=115). Serum levels of proteins were assayed using multiplex immunoassays on a Luminex Platform. Differences between the two groups were made by univariate analysis. Multivariate logistic regression was used to ascertain the potential of proteins as risk factors for CAD. Influence of age, duration of diabetes, sex, hypertension, and dyslipidemia was determined in a stepwise manner. Serum levels of 22 proteins were combined into a composite score using Ridge regression for risk-based stratification.
Results: Mean levels of CRP, IGFBP1, IGFBP2, IGFBP6, MMP1, SAA, sTNFRI, and sTNFRII were elevated in CAD patients (n=115) compared to T1D patients without CAD (nCAD, n=1107). After adjusting for age, duration of diabetes, sex, hypertension, and dyslipidemia, higher levels of sTNFRI (OR=2.18, 1.1x10-3), sTNFRII (OR=1.52, 1x10-2) and IGFBP6 (OR=3.62, 49 1.8x10-3) were significantly associated with CAD. The composite score based on Ridge regression was able to stratify CAD patients into low, medium, and high-risk groups.
Conclusion: The results show activation of the TNF pathway in CAD patients. Evaluating these markers in serum can be a potential tool for identifying high-risk T1D patients for intensive anti-inflammatory therapeutic interventions.

Fredrickson Lipid Research Conference 2023 Abstract

Duodenal and plasma lipidomics analysis of undernourished children with refractory environmental enteric dysfunction revealed lipid metabolism alteration

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INTRODUCTION

Undernutrition is a critical factor associated with high mortality rates along with the impairment of physical and cognitive development in children in low- and middle-income countries. The complex biomolecular perturbations induced by undernutrition at this critical window are poorly understood, especially when poor nutrition is caused by environmental enteric dysfunction (EED) or associated clinical conditions. Therefore, a major challenge is to understand key biochemical pathways in infants and children with EED who are stunted refractory to nutritional interventions and at high risk of developmental impairment. Mass spectrometry (MS)-based lipidomics is a systems approach that seeks to comprehensively profile lipids and metabolites of an individual to understand biochemical disease mechanisms and disease specific biomarker discovery.

OBJECTIVES

The primary objective of this study was to identify specific biomarkers from the duodenal and plasma lipidome of malnourished children with EED that were refractory to nutritional intervention using MS-based lipidomics analysis. We also performed an in-depth analysis of lipidomic profiles in correlation to histopathology features and other omics data from refractory EED patients.

METHODS

From 400 children from Pakistan enrolled for the Study of Environmental Enteropathy and Malnutrition in Pakistan (SEEM), a subset of this cohort (n=63) who were refractory to multiple nutritional interventions underwent esophagogastroduodenoscopy (EGD) to evaluate enteropathies. Paired samples of duodenal aspirates and plasma were collected at the time of endoscopy. The duodenal and plasma lipidomes were extensively characterized to determine biochemical phenotypes in EED. Due to ethical constraints and the rarity of upper endoscopy performed in healthy children living in Pakistan, we enrolled a pediatric control group (n=26) at Cincinnati Children's Hospital Medical Center (CCHMC) who received endoscopy for nonspecific GI problems and subsequently had no histopathological abnormalities detected. Untargeted lipidomics analysis was conducted on a UHPLC-HRMS system with Q Exactive™ plus hybrid quadrupole-Orbitrap™ mass spectrometer interfaced with Vanquish ultra-high performance liquid chromatography (UHPLC) system (Thermo Scientific, Waltham, MA). An Acquity CSH C18 UPLC column (2.1 × 100 mm, 1.7 µm, Waters, Milford, MA) was used in separation. Untargeted data were pre-processed by Progenesis QI (Waters Corp.) for peak picking, alignment, deconvolution, and preliminary metabolite/lipid annotation by in-house and on-line available databases, i.e., HMDB and LipidMaps, and identity of potential biomarkers was confirmed by both accurate mass and retention time as well as fragmentation patterns if available. Dysregulated biochemical pathways discovered were also correlated and confirmed with transcriptomics and histopathological results.

RESULTS

Untargeted lipidomics analysis revealed an altered lipid profile in both duodenal aspirate and plasma of malnourished EED children. A reduced level of intraluminal and plasma sphingolipids, i.e., sphingomyelins were observed, along with an increase in phospholipids, including phosphatidylcholine (PC) and lysophosphatidylcholine (LPC), indicating disrupted sphingolipid and phospholipid metabolisms that may underlie the enteric dysfunction. Ceramides, phosphatidylinositol (PI) and lysophosphatidylinositol (LPI) were increased in plasma but decreased in duodenal aspirate of the refractory EED patients. The suppression of the sphingolipid metabolic pathway and altered lipid metabolism were also revealed and confirmed by transcriptomics analysis. Plasma triacylglycerol (TAG) with polyunsaturated fatty acyl chains were decreased, while TAG with saturated and monounsaturated fatty acyl chains were increased in the children with EED. We also observed that plasma fatty acids and cholesterol esters levels were decreased in EED children compared to their healthy counterparts. Conjugated plasma bile acids, including glycocholic acid (GCA) and glycochenodeoxycholic acid (GCDCA), were elevated in the undernourished children, which was consistent with our previous findings and targeted bile acid analysis. The levels of bile acids in duodenal aspirate were relatively lower in EED children. The increased bile acids in the systemic circulation suggest reduced hepatic bile acid uptake and biliary secretion in these children.

CONCLUSIONS

This MS-based lipidomics analysis has provided valuable insights into the biochemical changes in children with early-life malnutrition and EED. Our results suggests a dysregulated sphingolipid metabolism, particularly a deficiency of sphingomyelin in children with refractory EED. Importantly, the plasma and duodenal lipidome data showed different trends in intraluminal fluid and plasma samples for some lipid subclasses indicating their different lipid absorption and uptake due to refractory EED. Prior and ongoing transcriptomics analyses are consistent with these patterns of dysregulation and indicate targeting specific lipid metabolisms as a potential approach to EED treatment.

Development of Synthetic High-Density Lipoproteins for Treatment of Infection Complications

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In the challenging landscape of sepsis, a systemic inflammatory condition triggered by infections, available therapeutic avenues are limited, resulting in consistently high mortality rates. This study centers on the promising therapeutic capabilities of synthetic high-density lipoprotein (sHDL) nanodiscs.

Aim: Our investigation focused on discerning the critical process parameters for nanodiscs manufacturing and assessing the influence of phospholipid components on the anti-sepsis properties of sHDL.

Results: In the pursuit to determine the critical parameters for the production of nanodiscs in PBS at a concentration of 15 mg/mL, we closely assessed the time required to achieve the formation of sHDL with an approximate size of 10 nm, as gauged through Dynamic Light Scattering. Key insights revealed that employing an UltraTurrex homogenization technique on the initial 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) suspension and subsequently on its combination with the 22A solution (under homogenization pressures of 10,000 and 15,000 psi), or heating up to 50°C did not expedite the generation of sHDL particles. The critical manufacturing component, specifically the mixing speed, was established to be a minimum of 150 rpm. When juxtaposed against the smaller synthesis scales (5-20 mL) at 2 mg/mL concentrations, a scale of 100 mL with a 15 mg/mL concentration demanded a substantially protracted synthesis duration, clocking in at 19-20 hours.

The anti-inflammatory effects and mechanisms of different sHDLs were investigated *in vitro* and *in vivo* on lipopolysaccharide (LPS)-induced inflammation models. Our results highlight the superior anti-inflammatory efficacy of nanodiscs developed using DMPC and 22a. These specific DMPC-22a formulations showcased an array of mechanisms to mitigate LPS-induced inflammation effectively. Firstly, they demonstrated a robust capability to neutralize LPS. Additionally, they prevented the migration of toll-like receptor 4 into lipid rafts, thereby hindering a crucial step in the inflammatory signaling pathway. On the molecular signaling front, these nanodiscs suppressed the activities of nuclear factor kappa B, a major pro-inflammatory signaling molecule. Notably, they also facilitated the activation of activating transcription factor 3, known for its anti-inflammatory actions.

Conclusion: The compositional choice of phospholipids, particularly the DMPC-22a combination, plays a cardinal role in the anti-inflammatory prowess of sHDL. Such nanodiscs hold substantial promise in counteracting LPS-induced sepsis, potentially revolutionizing therapeutic approaches in sepsis management. By bridging advanced manufacturing insights with therapeutic potentialities, this study lights the way for the next epoch in sepsis treatment innovations.

Investigating the lipid-lowering potential of Dabigatran

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Dabigatran etexilate is a prodrug that upon ingestion becomes converted into its active form and serves as a direct, reversible thrombin inhibitor. Dabigatran is an anticoagulant commonly prescribed in patients with deep vein thrombosis and pulmonary embolism therapeutically, or in patients with atrial fibrillation prophylactically to prevent the risks of myocardial infarction and stroke. Dyslipidemia is a frequent comorbidity of thrombotic cardiovascular diseases, and many patients on dabigatran have dyslipidemia. However, the impact of dabigatran on atherogenic LDL-cholesterol levels has not been investigated. Intriguingly, a study found that dabigatran decreased apolipoprotein B levels in the serum (Joseph et al., 2016, Heart). Therefore, we propose to investigate whether dabigatran would have LDL-cholesterol-lowering properties.

To study this, we extracted de-identified data from the Medical College of Wisconsin Data Warehouse. Based on the nature of this retrospective study and clinically available information, we collected information on LDL-cholesterol levels, medication history and demographics. We examined patients who were prescribed dabigatran alone (n=13) with no other lipid-modifying agents (fibrates, PCSK9 inhibitors, bile acid sequestrants, and selective cholesterol-absorption inhibitors) as well as patients who take dabigatran with statins (n=94).

The demographics of dabigatran alone versus dabigatran-statin double treatment were as indicated: age (mean, 71 vs. 77, p=0.08) and sex distribution (male, 38% vs. 55%). The LDL-cholesterol levels were collected within 3 months before and after dabigatran treatment. The mean levels of change in LDL-cholesterol levels post-dabigatran were similar between dabigatran alone or prescribed together with pre-existing statin treatment (mean, 5.54 vs. 5.34, p=0.98). Patients were then organized based on whether they had an overall increase or decrease in LDL-cholesterol levels post dabigatran treatment with or without prior statin treatment. In patients with prior statin treatment, post-dabigatran LDL-cholesterol levels were higher in those with lower pre-dabigatran LDL-cholesterol levels. The opposite was also observed - post-dabigatran LDL-cholesterol levels were lower in those with higher pre-dabigatran LDL-cholesterol levels. Similar trends were seen in patients taking only dabigatran without statistical significance. These results suggest that patients, who had prior statin treatment and started with higher LDL-cholesterol levels before dabigatran treatment, may have reduced LDL-cholesterol levels post-dabigatran treatment.

This retrospective study found that in patients with prior statin treatment, those with higher pre-dabigatran LDL-cholesterol levels had lower post-dabigatran LDL-cholesterol levels. Therefore, prospective studies are required to investigate dabigatran’s role in lowering LDL-cholesterol levels to further unveil the underlying mechanism.

2-Hydroxybenzylamine enhances atherosclerosis regression via the modulation of inflammation and HDL function in *Ldlr*$^{-/-}$ mice

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Background: Inflammation and hypercholesterolemia play major roles in the pathogenesis of atherosclerosis. Modification of lipoprotein and cellular components by lipid reactive carbonyls promotes macrophage foam cell formation and inflammation. 2-Hydroxybenzylamine (2-HOBA) is a natural compound that reacts with reactive dicarbonyls thereby preventing their adduct formation with proteins, lipids and nucleic acids. Therapies that will promote the regression and anti-inflammatory remodeling of existing atherosclerotic plaques are needed. We examined the ability of 2-HOBA to reduce and remodel existing atherosclerotic plaques in the *Ldlr*$^{-/-}$ mouse model of familial hypercholesterolemia.

Methods: Eight-week-old *Ldlr*$^{-/-}$ mice were fed a Western Diet for 16 weeks to establish existing atherosclerotic lesions(baseline). Then the mice were switched to a Chow diet and treated with 2-HOBA (1 g/L) or water alone for 6 weeks. The extent of proximal aortic lesions was measured by Oil-Red-O staining. The plasma lipids and lipoprotein profiles were determined by enzymatic assays and FPLC. The plaque cell populations (M1- and M2-like macrophages, smooth muscle cells), cellular apoptosis, necrotic core size, collage content, fibrous cap thickness, dicarbonyl content, and inflammatory gene expressions were examined by immunofluorescence staining, trichrome staining, and real time PCR. Serum HDL was isolated and tested for its ability to reduce macrophage cholesterol and promote efferocytosis.

Results: 2-HOBA treatment of *Ldlr*$^{-/-}$ mice significantly promoted regression of atherosclerosis as evidenced by a 18.1% and 21.1% ($p<0.05$) decrease in proximal lesion area compared to baseline and vehicle treated *Ldlr*$^{-/-}$ mice, respectively. 2-HOBA did not impact total plasma cholesterol or triglyceride levels, or lipoprotein profiles compared to vehicle treated *Ldlr*$^{-/-}$ mice. Importantly, 2-HOBA versus water alone treatment promoted substantial anti-inflammatory remodeling of existing plaques including decreased necrotic core (-54.1%), malondialdehye (-64.2%), total

macrophages (-29.9%) and proinflammatory CD38+ macrophages (-56.0%). Furthermore, 2-HOBA promoted pro-resolving features of existing plaques including increased fibrous cap thickness (+50.7%), collagen (+56.3%), smooth muscle cells (+64.7%), and anti-inflammatory Arginase 1+ macrophages (+151.0%). Consistent with 2-HOBA improving the pro-resolving functions of HDL, HDL from 2-HOBA versus vehicle treated *Ldlr*$^{-/-}$ mice had increased capacity to reduce macrophage cholesterol and promote efferocytosis of apoptotic cells. In addition, in vitro, 2-HOBA prevented the macrophage inflammatory response to the potent carbonyl, isolevuglandin, as evidenced by decreased expression of IL-1β, TNF-α, and IL-6.
Conclusions: Dicarbonyl scavenging with 2-HOBA promotes atherosclerosis regression and enhances anti-inflammatory/pro-resolving features of existing plaques thereby supporting the therapeutic potential of 2-HOBA in treating humans with coronary artery disease.

The Role AICAR Transformylase in Preadipocyte Differentiation

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Over the past fifteen years, there has been growing interest in brown adipose tissue (BAT) within the field of obesity research. In contrast to white adipose tissue (WAT), which mainly functions as an energy storage depot, BAT is metabolically active and contributes to energy dissipation through heat generation. Research indicates that individuals with higher levels of brown fat tend to have a lower body mass index (BMI) and display greater resistance to weight gain. Therefore, comprehending the mechanisms that regulate and activate brown fat holds promise for addressing obesity and exploring novel therapeutic approaches. In our recent investigations, we have identified 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/inosine monophosphate cyclohydrolase (ATIC), an enzyme involved in purine biosynthesis, as a promising molecular target for maintaining and regulating brown adipose tissue (BAT) activity. The present study aimed to explore the impact of modulating ATIC activity on the differentiation of white and brown adipocytes. To achieve this, we employed a lentivirus-mediated CRISPR-Cas9 method to generate ATIC-deficient precursor cells of both white and brown adipocytes. Additionally, we treated a subset of these cells with a specific pharmacological inhibitor targeting ATIC. Following the differentiation of both white and brown precursor cells, we examined the expression of crucial proteins involved in adipogenesis. Furthermore, we investigated the effects of ATIC deficiency and pharmacological inhibition on the activation of the AMPK signaling pathway and lipid accumulation through Western blotting and Oil Red O staining, respectively. Our findings revealed that ATIC deficiency and pharmacological inhibition led to increased phosphorylation and activation of AMPK. Moreover, both ATIC deficiency and pharmacological inhibition facilitated the conversion of white adipocytes into brown-like cells and enhanced the expression of brown adipogenic markers in mature brown adipocytes. Although these results are preliminary, they indicate that ATIC could potentially function as a regulator of white and brown adipocyte differentiation and represent a target for the development of novel therapeutic strategies against obesity.

Title: Cholesterol Excretion in Whole-body and Liver-specific ABCG5 ABCG8-deficient Mice

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Background: The ABCG5 ABCG8 sterol transporter is the body's primary defense against the accumulation of dietary sterols. Loss of function mutations in ABCG5 or ABCG8 result in Sitosterolemia, a rare disorder that presents as familial hypercholesterolemia, but is distinguished by recessive genetics and the accumulation of phytosterols in plasma and tissues. Biliary cholesterol is reduced by greater than 80% in ABCG5 ABCG8-deficient mice, but fecal neutral sterols are unaffected, suggesting a G5G8-independent pathway for cholesterol excretion. The objectives of the present study were to determine the ability of whole-body and liver-specific G5G8-deficient mice to maintain fecal neutral sterol output when challenged with a cholesterol-enriched diet and determine maximal rates of biliary cholesterol secretion.

Methods: Wild-type, whole-body and liver-specific ABCG5 ABCG8-deficient mice were maintained on cereal-based mixed diet, a phytosterol-free purified diet, or a purified diet supplemented with 0.2% cholesterol. Following acclimatization to each diet, fecal neutral sterol output was determined over a three-day period by gas chromatography/mass spectrometry. Maximal biliary cholesterol secretion rates were determined by biliary diversion with bile acid infusion over a range of 0 to 1000 nmol/minute. Biliary lipid secretion was determined by enzymatic colorimetric assays and molecular coupling of cholesterol to bile acid and phospholipid secretion calculated by linear regression.

Results & Conclusions: Whole-body G5G8-deficient mice exhibited a 40% reduction in fecal neutral sterol output compared to wild-type mice when maintained on cereal-based or purified, low cholesterol containing diets. Cholesterol feeding resulted in a 10-fold increase in fecal neutral sterols in wild-type mice, an effect not observed in G5G8-deficient mice. Biliary cholesterol secretion also demonstrated a 50-60% reduction in liver-specific knock-out mice when compared to wild-type. Basal and maximal cholesterol secretion rate in LKO mouse on cholesterol containing diet are 25% that of control mice. Molecular coupling of cholesterol to bile acid secretion failed to reach statistical significance in LKO mice. These data indicate that ABCG5 ABCG8 is indispensable in opposing the accumulation of dietary cholesterol and that alternative pathways cannot compensate for whole body or hepatic G5G8 deficiency in mice challenged with a cholesterol-enriched diet.

Adipocyte-specific HuR overexpression impairs adipose tissue lipid storage

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Recent work from our lab and others has established a functional role for the RNA binding protein Human antigen R (HuR) in adipose tissue. Li et al suggested that HuR regulates lipolysis in white adipose tissue (WAT), while we demonstrated that adipocyte-specific deletion of HuR (Adipo-HuR$^{-/-}$) results in an impairment of brown adipose tissue (BAT)-mediated thermogenesis through HuR-dependent regulation of calcium cycling. HuR expression in adipose tissue has also been shown to decrease with obesity and age, and our results have additionally linked the loss of HuR expression in adipose tissue to cardiac pathology.

The objective of this work is to determine the functional consequence of adipocyte-specific overexpression of HuR (Adipo-HuR^{OE}). Despite no changes in total food intake, activity, or body weight, Adipo-HuR^{OE} mice show a significant decrease in fat mass driven predominately by a loss of WAT mass, with a concomitant increase in muscle mass. When placed on a 45% kcal/fat high fat diet (HFD), Adipo-HuR^{OE} mice exhibit minimal adipose tissue and lack a HFD diet-induced increase in WAT. Indirect calorimetry reveals an increase in respiratory exchange ratio along with a decrease in energy expenditure, suggesting an impairment in fatty acid oxidation in HFD-fed Adipo-HuR^{OE} mice. Consistently, Adipo-HuR^{OE} mice have an exacerbated increase in both serum and hepatic triglycerides following 16-weeks on a HFD, though no differences were observed between Adipo-HuR^{OE} mice and littermate controls on chow.

Taken together, these results suggest that adipocyte-specific overexpression of HuR impairs lipid storage in WAT leading to ectopic lipid accumulation in other tissues, such as the liver, but the underlying mechanisms remain unknown.

A Unique Polyherbal Blend Enhances Muscle Cell Differentiation and Alleviates Palmitate-induced Inflammation and Insulin Resistance

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Abstract: Metabolic syndrome (MetS) characterizes an array of metabolic abnormalities that include central obesity, high blood pressure, elevated blood sugar levels, and irregular lipid profiles. Although MetS is traditionally linked to cardiovascular diseases and type 2 diabetes mellitus (T2DM), emerging evidence indicates a significant correlation between MetS and muscle mass and function. Individuals with MetS commonly experience reduced muscle mass, strength, and endurance. Therefore, strategies to enhance muscle mass and function have the potential to improve overall health outcomes by reducing disability and enhancing quality of life in MetS patients. Our recent research focuses on a unique polyherbal blend (PHB), which exhibits potential health benefits such as decreased obesity and the prevention of T2DM through AMPK activation. However, the specific effects of PHB on muscle mass and function have not been thoroughly investigated. Therefore, the objective of our current study is to determine the impact of PHB on skeletal muscle differentiation and glucose homeostasis. In this study, we treated C2C12 mouse myoblasts with PHB to evaluate changes in differentiation and insulin sensitivity. Our results demonstrate that PHB promotes C2C12 differentiation and mitigates palmitate-induced inflammation and insulin resistance through significant increases in AMPK phosphorylation and activity in differentiated C2C12 myotubes. Moreover, when combined with metformin, PHB synergistically stimulates AMPK activity and the expression of myogenic differentiation markers. These findings underscore the potential of PHB as a preventive and complementary approach to alleviate the metabolic complications associated with MetS, therfore, warrants additional investigation.

The Gut Microbe-Derived Metabolite Trimethylamine Shapes Host Circadian Metabolic Rhythms of Brown Adipose Tissue Via the Host G Protein-Coupled Receptor TAAR5

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The hypothalamus is considered the center for homeostasis due to its control over circadian rhythms, eating behavior, and thermogenesis. Disruption of these outcomes are often associated with metabolic diseases such as obesity and cardiovascular disease. Cardiometabolic disease (CMD) affects nearly one third of the adult population globally and increases the propensity to develop coronary heart disease—the leading cause of death worldwide. Current therapies aimed at reducing the burden of CMD fall short in practice due to undesirable side effects. Our previous work has shown that inhibiting the production of the gut-derived metabolite trimethylamine (TMA) improves glucose tolerance and prevents weight gain in both healthy and obese mice. We had previously postulated that these results were related to TMA being converted to TMAO via FMO3, which has been implicated in many CMDs. However, our new preliminary data show that knocking out *Taar5*, a GPCR for TMA which has recently been discovered in the hypothalamus and other brain regions, improves cold-induced thermogenesis and decreases latency to scavenge for food. Additionally, there are circadian changes in metabolic tissues and the gut microbiota when *Taar5*$^{-/-}$ mice were compared to wildtype controls, further supporting evidence of hypothalamic perturbation. Interestingly, *Taar5*$^{-/-}$ mice show improvements in energy balance and thermogenesis when compared to wildtype controls, suggesting a metabolic role for *Taar5* in associated adipose tissues such as inguinal and brown fat. With this new data, we have hypothesized that TMA-*Taar5* signaling influences hemostatic regulation of eating behavior, circadian rhythms, and thermogenesis in the hypothalamus. We propose that *Taar5* activation plays a restrictive role in hypothalamic activation of brown adipose tissue, thus improving thermogenesis when *Taar5* is not activated. The short-term impact of this project is the identification of a novel pathway that influences thermogenesis, circadian rhythms, and eating behaviors. The long-term impact of this project is the development of a potential therapeutic to target *Taar5* to change eating behaviors, circadian rhythms, and other metabolic measures in order to improve conventional CMD treatment outcomes.

Carnitine Palmitoyltransferase 1a Modulates Hepatic Lipid and Lipoprotein Metabolism

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

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

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

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

Title: Evaluation of synthetic high-density lipoprotein for the protection of endothelial function

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Nearly 17.9 million people die every year from cardiovascular-related diseases, which accounts for 31% of all deaths worldwide. Endothelial dysfunction is implicated in the development of many types of cardiovascular disorders such as atherosclerosis. Endothelial dysfunction can be characterized by a decrease in vasodilation, reduction of NO production, and elevated pro-inflammatory and prothrombotic states. Previous studies have shown that endogenous high-density lipoprotein (HDL) displays a variety of protective properties on the vascular endothelium, including modulating vascular tone and enhancing endothelial monolayer integrity. To mimic the natural cardioprotective behaviors of endogenous HDL, synthetic high-density lipoproteins (sHDL) have been developed in the present study. sHDLs were prepared using ApoA-1 mimetic peptide and different phospholipids, including 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-dimyristoyl-sn-glycero-3-phosphorylcholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). Prepared by the co-lyophilization method, all sHDLs present homogenous particle distribution ranging from 9-12 nm in size. sHDLs showed the ability to increase nitric oxide (NO) release from human umbilical vein endothelial cells (HUVEC). In lipopolysaccharide (LPS) and TNF-α activated HUVECs, sHDL decreased the expression of adhesion molecules, including VCAM-1, ICAM-1, and E-selectin. In a murine model of traumatic brain injury, infusion of sHDL reduced endothelial permability to Evan's Blue dye, suggesting positive effects of sHDL on endothelial integrity.On the basis of these findings, further studies will need to be completed in order to determine the optimal composition of sHDL and to explore its potential in treating endothelial dysfunction.

Commensal gut bacteria derived *N*-acyl serinols can regulate host's postprandial metabolic homeostasis

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Commensal gut bacteria expressing the *N*-acyl synthase (*NAS*) type VI gene produce *N*-acyl serinols, an endocannabinoid-like lipid that signal through the host G-coupled protein receptor, GPR119. Since GPR119 is vital for maintaining postprandial incretin production and systemic host hormone action, a study was designed to understand the role of *N*-acyl serinols in the regulation of post-prandial metabolic response. Here wild-type C57BL/6J male mice were subcutaneously implanted with slow-release pellets containing vehicle, 1-palmitoyl (16:0) serinol (PS) or 1-oleoyl (18:1) serinol (OS), and were maintained at *ad libitum* for 7 days. Thereafter, mice were either necropsied at fed, 12 h fasted, or refed (12 h fasted followed by 3 h of refeeding) states. LC-MS analysis of plasma and liver showed elevated levels of PS and OS in mouse implanted with these pellets, respectively. However, the levels of endogenous *N*-acyl amides remained unchanged. *N*-acyl serinol treated mice showed significantly elevated plasma insulin and leptin levels in response to refeeding. Hepatic gene expression analysis revealed that *N*-acyl serinol treated mice had significantly decreased expression of genes involved in *de novo* lipogenesis and cholesterol metabolism, in the refed state. Additionally, monocolonization of germ-free mice with *NAS* expressing *Escherichia coli* showed accumulation of *N*-acyl serinol in the left ventricle of the heart. We also found that circulating levels of gut microbe-derived PS and OS in patients with heart failure with preserved ejection fraction were significantly reduced compared to healthy controls, suggesting a possible correlation of *N*-acyl serinols in maintenance of cardiometabolic health. Collectively, we conclude that metabolic consequences of gut microbe derived *N*-acyl serinols in the host may be an effective therapeutic approach to ameliorate postprandial hormonal response dysregulation in metabolic diseases including type 2 diabetes and obesity, and related heart failure.

Keywords: *N*-acyl serinol, GPR119, metabolism, insulin, lipogenesis, HFpEF

Cross talk between cholesterol, PIP2, and LXR-RXR to maintain cellular lipid homeostasis.

Introduction: Excess plasma cholesterol, especially in the form of oxidized low-density lipoprotein (LDL), promotes inflammation and atherosclerotic plaque growth. Cholesterol homeostasis is tightly regulated by the nuclear liver X receptor-Retinoid X receptor (LXR-RXR) heterodimer complex. Cholesterol efflux pump ABCA1 serves as an atheroprotective protein, via removing excess lipids from arterial foam cells and dampening inflammation. In presence of excess cholesterol LXR-RXR is activated, leading to induction in expression of genes involved in cholesterol efflux, such as ABCA1. Phosphatidylinositol 4,5-bisphosphate (PIP2) promotes ABCA1-mediated cholesterol efflux, but it's not clear if PIP2 plays a direct role in regulating cellular cholesterol homeostasis.

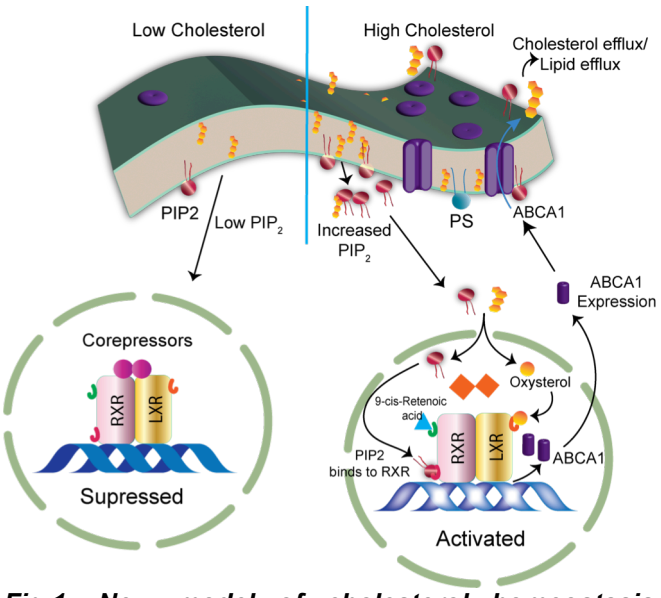

Fig.1: New model of cholesterol homeostasis. Cholesterol loading increased cellular PIP2 and induced PIP2 nuclear localization, allowing binding of PIP2 to LXR-RXR via a novel PIP2 binding domain. PIP2 activates the LXR-RXR complex in conjunction with cholesterol derivatives, promoting the induction of ABCA1 and cholesterol efflux. In low cholesterol conditions, nuclear PIP2 levels drop, leading to reduction in ABCA1 levels and dampened cholesterol efflux. *This project received funding from American Heart Association TPA award to K.G (2023-26)*

Objective: We aim to decipher the mechanism-based role of PIP2 in cholesterol homeostasis.

Methods and Results: Using cholesterol-loaded/depleted and PIP2 loaded/depleted conditions, we made several exciting findings showing cross talk between cholesterol, PIP2, LXR-RXR, and ABCA1. These findings are: 1) cholesterol loading in macrophages and hepatocytes leads to PIP2 redistribution from plasma membrane to the endoplasmic reticulum-plasma membrane (ER-PM) junctions and increased nuclear PIP2 levels, 2) expression of ABCA1 correlated with levels of cellular PIP2, and 3) PIP2 interacted with LXR-RXR complex in a cooperative fashion to regulate ABCA1 expression.

Conclusions: PIP2 levels are directly related to cellular cholesterol load, and PIP2 modulates ABCA1 levels to maintain cholesterol homeostasis.

Impact: We propose a new model of lipid homeostasis, where cholesterol regulates PIP2 levels and localization, and PIP2 in-turn regulates ABCA1 expression via LXR-RXR (***Figure 1***). Understanding mechanistic details of pathways regulating cholesterol homeostasis may allow design of novel anti-atherosclerotic therapeutics.

Title: Novel Mechanism for Extrahepatic Inflammation in Progressive Familial Intrahepatic Cholestasis 1 (PFIC1).

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ABSTRACT

Introduction: Human mutations in ATP8b1, a member of the P4-type ATPase family, causes Progressive Familial Intrahepatic Cholestasis I (PFIC1). PFIC1 patients also show extrahepatic manifestations such as pancreatitis, hearing loss, and atherosclerosis. Even after liver transplantation, which rescues PFIC1 hepatic symptoms, the extrahepatic inflammatory symptoms persist. Membrane pore-forming protein Gasdermin D (GsdmD) has emerged as a major player in the inflammasome field. GsdmD can be cleaved via a caspase-1/Nlrp3 dependent canonical inflammasome pathway, or a caspase-11 (in mice) or caspase-4/5 (in humans) dependent non-canonical inflammasome pathway.

Objectives: To decipher the mechanism for ATP8b1-mediated regulation of inflammasome activity in immune cells.

Methods & Results: We employed Crispr-Cas9 generated homozygous ATP8b1 knockout human monocytes/macrophages to determine the status of inflammasome activity. Interestingly, ATP8b1$^{-/-}$ cells showed GsdmD cleavage upon LPS priming, and markedly increased IL-1β release vs. WT cells. Mechanistically, GsdmD is cleaved in ATP8b1$^{-/-}$ via an Nlrp3-independent non-canonical inflammasome pathway. Atomic force microscopy of ATP8b1$^{-/-}$ cells showed an altered membrane environment, allowing LPS entry into cytoplasm vs. WT cells. ATP8b1$^{-/-}$ cells showed impaired lysosomal acidification, altered mitochondrial membrane potential, and defective phagocytosis/efferocytosis activities.

Conclusion: Our data identify ATP8b1 as the first known negative regulator of GsdmD cleavage. Macrophages/monocytes lacking ATP8b1 cleave GsdmD via a non-canonical inflammasome pathway.

Impact: Humans are always exposed to small doses of LPS, and this exposure may induce chronic GsdmD cleavage in PFIC1 immune cells. Our data's clinical implication is identifying GsdmD cleavage as a therapeutic target for resolving inflammation in PFIC1 patients.

A novel non-coding genetic variant affects blood lipids by regulating a human-specific long non-coding RNA

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Elevated blood cholesterol and triglyceride, or hyperlipidemia, is a common human disorder associated with an increased risk of coronary artery disease (CAD). Genetic variants significantly contribute to the development and progression of hyperlipidemia. Recent genome-wide association studies (GWAS) have identified hundreds of significant loci associated with hyperlipidemia risk. However, only a few of these GWAS loci have been functionally characterized. For most hyperlipidemia-associated GWAS loci, their mechanisms of action and downstream target genes are unknown, thus compromising our capacity to take advantage of these valuable genetic data to discover novel therapeutic targets for the optimal treatment of hyperlipidemia and CAD. The major hurdles that stop us from understanding blood lipid-associated GWAS loci include: (i) most GWAS SNPs (single nucleotide polymorphisms) are within non-coding regions of the genome, which are poorly conserved among common animal models like mice. The lack of conservation makes it challenging to study mechanisms of action for blood lipid-associated GWAS variants in key tissues/organs like the liver, which plays a central role in regulating blood lipid levels by balancing endogenous lipid production with dietary intake; (ii) novel genes that harbor disease-associated SNPs, such as long non-coding RNAs (lncRNAs), are important regulators of many biological processes, but their roles in mediating the effects of GWAS variants are understudied; (iii) most human lncRNAs are also not conserved even among other mammals; (iv) key metabolic processes, including lipoprotein metabolism, display features that are unique to humans.

In this study, we overcome these limitations by providing evidence supporting a blood lipid-associated non-coding genetic variant (rs9653945) functions by regulating a human-specific lncRNA in the liver. We first analyzed published human genetic data and found that rs9653945 associates with blood low-density lipoprotein cholesterol and triglyceride levels, as well as the expression of a human-specific lncRNA, LOC100507389, in human liver tissues. We then demonstrated that rs9653945 acts as an enhancer affecting sterol regulatory element binding transcription factor 1c (SREBP1c) activity. CRISPR base editing in cultured human hepatocytes showed that rs9653945 risk allele (G) is essential for SREBP1c to induce the expression of LOC100507389. To test the role of this rs9653945 (G)-SREBP1c-LOC100507389 pathway in affecting blood lipids in vivo, we prepared a humanized liver mouse model where mouse hepatocytes were replaced by primary human hepatocytes that are homozygous for rs9653945-G. In line with our in vitro data, we found that the expression of LOC100507389 is induced by feeding when SREBP1c activity is high. More importantly, knocking down of LOC100507389 in humanized liver mice resulted in a broad downregulation of human genes in the glycolysis, lipogenesis, and cholesterol synthesis pathway. Furthermore, cholesterol levels in human Apolipoprotein B-containing lipoproteins purified from the blood were significantly lower in humanized mice with knocking down of LOC100507389. Finally, we found that hLMR1 and its downstream genes involved in lipid synthesis were significantly upregulated in the liver tissue of CAD patients. In summary, using in vitro CRISPR base editing and in vivo functional analysis in humanized liver mice, our work supports a novel rs9653945 (G)-SREBP1c-LOC100507389-hepatic lipid synthesis pathway, thus providing an example showing how a non-coding genetic variant functions by regulating a human-specific lncRNA to affect blood lipids. Our study also suggests that LOC100507389 is a promising therapeutic target for hyperlipidemia-associated human diseases, including CAD.

Unraveling the Role of NAPEPLD in Macrophage Function

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Macrophage carry out critical functions including reverse cholesterol transport and phagocytosis of apoptotic cells (efferocytosis). These functions are impaired during the development of atherosclerosis, a prevailing cause of mortality in the United States. N-acyl phosphatidylethanolamine hydrolysing phospholipase D (NAPEPLD) is responsible for the hydrolysis of N-acyl phosphatidylethanolamines (NAPEs) into bioactive N-acylethanolamines (NAEs), with palmitoylethanolamide (PEA) being one of the prominent NAEs. Diminished expression of NAPEPLD has been reported in human atherosclerotic lesions and in mice fed atherogenic diets, leading us to hypothesize that reduced macrophage NAPE-PLD activity contributes to the progression of atherosclerosis.

To test this hypothesis, bone-marrow derived macrophages (BMDMs) were isolated from wild-type (WT) and NAPEPLD-/- mice and the capacity of these macrophages to carry out efferocytosis was measured by incubating them with labeled apoptotic cells (Jurkat T cells exposed to UV) for 6 h, then measuring the extent of apoptotic cell uptake by flow cytometry. Compared to WT BMDM, significantly fewer Napepld-/- BMDM had taken up apoptotic cells, supporting an essential role for Nape-pld in efferocytosis. RNA sequencing of these BMDMs revealed 30 genes with significant differential expression (greater than a 2-fold change, $p \leq 0.05$). qPCR analysis validated differential expression of many of these genes including Ornithine decarboxylase 1 (Odc1), Heme Oxygenase 1 (Hmox1), Acid phosphatase 1 (Acp1), Adhesion GPCR G1 (Adgrg1), and Lipin-1 (Lpin1) Of the genes reduced in Napepld-/- BMDM, only a few such as Ornithine decarboxylase 1, have previously been implicated in efferocytosis. Downregulated genes included Heme Oxygenase 1 (Hmox1), Acid phosphatase 1 (Acp1), Adhesion receptor ADGRG1, and Lipin-1 (Lpin1). Treatment of Napepld-/- BMDMs with 10 uM PEA (at 10uM) for a duration of 6 hours upregulated the expression of Hmox1, Acp1, and Lpin1. In the case of VU534-treated BMDMs, RNA sequencing was conducted, while veh/PEA-treated BMDMs had 200ug of RNA reverse-transcribed into cDNA, which was subsequently diluted five-fold and utilized for qPCR. Statistical analysis of the data (n = 6-12 replicates) was carried out using ANOVA with a significance level set at $P \leq 0.05$.

The RNA sequencing results qPCR analysis also confirmed downregulation of Acp1, Odc1, Lpin1, and Hmox1 mRNA. Interestingly, PEA treatment upregulated all four genes, except Odc1, indicating that the latter might not be involved in PEA-mediated macrophage function. Further

research endeavors will delve into the protein expression of these genes and explore their interactions with the localization and function of the NAPEPLD enzyme.