September 7-10, 2022

FREDRICKSON LIPID RESEARCH CONFERENCE

Durham, NC

INSTITUTIONAL HOST:
Duke University and University of North Carolina at Chapel Hill
Wednesday, September 7
Welcome Reception
21C Museum Hotel, Durham
Second Floor Gallery
6:00 pm – 8:00 pm

Thursday, September 8
FLRC Day 1
21C Museum Hotel, Durham
Main Gallery and Second Floor Gallery

8:30  Check-in and Continental Breakfast | Second Floor Gallery

9:00  Welcoming Comments | Main Gallery
Saskia Neher, PhD | University of North Carolina at Chapel Hill
John Rawls, PhD | Duke University

Session I: Thinking Outside the Sphere: Weird and Wonderful Functions of Lipoproteins
Chair: Sarah Cohen, PhD | University of North Carolina at Chapel Hill
9:05  Sarah Cohen, PhD | University of North Carolina at Chapel Hill
APOE Moonlights as a Lipid Droplet Protein in Astrocytes
9:25  Lance Johnson, PhD | University of Kentucky
APOE, Immunometabolism, and Lipid Droplet Dynamics in Microglia
9:45  Selected Abstract - William Clarkson | University of South Carolina
Association between Cholesterol Efflux Capacity and HDL-Sized and Whole Plasma Proteins in the HERITAGE Family Study
10:00 Selected Abstract - Alexander Bashore, PhD | Columbia University
High Plasma Lp(a) Levels Associate with a Specific Transcriptomic Signature in Monocytes in African Americans but not in Whites

10:15  Break
Thursday, September 8
FLRC Day 1
Continued

Session II: A Celebration of Innovation: New Technologies, New Insights
Chair: Scott Gordon, PhD | University of Kentucky

10:45 Michael Airola, PhD | Stony Brook Cancer Center
Snapshots of Lipid Synthesis and Storage

11:05 Melissa Ellermann, PhD | University of South Carolina
Endocannabinoids Modulate Commensal and Pathogenic Enterobacteriaceae in the Gut

11:25 Meng Wang, PhD | Baylor College of Medicine
Lysosomal Lipid Signals in Longevity Regulation

11:45 Selected Abstract - Shunxing Rong, PhD | UT Southwestern Medical Center
Increasing Phosphatidylethanolamine Content in the Endoplasmic Reticulum

12:00 Selected Abstract - Ian Williamson, PhD | Duke University
Glycerate Production from Enteric Fructose Metabolism is Elevated by Dietary Fat, Inducing Glucose Intolerance through β-cell Damage

12:15 Lunch

Faculty and Staff – Lunch on Your Own

Trainees – Career Development Session (Prior Registration Required)

Option 1 | Gallery Three
How to Publish in High-Impact Journals: A Panel Discussion
Alan Daugherty, PhD, DSc | University of Kentucky
Suzanne Barbour, PhD | Duke University
Scott Gordon, PhD | University of Kentucky
Deb Muoio, PhD | Duke University

Option 2 | Gallery Five
Transitional Awards from NIH
Karin Lidman, PhD | Program Director, Office of Research Training and Career Development, Division of Cardiovascular Sciences, NHLBI, NIH
Ian Williamson, PhD | Postdoctoral Research Associate, Division of Gastroenterology, Duke University School of Medicine
Sarah Cohen, PhD | Assistant Professor, Cell Biology and Physiology, University of North Carolina at Chapel Hill
Thursday, September 8
FLRC Day 1
Continued

2:00 Keynote Speaker
Karen Mohlke, PhD | University of North Carolina at Chapel Hill
Molecular Genetic Mechanisms Underlying Inter-Individual Variation in Lipid Metabolism

2:45 Break

3:15 Larry Rudel Award Lectures
Chair: Ryan Temel, PhD | University of Kentucky
3:15 Natalia Do Couto, PhD | University of Illinois at Chicago
Postdoctoral Fellow
Cholesterol-Induced Suppression of Kir2.1 Contributes to Atherosclerotic Plaque Development
3:30 Kathryn Gunn, PhD | University of North Carolina at Chapel Hill
Postdoctoral Fellow
Active Lipoprotein Lipase Dimer Revealed by CryoEM
3:45 Clint Upchurch | University of Virginia
Graduate Student
AAV8-Mediated Expression of Scfv-E06 to Target Oxidized Phosphatidylcholines is an Effective Therapeutic Intervention to Prevent Progression to Hepatic Fibrosis

Session III: I’m No Model: Lipid Metabolism in Unconventional Systems
Chair: Robert Bauer, PhD | Columbia University
4:00 Meredith Wilson, PhD | Carnegie Institute for Science
Imaging Intestinal Lipid Droplet Dynamics in Vivo with Fluorescent Perilipin 2 and Perilipin 3 Knock-In Zebrafish
4:20 Marina Blanco, PhD | Duke Lemur Center
From Fruits to Fats: High Sugar Diets Predict White Adipose Tissue Deposition and Depletion in a Hibernating Lemur
4:40 Eva Hurt-Camejo, PhD | Astra Zeneca
Absence of Atherogenesis Associated with Vasculo-Protective Properties of Lipoproteins despite Elevated Total Plasma Lipids in Brown Bears (Ursus arctos)
5:00 Selected Abstract - Madison Kirk | Wake Forest University
The Effect of Hydrogel Materials on Lipid Metabolic Capacity of Three-Dimensional Primary Human Hepatocyte Cultures
Thursday, September 8
FLRC Day 1
Continued

6:00  Depart hotel lobby for the short walk to Durham Bulls Baseball Stadium

6:15  Dinner and Baseball (Prior Registration Required)
   Durham Bulls vs Scranton/Wilkes Barr RailRiders
   Pinnacle Party Deck
   Durham Bulls Athletic Park
Friday, September 9, 2021
FLRC Day 2
The North Carolina Diabetes Research Center
Annual Symposium
Haw River Ballroom

8:00  Buses depart 21C Museum Hotel lobby for transportation to the venue

9:00  Welcoming Comments

9:15  NCDRC Keynote Speaker
Scott Summers, PhD | University of Utah
Ceramides and the Two Phases of Lipotoxicity

10:15 Presentation
Herman Pontzer, PhD | Duke University
Evolution, Activity, and Aging in Human Energy Expenditure

11:00  Break

11:15 Presentation
Beth Mayer-Davis, PhD | University of North Carolina at Chapel Hill
Nutrition for Precision Medicine Study

12:00  Lunch
Friday, September 9, 2021
FLRC Day 2
Continued

1:00 Afternoon Presentations

David Herrington, MD | Wake Forest University
Understanding Cardiometabolic Heterogeneity in the Age of Big Data, Advanced Analytics and Complexity Theory

Anna Lee, PhD | North Carolina Agricultural and Technical State University
Examining the Influence of Social Determinants of Health on Obesity and Diabetes among Aging African Americans

Abbie Smith-Ryan, PhD | University of North Carolina Chapel Hill
Isoenergetic High Intensity Interval Training and Moderate Intensity Training in Adults with Type 1 Diabetes

Morgana Mongraw-Chaffin, PhD | Wake Forest University
Meals for Moms: A Postpartum Medically-Tailored Meal Program to Promote Weight Loss and Blood Glucose Control Among Women with Hyperglycemia in Pregnancy

Svati Shah, MD, MHS | Duke University
Omics Biomarkers in Obesity and Diabetes: A Step Towards Precision Health?

4:15 Invitation to Poster Session

Elimelda “Moige” Ongeri, PhD | North Carolina Agricultural and Technical State University

4:30 Poster Session | Cash Bar

5:45 Buses depart Haw River Ballroom for 21C Museum Hotel
Saturday, September 10
FLRC Day 3
21C Museum Hotel, Durham
Main Gallery and Second Floor Gallery

8:30  Continental Breakfast | Second Floor Gallery

9:00  Virgil Brown Lecture | Main Gallery
Deb Muoio, PhD | Duke University
Mitochondrial Fitness and Failure in Health and Disease

Session I: The Big Picture: Systems, Omics, and Whole-body Studies
Chair: Svati Shah, MD, MHS | Duke University

9:45  Keshari Thakali, PhD | University of Arkansas Medical Sciences
Sex Differences in Maternal Programming of Perivascular Adipose Tissue

10:05  Jessica Alvarez, PhD, RD | Emory University
Nutritional Metabolomics to Identify Novel Pathways Linked to Body Composition

10:25  Opeyemi Olabisi, MD, PhD | Duke University
A Friend of Lipid but a Toxin to Kidney Cells: The role of APOL1 in the pathogenesis of kidney failure in African Americans

10:45  Break

Session II: Adios Adipose: Lipids in Other Tissues
Chair: Rebecca Haeusler, PhD | Columbia University

11:00  Arion Kennedy, PhD | North Carolina State University
Investigating H2-Kb Restricted Peptides in Fatty Liver Disease

11:20  Taku Kambayashi, MD, PhD | University of Pennsylvania
Control of Lipids through the Skin by the Immune-Sebum Axis

11:40  Phillip White, PhD | Duke University
A New Homeostatic Regulatory Node at the Intersection of Amino Acid and Lipid Metabolism

12:00  Robert Helsley, PhD | University of Kentucky
Carnitine Palmitoyltransferase 1a Serves as a Gatekeeper of Sexual Dimorphic NAFLD

12:20 – Closing remarks and meeting adjourned
Virgil Brown Lecture

Deb Muoio, PhD
Professor, Medicine
Director, Basic Research
Duke Molecular Physiology Institute and Sarah W. Steadman Nutrition and Metabolism Center
Duke University

Mitochondrial fitness and failure in health and disease

Fredrickson Keynote Speaker

Karen Mohlke, PhD
Professor, Genetics
Associate Chair for Research
University of North Carolina

Molecular genetic mechanisms underlying inter-individual variation in lipid metabolism

NCDRC Keynote Speaker

Scott Summers, PhD
Co-Director, Diabetes & Metabolism Research Center
Department Chair and Professor, Nutrition & Integrative Physiology
University of Utah

Ceramides and the two phases of lipotoxicity
Event Organizers

2022 Conference Organizers

Saskia Neher, PhD
Associate Professor
Biochemistry and Biophysics
University of North Carolina at Chapel Hill

John Rawls, PhD
Professor
Molecular Genetics and Microbiology; Medicine
Duke Cancer Institute
Duke University

2022 Session Chairs

Sarah Cohen, PhD
University of North Carolina at Chapel Hill

Scott Gordon, PhD
University of Kentucky

Robert Bauer, PhD
Columbia University

Svati Shah, MD, PhD
Duke University

Rebecca Haeusler, PhD
Columbia University

Ryan Temel, PhD
University of Kentucky
Invited Speakers

Michael Airola, PhD
Stony Brook Cancer Center

Karin Lidman, PhD
National Institute of Health

Jessica Alvarez, PhD, RD
Emory University

Beth Mayer-Davis, PhD
University of North Carolina at Chapel Hill

Suzanne Barbour, PhD
Duke University

Morgana Mongraw-Chaffin, PhD
Wake Forest University

Marina Blanco, PhD
Duke Lemur Center

Priyanka Narayan, PhD
NIDDK

Alan Daugherty, PhD, DSc
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Opeyemi Olabisi, MD, PhD
Duke University

Melissa Ellermann, PhD
University of South Carolina

Herman Pontzer, PhD
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Wake Forest University

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Eva Hurt-Camejo, PhD
Astra Zeneca

Meng Wang, PhD
Baylor College of Medicine

Lance Johnson, PhD
University of Kentucky

Phillip White, PhD
Duke University

Taku Kambayashi, MD, PhD
University of Pennsylvania

Ian Williamson, PhD
Duke University

Arion Kennedy, PhD
North Carolina State University

Meredith Wilson, PhD
Carnegie Institute for Science

Anna Lee, PhD
North Carolina Agricultural and Technical State University
**Larry Rudel Award Lectures**

**Natalia Do Couto, PhD | University of Illinois at Chicago**  
Cholesterol-Induced Suppression of Kir2.1 Contributes to Atherosclerotic Plaque Development

**Kathryn Gunn, PhD | University of North Carolina at Chapel Hill**  
Active Lipoprotein Lipase Dimer Revealed by CryoEM

**Clint Upchurch | University of Virginia**  
AAV8-Mediated Expression of Scfv-E06 to Target Oxidized Phosphatidylcholines is an Effective Therapeutic Intervention to Prevent Progression to Hepatic Fibrosis

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**Selected Abstract Presentations**

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**Alexander Bashore, PhD | Columbia University**  
High Plasma Lp(a) Levels Associate with a Specific Transcriptomic Signature in Monocytes in African Americans but not in Whites

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**Madison Kirk | Wake Forest University**  
The Effect of Hydrogel Materials on Lipid Metabolic Capacity of Three-Dimensional Primary Human Hepatocyte Cultures
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NCDRC SYMPOSIUM 2022
POSTER ABSTRACTS
**Poster 1:**

**Interaction of iron and hypoxia pathways in determining diabetes risk.**

Alexandria V. Harrison, Philip Lorenzo and Donald A. McClain

Wake Forest University School of Medicine

**Abstract:**
The micronutrient iron is a risk factor for type II diabetes (T2D). The effects of iron are, in part, mediated by the iron-sensing hypoxia inducible factor (HIF) pathway. The interactions of iron and hypoxia in the progression of T2D are not fully understood. We therefore fed mice a fast-food (FF) diet supplemented with either normal iron (35 mg/kg) or high iron (2000 mg/kg) under normoxic (21% O2) and hypoxic (12% O2) conditions. There was an interactive effect of iron and hypoxia on fasting glucose and glucose tolerance (p=0.016, p=0.41 respectively) in which the combination of hypoxia and normal iron yielded a significant improvement as compared to all other groups. Direct measurement of insulin sensitivity showed no additional change with hypoxia, suggesting non-insulin mediated glucose uptake mechanisms. Transcriptional profile of mouse eWAT shows significant upregulation of classic HIF target genes involved in glycolysis, mitochondrial metabolism, and intracellular iron homeostasis in hypoxic mice and interestingly also in normoxic high iron mice. Lastly, protein expression of HIF regulators FIH1 and PHD2 were significantly reduced (20-30% reduction, p<0.05 for FIH and p<0.01 for PHD2) under hypoxia, while GLUT1 protein expression was significantly increased under hypoxia with a trend of higher GLUT1 expression in the normal iron hypoxic group. We conclude that both iron and hypoxia affect glucose tolerance in mice on a FF diet, and their synergistic effects may be driven by the two regulatory arms of the hypoxia inducible factor (HIF) pathway through non-insulin mediated glucose uptake mechanisms.
Initiation of Statin Use for Primary Prevention According to Age in the Women's Health Initiative

Michael P. Bancks, Chris Gillette, Dan Beavers, Lindsay M. Reynolds, Aladdin H. Shadyab, Longjian Liu, Bernhard Haring, David J. Maron, Adam Bress, Mara Vitolins

Wake Forest University School of Medicine

Abstract:

Background: Over half of US adults age ≥75 live without prior cardiovascular disease (CVD) and diabetes mellitus. Evidence to guide statin initiation and use for CVD primary prevention for adults age ≥75 years is inadequate.

Objective: To assess incidence of statin initiation for primary prevention of CVD according to age among participants in the Women's Health Initiative (WHI) free of clinical CVD and diabetes at baseline.

Methods: Self-reported prescription medication use was assessed at baseline (1993) and over follow-up (1996 and 2008) among all WHI participants. Statin medication and dosage was identified using National Drug Codes and dose intensity was defined by expected LDL-cholesterol lowering low (<30%), moderate (30-49%), and high (≥50%). Baseline age groups were defined as: age <65, 65-74, and ≥75 years. Women who at baseline had prior CVD (n=33,992), prevalent diabetes (n=5,972), or current statin use (n=6,811), or were missing follow-up information (n=452) were excluded from analysis. Follow-up time was censored at date of statin initiation, incident CVD, or date of last medication follow-up.

Results: Among 114,581 WHI participants, 27% started statin medications over 15 years. Incidence of statin medication use was lower with older age. Moderate-intensity dose was most common at statin initiation and dose intensity differed by age.

Conclusion: Among women without CVD and diabetes, initiation of statin medications is lowest for women age ≥75 years. Women ≥75 years are more likely to be prescribed a low-dose intensity statin at initiation than younger women.
Poster 3:

Histological Assessment of Nonhuman Primate Brown Adipose Tissue Importance of Sympathetic Innervation.

Abigail Williams, Masha Block, Kylie Kavanagh

Department of Pathology, Wake Forest University School of Medicine, Winston-Salem, NC, USA.

Abstract:
The objective of this study was to analyze key histological features in brown adipose tissue (BAT) functionally and how they relate to the clinical traits expressed by their nonhuman primate adipose tissue donors. Axillary adipose tissue biopsies were collected from a metabolically diverse nonhuman primate cohort with measured clinical data. Immunohistochemical analysis was performed to quantify expression of tyrosine hydroxylase (TH), uncoupling protein 1 (UCP1), cluster of differentiation 31 (CD31), cytochrome c oxidase subunit 4 (COX IV), and cell size. Computed tomography scans were performed to assess body composition. Tyrosine hydroxylase was significantly negatively correlated with whole body fat mass as a percentage of body weight, and was significantly positively correlated with the density of UCP1, COXIV, CD31, and BAT cell density of the BAT samples. Our findings highlight the disparity of innervation provided to BAT based upon body composition, as well as the importance of innervation in the functionality of brown adipose tissue. In effect, these results support the use of sympathetic nervous system stimulants in activating brown adipose tissue for the purpose of treating obesity.

This work was supported by the Peer Reviewed Medical Research Program under Award Nos. W81XWH2110565 and DOD W81XWH-15-1-0574 (to KK) and by the National Institutes of Health grants: ULTR001420, P40OD10954, and R01HL142930 (to KK).
Poster 4:

Retrospective Analysis of Caregiver Health Management Competence During an Occupation Therapy Coaching Telehealth Intervention.

Julia Shin EdD, OTR/L; Vanessa Jewell, PhD, OTR/L; Yongyue Qi, PhD, Andrea Valdez

University of North Carolina; Creighton University

Abstract:
Occupation-based coaching (OBC) is a collaborative, family-centered intervention which can empower caregivers of children with T1D to establish independence with diabetes management. A RCT involving telehealth OBC intervention was piloted with rural caregivers with a child (2-12 years) diagnosed with T1D. Ten caregivers received 1-hour weekly sessions across 12 weeks, which were digitally recorded and transcribed verbatim. Six session per family (N=60) were randomly selected from each of the beginning, middle, and end phases. The average word count and rating at each phase were calculated and repeated measures ANOVA were used to evaluate how caregiver problem-solving progressed. The percentage of parent mean word count increased significantly from 67.2 at the beginning to 67.9 at middle phase and then increased to 72.4 at the end (p=0.034) demonstrating a significant linear trend (p=0.019). The percentage of interventionist word count significantly decreased from beginning to end phases (beginning: Mean=32.8, SD=15.0; middle: Mean=32.1, SD=17.8; end: Mean=27.6, SD=12.9, p=0.034) and showed significant linear trend (p=0.019). Average rating on the Evidence of Independent Capacity Rating Scale with an “A” indicates high level of independence and “C” indicates low level of independence from the interventionist improved significantly from a rating close to B and rating of B at the beginning and middle phases, respectively, and continued to improve to a rating close to A at the end phrase (p<0.001); significant linear trend of improvement was also noted (P<0.001). Preliminary evidence suggests that OBC supports rural caregivers with developing problem-solving skills to manage their child’s diabetes cares.
Poster 5:

Interplay between life extenders: Cardiorespiratory fitness vs. caloric restriction on clinical and molecular phenotypes.

Johanna Y. Fleischman, Nathan Qi, Mary K Treutelaar, Lauren G Koch, Steve L Britton, Charles F Burant

Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor

Abstract:
Caloric restriction (CR) is a highly conserved mechanism to increase longevity across many species and shares many phenotypical parallels with high cardiorespiratory fitness (CRF) including reduced adiposity, increased cardiometabolic health, and increased longevity. CRF is a highly heritable trait in humans and can be studied in a genetic rat model selectively bred for high (HCR) and low (LCR) CRF, in which the HCRs live longer and have reduced bodyweight. This study addresses whether the high CRF phenotype occurs through similar mechanisms by which CR promotes health and longevity. Generation 22 HCR and LCR male rats were fed ad libitum (AL) or were calorically restricted to 60% of AL calories (CR) for 1 year starting at 4 weeks old. Animals were intermittently tested for running capacity, metabolic cage activity, body composition, plasma lipidomics, and liver and muscle transcriptomics. LCRs under CR developed a clinical and metabolic profile that mirrors the high-CRF phenotype, including reduced adiposity and increased insulin sensitivity. Linear mixed modeling analysis of whole-body metabolism suggests HCRs have a higher oxygen consumption than LCRs and that CR increases fat oxidation in both lines. Transcriptomic analysis indicates that HCR and LCR muscle responds similarly to CR, but that a differential response is induced in the liver. Evaluation of changes in clinical parameters in both lines indicate that LCR-CR rats experience a health-associated positive effect on clinical parameters whose values are indistinguishable from HCR animals. However, the HCRs experienced a CR response in the muscle transcriptome, suggesting that not all CR and CRF pathways overlap. In conclusion, LCRs experience significant positive health outcomes under CR that are observed to a lesser degree, or are already apparent, in HCRs, supporting the hypothesis that a portion of high CRF-associated pathways in the HCRs overlap with those induced by CR.
Decellularized human pancreatic extracellular matrix-based physiomimetic microenvironment for human islet culture.

Amish Asthana1,2,3, Lori N. Byers1,2,3, Emmanuel C. Opara2,3, Stephen J. Walker2,3, Sang Jin Lee2,3, Giuseppe Orlando1,2,3

1Department of Surgery, Wake Forest Baptist Medical Center, Medical Center Boulevard, Winston Salem, USA.
2Wake Forest Institute for Regenerative Medicine, Winston Salem, USA.
3Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, USA

Abstract:
Maintenance of human islet viability and function ex vivo can be a significant bottleneck for both regenerative medicine and tissue engineering applications. A strategy that seeks to combine the biophysical and mechanical properties of inert encapsulation materials like alginate with the periislet niche provided by extracellular matrix (ECM)-derived biomaterials, could provide a physiomimetic pancreatic microenvironment for maintaining long-term islet viability and function in culture. We have recently developed an innovative, detergent-free, DI water-based protocol for the decellularization of human pancreas and production of human pancreatic decellularized (dECM) as well as solubilized (dsECM) extracellular matrix. Herein, primary human islets were embedded in alginate capsules supplemented with either dECM or dsECM and cultured for 58 days. Incorporating dsECM (0.1 mg/ml) within alginate microcapsules provided biochemical cues essential for maintaining long-term islet morphology, viability and function. Functional results indicated a significant increase in Glucose Stimulation Index (GSI) and total secreted insulin in islets encapsulated in dsECM-alginate capsules, compared to control groups - free islets and islets encapsulated in only alginate, starting on day 33 of culture. Moreover, islets in dsECM-alginate capsules maintained GSI levels similar to that observed in free islets at the first time point. At early time points in culture, the dsECM stimulated gene expression changes through ECM- and adhesion-mediated pathways, while it demonstrated a mito-protective effect in the long-term. It can be concluded that the addition of human pancreatic dsECM to alginate capsules preserves the ability of human islets to produce insulin in a glucose-responsive manner over long-term culture.
Poster 7:

**Ethnic Difference in Systemic Inflammation, A1C and Cognition.**

Cassandra M. Germain

North Carolina A&T State University

**Abstract:**
Clinical and animal studies have provided some evidence that obesity-related peripheral inflammation is associated with insulin resistance and T2D. Emerging evidence also suggests that inflammatory pathways are similarly activated in diabetes and AD. Thus, it is plausible that obesity-related systemic inflammation may serve as a mechanism for cognitive decline, and subsequent Alzheimer’s disease among the chronically obese and those with T2D. Data from the 2006 wave of the population-based Health and Retirement Study will was used for this analysis. We examined race/ethnic differences in diabetes status (A1C) and CRP levels in N= 5,226 adults aged 50+ (M=66.8. SD=10.7). Associations between systemic inflammation (CRP), A1C and cognitive status were also examined. Preliminary results indicate that non-Hispanic Blacks and those who identified as non-White Other had significantly higher A1C levels than Whites (p <.001). Non-Hispanic Blacks also had significantly higher CRP levels than Whites (p <.001), but not Other race/ethnicities (p=.662); nor were there significant differences in CRP levels between non-Hispanic Whites and Others (p=.447). The association between systemic inflammation and risk for cognitive decline (prodromal Alzheimer’s Disease) will be discussed.
Analysis of gene expression in diabetic foot ulcers by spatial transcriptomics.

Jeannie Chan, Lucian Vlad, Ge Li, Elizabeth Forbes, Tammy Sexton, Wencheng Li, Joseph Molnar, Laura Cox.

Wake Forest University School of Medicine

Abstract:
The purpose of this project was to identify gene expression signatures in diabetic foot ulcers (DFU) that could predict the healing outcome of treatment. Tissue samples were collected from patients (n=6) at their first and second debridements. Spatial RNA-Seq enables us to study gene expression in the context of its cellular location within tissues. We used this technology to profile the transcriptomes of DFU for identification of genes that were differentially expressed after two weeks of treatment and to relate the findings to the healed/non-healed status of DFU at week 12. We were particularly interested in studying gene expression in the basal layer of the epidermis, where new epithelial cells are regenerated for wound closure. We observed a vast majority of the differentially expressed genes were up-regulated in healed DFU, but were down-regulated in non-healed DFU. Signaling pathways involving integrins, chemokines, IL-17, IGF-1 and N-formyl-Met-Leu-Phe in neutrophils were not only significantly enriched with differentially expressed genes in healed DFU, but the pathways were also predicted to be activated based on gene expression patterns and were consistent with wound healing. The HMGB1 gene, encoding a non-histone protein that also functions as a cytokine in injured tissues, appeared to play a key role in coordinating wound healing and may have potential as a biomarker for predicting the healing status of DFU. This study demonstrated the power of spatial transcriptomics for quantification of gene expression by cell type most relevant to healing, which is not feasible with standard transcriptome analysis tools.
Poster 9:

Metabolomic analyses identifies insulin resistance-associated plasma metabolites in African Americans

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Abstract:
Metabolic changes initiated by obesity and insulin resistance (IR) are risk factors for type 2 diabetes (T2D), a disease diagnosed by increased plasma glucose levels. Analyses of plasma metabolite levels has facilitated the understanding of biochemical mechanisms of obesity and IR, but such studies in non-European ancestry cohorts remain limited. In this study we integrated measures of insulin sensitivity (Matsuda index from OGTT, and Si from FSIGT) and obesity (BMI), with fasting plasma metabolite profiles (liquid chromatography-mass spectrometry, Metabolon) in 253 non-diabetic African Americans (AA) from AAGMEx cohort.

Among the 1124 plasma metabolites evaluated in AAGMEx participants, 166, 153 and 60 were significantly associated (FDR-P<0.05) with BMI, Matsuda index and Si, respectively. Lipid metabolites 1-oleoyl-GPC and cortolone glucuronide and amino acids hypotaurine and glycine were among the top ranked BMI and IR-associated metabolites in AAGMEx. Analyses were similarly undertaken in AAs from the IRASFS cohort (N~565) which replicated directionally consistent significant association of 100 and 36 metabolites for BMI and Si, respectively. Glycine was positively associated, while palmitoyl-linoleoyl-glycerol (a Diacylglycerol) was inversely associated with Si in both cohorts. GWAS of 218 gluco-metabolic trait-associated metabolites identified SNPs in genetic loci putatively regulating plasma levels of a subset of these metabolites in AAGMEx.

In summary, this study identifies obesity and IR-associated metabolites in African Americans, and also suggests a role for genetic variants in determining the plasma level of at least a subset of these metabolites.
A novel approach to isolate and characterize adipose tissue-derived extracellular vesicles from blood

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Abstract:
Obesity is associated with an increased risk for multiple metabolic disorders and diseases. Therefore, numerous studies have focused on understanding the role of adipose tissue (AT) in obesity-related comorbidities. However, currently anthropometric and imaging approaches are mostly used to assess obesity, and there is a dearth of techniques to assess the changes in AT at the molecular level, especially in visceral AT (VAT). Lately, extracellular vesicles (EVs) have offered a novel opportunity to characterize hard-to-access tissues/organs in a less invasive manner. In fact, the isolation of cell-specific EVs from biofluids is emerging as 'liquid biopsies' providing valuable molecular information. In this study, we isolated and characterized small EVs (sEV) from the subcutaneous AT (SAT) and VAT of lean and diet-induced obese (DIO) mice. We identified several unique proteins present on the surface of sEV by mass spectrometry (MS), and subsequently validated those by immunogold labeling-transmission electron microscopy and flow cytometry. Based upon these studies, we developed a signature of 5 unique proteins present on the surface of sEV secreted by AT. Next, using these unique signatures, we pulled out AT specific sEV from the blood of mice using biotin-tagged antibodies and streptavidin coated beads. Next, we validated the AT specific sEV for adiponectin level, adipokine array and a panel of AT specific miRNAs. Altogether, we have identified novel surface markers to isolate AT-derived sEV from blood, which could provide relevant molecular information related to various obesity-associated diseases.
Effect of Sampling Location and Diabetes on Transport Proteins in Human Placenta Measured by Quantitative Proteomic Analysis


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Abstract:
Gestational diabetes mellitus (GDM) is associated with adverse outcomes, such as stillbirth, large-for-gestational age infants, and neonatal hypoglycemia. Key components that maintain the placental barrier function include transport proteins (e.g., multidrug resistance protein, MDR; breast cancer resistance protein, BCRP; organic anion transporting polypeptide, OATP; organic cation transporter, OCT; glucose transporter, GLUT). Studies have shown that blood flow across the placenta is not uniform and is reduced in people with diabetes. However, the effects of sampling location and diabetes on placental transport protein levels are not well characterized. Therefore, the aim of this study was to extract membrane protein from three regions (peripheral, P; intermediate, I; medial, M) of healthy (n=10) and GDM (n=4) placentas by ultracentrifugation and to quantify the concentrations of MDR1, BCRP, OATP2B1, OCT3, and GLUT-1 by nanoLC-MS/MS-based quantitative targeted absolute proteomic analysis. Region and diabetes effects were estimated with a two-way repeated-measures ANOVA model. Protein levels among the three regions were similar (all within 20% of the overall mean) for all five placental transport proteins for healthy controls and also for GDM. Relative to controls, placental transport protein levels for GDM were 1.55-fold higher for OATP2B1 and 1.71-fold higher for OCT3. The mean±SD for GDM vs. controls were, respectively: OATP2B1 (0.76±0.22 vs. 0.49±0.20); OCT3 (1.88±0.87 vs. 1.10±0.90); MDR1 (1.94±0.62 vs. 1.68±0.88); BCRP (0.86±0.43 vs. 0.76±0.25); GLUT-1 (258±46 vs. 289±82). These results provide critical information on sampling location and on changes in placental transport proteins that could impact drug safety and efficacy in pregnant people with diabetes.

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Feeding the Diabetic Brain: Metabolic Risk for Alzheimer's Disease in Diabetic Non-Human Primates

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Abstract:
The Alzheimer’s Association projects Alzheimer’s Disease (AD) to more than double by 2050, fueled by the parallel increases in older people and prevalence of Type 2 Diabetes (T2D). We observed cerebrospinal fluid (CSF) metabolite shifts to lower amino acids and ketones as glucose increases in nonhuman primates (NHPs). We hypothesized that T2D augments brain reliance on biochemical alternatives to glucose for metabolism and exacerbation of high levels of CSF glucose relates to AD in rodent models. Ketogenic diets match the fuel shift observed in T2D NHPs. We aimed to evaluate brain fuel uptake in T2D NHPs by positron emission scanning (PET) and evaluated outcomes of a T2D NHP with chronic consumption of ketogenic diet.

We evaluated 6 T2D NHPs for glucose and ketone uptake by PET and a pair (control and ketogenic diet) for 2 months. CSF metabolomics profiles were evaluated in all NHPs.

Urine and blood ketones were highly related as expected (r=0.89, p=0.02). In T2D NHPs, there’s a negative correlation between glycemic control (A1c%) and brain glucose uptake (r=0.82, p=0.04), while ketone uptake, acetoacetate, correlated positively with glucose uptake. During ketogenic diet, CSF ketone levels were increased while glucose and acetoacetate uptake in the brain were both decreased. Worsening ketosis correlated with worsening brain activity by FDG (r=0.71, p=0.11). CSF ketones increase with measures of peripheral ketosis, as expected. Diminished uptake of glucose and acetoacetate during ketosis is concerning for global hypometabolism. Further hypometabolism with the ketogenic diet suggests the brain is inflexible with regards to fuel substrate.
FREDRICKSON LIPID CONFERENCE
POSTER ABSTRACTS
High Plasma Lp(a) Levels associate with a specific transcriptomic signature in monocytes in African Americans but not in Whites

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Post Doc

Genome-wide association, epidemiological, and clinical studies have established high Lipoprotein(a) [Lp(a)] as a causal risk factor for atherosclerotic cardiovascular disease (ASCVD). Lp(a) levels and isoforms vary by race with markedly higher levels in African Americans than Whites. Lp(a) is an apoB100-containing lipoprotein covalently bound to apolipoprotein(a) [apo(a)]. High plasma levels of Lp(a) are linked to atherosclerosis, thrombosis, and arterial calcification. Its link to CVD may also be regulated by pro-inflammatory effects. A recent study utilizing bulk RNA sequencing of circulating CD14+ classical monocytes identified a distinct pro-inflammatory gene expression profile in individuals with high Lp(a), suggesting a potential connection between high Lp(a) and inflammation, leading to increased ASCVD. However, data are lacking on monocyte distinct subpopulations and on diverse populations. We performed scRNA-seq on circulating monocytes from 24 individuals, 12 African Americans (6 males and 6 females 30-47 years old) individuals – six with high (188.1±75.03 nmol/L) and six with low plasma Lp(a) (19.44±12.56 nmol/L) and 12 white individuals (6 males and 6 females 31-73 years old) – six with high (125.62±47.62 nmol/L) and six with low plasma Lp(a) (14.6±9.8 nmol/L). Subjects were not on lipid-lowering therapies and were otherwise considered healthy volunteers. Our analysis identified six major monocyte subpopulations: three classical, one MHCIIhi, one interferon (IFN)-responsive, and one non-classical. We performed differential expression (DE) analysis for each subpopulation comparing Lp(a) high vs. Lp(a) low subjects for each racial group, followed by Canonical Pathway analysis using Ingenuity Pathway Analysis (IPA) to identify the enriched pathways in the DE genes. In African American subjects, “Th1 pathway” was enriched and predicted to be activated in Lp(a) high subjects in the non-classical, MHCIIhi, and IFN-responsive subpopulations, which was driven by the higher expression of number of HLA genes. In the MHCIIhi subpopulation, “interferon signaling” was enriched and predicted to be activated in African Americans with high Lp(a) high. In contrast, these same pathways were not activated in white individuals with high Lp(a). These findings suggest that high Lp(a) may drive a specific transcriptomic signature in specific monocyte subpopulations and functions and that this might vary by sociodemographic or genetic factors associated with race and CVD risk. Confirmatory and larger studies are required to replicate and extend these finding and define potential mechanisms.
Poster 13:

Role of cholesterol-induced suppression of endothelial Kir channels in CVD: from molecular dynamic simulations of cholesterol-Kir interactions to improving endothelial function in murine and human arteries

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Post Doc

Plasma hypercholesterolemia is one of the main risk factors for the development of cardiovascular disease (CVD). A key early stage in CVD is endothelial dysfunction. Our studies show that a major role in hypercholesterolemia-induced endothelial injury can be attributed to cholesterol-induced suppression of endothelial inwardly-rectifying K⁺ channels (Kir2.1), a major endothelial ion channel. Our earlier studies showed that elevation of cellular cholesterol in vitro and exposure to plasma dyslipidemia in vivo suppresses Kir channels in aortic endothelial cells in several animal models of hypercholesterolemia, including mice and pigs. Then, using a combination of modeling, site-directed mutagenesis and biophysical approaches, we established that cholesterol suppresses Kir channels by direct binding of an ensemble of cholesterol molecules to hydrophobic pockets in between the transmembrane domains of the channel protein. Furthermore, we identified several single-point mutations that render Kir channels insensitive to cholesterol whereas not interfering with the normal activity of these channels. More recently, we determined that endothelial Kir channels are essential for flow-induced activation of eNOS, and for flow-induced vasorelaxation (FIV), and that cholesterol-induced suppression of endothelial Kir channels is a driver of impairment of arteriolar flow-induced vasodilation in hypercholesterolemic ApoE⁻/⁻ mice and in hypercholesterolemic humans. Rendering the channels to be cholesterol-insensitive by introducing a mutation identified in our structural studies of Kir-cholesterol interactions prevents hypercholesterolemia-induced reduction of FIV and alleviates atherosclerosis burden.
Post Doc

**Abstract:**
Lipoprotein lipase (LPL) is the enzyme responsible for hydrolyzing triglycerides from lipoproteins located in the capillaries to release free fatty acids. Recent structural work has shown that LPL’s oligomeric state can vary based on its location. When stored inside of vesicles, LPL adopts an inactive helical oligomer. However, when bound to partner protein glycosylphosphatidylinositol anchored high density binding protein 1 (GPIHBP1) in the capillary, LPL is an active monomer. Previous work has also indicated that LPL can be active as a dimer, suggesting another oligomeric state of LPL could exist. Using cryogenic electron microscopy (cryoEM), we have resolved the structure of an active LPL dimer to 3.9 Å resolution. The structure of dimeric LPL reveals an unexpected dimerization interface that bears significant overlap with the GPIHBP1/LPL interface. These data suggest that LPL in complex with GPIHBP1 would be unable to form an LPL homodimer. Analysis of the active site pore reveals a shift in conformation to create a channel for hydrolysis of triglycerides. We have crosslinked LPL to unambiguously show the formation of dimers by both western blotting and mass photometry. Based on this novel structure, we hypothesize that LPL may dimerize when associated with free-floating lipoproteins in the capillary following dissociation from GPIHBP1.
Modulation of the PKM2/Beta Catenin Axis Alters White Adipocyte Differentiation and Promotes a Thermogenic Program

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The obesity epidemic continues to be a growing concern as it closely parallels with the trending rise of associated co-morbidities including cardiovascular disease, type 2 diabetes, and other metabolic disorders. Furthermore, this epidemic has harnessed a wide interest to investigate the variable dynamics of adipose tissue and its function. Although pharmaceuticals targeting weight loss and obesity are currently available, safety concern and off-target side effects propel the need for novel prevention and treatment options. In the last decade, development and regulation of brown adipose tissue (BAT) has become an attractive therapeutic strategy to promote weight loss and improve metabolic homeostasis. Additionally, our recent studies have identified the glycolytic enzyme, pyruvate kinase M2 (PKM2), as a novel regulator of brown adipogenesis and the browning of white adipose tissue (WAT). However, the molecular mechanisms remain to be elucidated. The current study investigates the role of β-catenin and its downstream targets in mediating PKM2’s role in the browning of WAT. We demonstrate that the expression of PKM2, β-catenin, and their downstream targets increase in the adipose tissue of diet-induced obese mice. Similar findings were observed in the adipose tissue of adult humans with overweight or obesity. Using shRNA mediated lentiviral gene editing technique, we demonstrate that PKM2 deficiency promotes a thermogenic program characterized by the browning of white primary pre-adipocytes. Our mechanistic studies also indicate that the effects of PKM2 deficiency on thermogenesis were mediated, at least in part, through the modulation of β-catenin signaling pathway. Notably, the disruption of the β-catenin signaling pathway abolished the effects of PKM2 deficiency on the browning of white adipocyte. While these findings are still preliminary, our study suggests that targeting the PKM2/β-catenin axis could constitute a strategy to prevent excess fat accumulation, enhance whole body energy expenditure, and potentially, improve glycemic control.
Our previous studies demonstrated that cholesterol suppresses inwardly-rectifying K+ channels (Kir2.1) in endothelial cells \textit{in vitro} and \textit{in vivo}. Additionally, we have recently established that a global deficiency of Kir2.1 leads to exacerbated lesion formation in ApoE\textsuperscript{-/-} mice, particularly in the descending aorta that is relatively atheroresistant. Here, we investigate whether the cholesterol sensitivity of Kir2.1 channels play a role in plaque development in hypercholesterolemic mice. In this study we developed a new CRISPR mouse model, originated from our molecular dynamics research for the Kir2.1 channel. This model Kir2.1\textsuperscript{L222I}, has a point mutation (L222I) that renders the channel cholesterol insensitive. These mice were then crossed with dyslipidemic ApoE\textsuperscript{-/-} mice resulting in ApoE\textsuperscript{-/-}/Kir2.1\textsuperscript{L222I} mice. We evaluated Kir2.1 channel function with patch clamp. We then placed these mice on a high fat (42% kcal from fat) diet for 12 -14 weeks and measured atherosclerotic lesions in cross sections of carotid arteries and \textit{en face} aortas stained with Oil Red O. ApoE\textsuperscript{-/-} mice were used as controls. The lack of cholesterol sensitivity of Kir2.1\textsuperscript{L222I} was functionally confirmed in endothelial cells freshly isolated from mesenteric arteries of ApoE\textsuperscript{-/-}/Kir2.1\textsuperscript{L222I} mice. We found that ApoE\textsuperscript{-/-}/Kir2.1\textsuperscript{L222I} male mice (n=8) have significant reduction in the atherosclerotic lesions in their carotid arteries, as compared to ApoE\textsuperscript{-/-} mice (n=8). Specifically, we observed a decrease of 69.2\% in plaque size in ApoE\textsuperscript{-/-}/Kir2.1\textsuperscript{L222I} mice when compared to the control ApoE\textsuperscript{-/-} (413.5µm\textsuperscript{3} and 1353.4µm\textsuperscript{3}, respectively). This phenomenon was also observed both in the aortic arch and in the descending aorta. Measurement of maximum stenosis in carotid arteries also revealed the atheroprotective effect in the cholesterol-insensitive animals, where the reduction in stenosis reaches nearly 30\%. Interestingly, in carotid arteries, these effects were restricted only to male mice as no differences in plaque size nor stenosis were found between ApoE\textsuperscript{-/-}/Kir2.1\textsuperscript{L222I} and ApoE\textsuperscript{-/-} females (n=5). When investigating sex differences, ApoE\textsuperscript{-/-}/Kir2.1\textsuperscript{L222I} male mice display a significant reduction in maximum stenosis when compared to their female counterparts. It is important to note that no differences were found in the levels of LDL, HDL and total cholesterol in any of these mice. Our data suggest that cholesterol-induced suppression of Kir2.1 is an important contributor to the development of atherosclerosis.
As the prevalence of obesity continues to grow in the U.S., adult obesity and its comorbidities are now a major health threat to our population. Obesity is caused by the chronic imbalance between energy intake and energy expenditure resulting in adipose tissue expansion which can occur through adipocyte hypertrophy and/or hyperplasia (i.e., adult adipogenesis). Although both genetics and environment contribute to obesity, the genetic basis remains poorly understood. Data from our human cohort, an outbred rat model, and diet-induced obese mice demonstrated a positive correlation between the gene expression of G protein-coupled receptor (GPCR) kinase 5 (GRK5) in white adipose tissue and adiposity. Wang et al. has reported that whole body GRK5 knockout (KO) mice exhibited decreased adipogenesis, and protection from diet-induced obesity. However, the mechanism by which GRK5 regulates adipogenesis is unknown. To fill this knowledge gap, we created a GRK5 KO 3T3-L1 pre-adipocyte cell line. We found that, during adipogenic stimulation, GRK5 KO pre-adipocytes had decreased white adipogenesis and lipid accumulation. Similar results were observed in brown-like adipocyte differentiation. In pre-adipocytes, insulin of the adipogenic cocktail acts through insulin-like growth factor-1 receptor (IGF-1R), a receptor tyrosine kinase (RTK), which can activate two downstream pathways: Extracellular signal-regulated kinase (ERK) and AKT. We found that deletion of GRK5 suppressed insulin stimulated ERK, but not AKT, phosphorylation and activation. These findings indicate that adipogenesis is regulated by GRK5 potentially through insulin/IGF-1R/ERK pathways, suggesting that GRK5 is not only a serine/threonine GPCR kinase, but might also regulate RTK signaling in pre-adipocytes.
Poster 16:

LIPID DROPLET ACCUMULATION REGULATES HUMAN α AND β CELL ACTIVITY

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One of the biggest challenges in studying human islet biology is the limited access to healthy and disease human pancreas samples. Consequently, much of our insight has been obtained in rodent models despite notable morphological, molecular, and physiological differences with human islets. Distinctly, when transplanted in immunodeficient mice, only human islets, and not mouse, have the ability to effectively accumulate lipid droplets (LDs). LD numbers in transplanted human islets increased further when the host mice were raised on a high fat diet relative to regular diet, suggesting the LDs in human islet cells can be dynamically regulated by exposed free fatty acid (FFA) levels. LD depots serve numerous purposes including sequestering excess FFAs for metabolic and/or synthetic use in many cell types, such as hepatocytes and adipocytes. LDs also play a protective role in neurons and cancer cells by reducing oxidative and ER stress-induced inflammatory conditions. Importantly, while LD accumulation is difficult to detect in mouse pancreatic islets, LD buildup is found at roughly 11 years of age in human islet α and β cells, increasing throughout adulthood and is enriched in Type 1 diabetic (T1D) α cells and Type 2 diabetic (T2D) islets. By manipulating the levels of a key LD scaffolding protein, perilipin2 (PLIN2), I have gained mechanistic insight into the role of LDs on β cell function using the cultured EndoCβH2-Cre human β cell line. Reducing PLIN2 levels by knockdown (KD) impairs LD accumulation and produced stress response signatures characteristic of T1D and T2D, such as reduced β cell function, ER stress and loss of β cell identity. In contrast, enhancing LD accumulation by over-expressing (OE) PLIN2 was protective under lipotoxic condition and even enhanced cell activity. More recently, human pseudo islet culture system was applied to determine how PLIN2/LD status impact primary human islet function. Consistent with the human cell line, PLIN2KD compromised insulin secretion in β cell enriched pseudo islets. Interestingly, PLIN2KD in α cell enriched pseudo islet showed improved glucagon secretion. To gain mechanistic insight of this islet cell type-specific effect, single-cell transcriptomic studies in PLIN2KD/OE pseudo islets are currently under investigation. Collectively, these results strongly suggest that LDs are essential for adult human islet α and β cell activity. This study should provide molecular basis of how LD homeostasis regulates human islet function and health.

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Loss of the murine ALX/FPR2 receptor dysregulates the concentration of pulmonary oxylipins of inflammation initiation and resolution

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Graduate Student

The G-protein coupled receptor lipoxin A4/formyl peptide receptor type 2 (ALX/FPR2) controls the resolution of inflammation; however, little is known about its role in lung inflammation, which is timely given the current COVID-19 pandemic. Herein, we tested the hypothesis that the loss of ALX/FPR2 plays a role in controlling pulmonary inflammation. To test the hypothesis, we studied a newly generated ALX/FPR2 knockout mouse model relative to wild-type littermates. Initial metabolic characterization of the ALX/FPR2-/- mice, compared to controls, revealed an impairment in glucose tolerance and dysregulation of glucose and lipid oxidation, as measured with indirect calorimetry. Prior to lung injury, targeted lipidomic analysis using mass spectrometry revealed an increase in the concentration of polyunsaturated fatty acid-derived oxylipins, which regulate the initiation and resolution of inflammation. Subsequently, we employed a lipopolysaccharide-induced acute lung injury (ALI) model to assess inflammation and injury in bronchoalveolar lavage fluid (BALF). The concentration of total protein, levels of cytokines (IL-6, TNF-α, and IL-1β), and immune cell differentials in BALF were the same between wild-type and ALX/FPR2-/- mice at 24 and 48 hours of ALI. However, at 24 hours of ALI, ALX/FPR2-/- mice had a 6-fold and 4.5-fold respective increase in pulmonary gene expression of ccl2 and ccl3, which control recruitment and activation of immune cells during inflammation. Collectively, the data reveal an important role for the ALX/FPR2 receptor in controlling glucose metabolism, pulmonary oxylipins, and ccl2 and ccl3 gene expression, which warrants further investigation.
**Poster 18: Selected Abstract Presentation**

**Association between cholesterol efflux capacity and HDL-sized and whole plasma proteins in the HERITAGE Family Study**

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**Graduate Student**

**Background.** HDL particles contain numerous proteins that influence their function. Previous studies of proteomic determinants of cholesterol efflux capacity (CEC) mostly included patients with cardiometabolic disease.

**Purpose.** The purpose of this analysis was to examine the relationship between the abundance of HDL-sized and whole plasma proteins with CEC in a cohort of healthy adults.

**Methods.** We examined the HDL-sized (n=139) and circulating plasma proteomes (n=537) in Black (36%) and White men and women (56%) from the HERITAGE Family Study. The HDL-sized fraction was isolated from plasma via gel filtration chromatography and untargeted MS analysis was performed via nano-HPLC-MS/MS. The whole plasma proteome was measured using a modified aptamer (SOMAscan) assay. CEC was measured using J774 cells, radiolabeled cholesterol, and apoB-depleted plasma. Associations between CEC and 144 HDL-associated proteins and 4979 plasma proteins were examined using mixed linear models and FDR<0.05 was used to determine significance. Additionally, LASSO regression models were performed on nominally significant proteins to identify separate HDL-sized and whole plasma protein signatures of CEC.

**Results.** The abundance of 5 HDL-sized proteins (HRG, SEPP1, KV309, APOA2, TTHY) were significantly associated with CEC, with negative associations for all but TTHY. In whole plasma, 17 proteins were significantly associated with CEC, including APOC3, APOM, Cystatin C, and two interleukins ([Figure 1](#)). Abundance of 27/88 HDL-sized plasma proteins and 150/554 whole plasma proteins entered in separate LASSO regression models were retained in each protein signature of CEC. A few proteins retained in the HDL-sized signature include APOA1, APOC3, RET4, LCAT, and PLMN in the positive direction, and SAA4, HRG, and APOA2 in the negative direction. Proteins retained in the whole plasma signature include APOC1, SAA2, APOC3, APOM, and APOC2.

**Discussion.** The abundance of several proteins measured in HDL-sized and whole plasma were associated with levels of CEC. Further analyses are needed to determine whether HDL-sized or whole plasma proteins improve the prediction of CEC as compared to clinical measures and/or other features of HDL particles.
Figure 1. Volcano plot of the association of plasma proteins with cholesterol efflux capacity (CEC). (A) Association of HDL-sized plasma proteins with CEC. Models tested 144 proteins and adjusted for age, sex, ethnicity, and family membership. (B) Association of whole plasma proteins with CEC. Models tested 4979 aptamers and adjusted for age, sex, ethnicity, and family membership. FDR<0.05 shown in red, p<0.05 in blue, and p>0.05 in gray.
Heart disease continues to be an issue affecting many Americans today. There is a well-established relationship between plasma lipoprotein profiles and the risk of cardiovascular disease (CVD). Elevated levels of lipoproteins rich in triglycerides, including low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL), in the plasma, are associated with an increased risk of CVD. Conversely, circulating levels of high-density lipoprotein cholesterol (HDL-C) are inversely associated with atherosclerotic cardiovascular disease. Endothelial Lipase (EL, encoded by the LIPG gene) is a phospholipase A1 enzyme that plays a significant role in human cardiovascular health by controlling the level of circulating lipoprotein particles. EL directly remodels HDL particles and can mediate the efficient lipolysis of triglyceride-rich particles with its homolog lipoprotein lipase (LPL) in a synergistic manner. However, we do not fully understand the enzymatic activity of the purified protein on lipoprotein substrates, nor do we understand the mechanism by which LPL and EL mediate synergy when lipolyzing triglyceride-rich particles. Furthermore, EL is known to be inhibited by Angiopoietin-like 3 (ANGPTL3) to limit its hydrolysis of lipoprotein particles, but there is a critical need for biochemical and biophysical studies to elucidate this mechanism of interaction.

In this study, we will analyze the enzymatic activity of purified EL on different lipoprotein substrates and examine its relationship with LPL on the hydrolysis of these particles. Furthermore, we will explore the inhibition mechanism between EL and ANGPTL3 and explore whether ANGPTL3 can effectively inhibit both EL and LPL. Furthermore, we will explore the biophysical characteristics of the complex formed between EL and ANGPTL3. We will perform this study using advanced biophysical techniques like mass photometry, to explore the oligomeric nature of the complex, and Cryo-EM, to explore the quaternary structure of the complex. These aims will expand our view on how lipases work together to metabolize lipoprotein particles to reduce the burden of heart disease by providing critically needed biochemical and biophysical data of EL and LPL's activity on these particles. Lastly, it will elucidate the potential biological role of ANGPTL3 as an inhibitor of both EL and LPL.
Poster 20:

Sex-Specific Genetic Regulation of Adipose Mitochondria: Relationship to Metabolic Syndrome

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**Background and Objectives:** Mitochondria plays a major role in the pathophysiology of complex metabolic traits such as obesity, insulin resistance and fatty liver disease. However, the exact causal relationship between mitochondrial function and these traits is not completely understood. We have previously suggested a central role for mitochondria in the observed sex differences in metabolic traits. However, the mechanisms by which sex differences affect adipose mitochondrial function and metabolic syndrome are unclear. To understand the nature of the sex differences and causal relationships, we examined genetic factors contributing to mitochondrial function using a mouse reference population that were extensively phenotyped called hybrid mouse diversity panel. We have also used two different human cohorts namely STARNET (Swedish cohort) and METSIM (Finnish cohort) for testing human relevance.

**Results:** Here we show that in both mice and humans, adipose mitochondrial functions are elevated in females and are strongly associated with adiposity, insulin resistance and plasma lipids. Using a panel of diverse inbred strains of mice, we identified a genetic locus on mouse chromosome 17 that controls mitochondria levels and function in adipose tissue in a sex- and tissue-specific manner. It regulates the expression of at least 89 (10%) mitochondrial genes, many of them related to oxidative phosphorylation, as well as mitochondrial DNA levels, in female but not male mice. Overexpression studies indicate that the effects of the locus are mediated by the Ndufv2 gene that elevates mitochondrial ROS production, which generates a signal to increase mitochondrial biogenesis. The gene is activated by gonadal hormones and is regulated in cis only in females.

**Conclusion:** We report that adipose mitochondria are regulated by both genetic variation and sex hormones and that high levels are an important determinant of metabolic syndrome traits.
Microbiota suppression of the transcription factor HNF4A in the small intestinal epithelium is linked to altered protein-protein interactions

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Abstract
Over 40% of Americans are obese, leading to increasing incidences of heart disease, stroke, type 2 diabetes, and certain kinds of cancer. Obesity is caused by the imbalance between energy output and intake, but genetics and environmental factors significantly influence this process. Gut microbiota play a role in obesity as microbiota derived from humans and mice with obesity conveys the obesity phenotype to germ-free mice upon fecal transplantation, and germ-free mice are resistant to diet-induced obesity. However, the host transcriptional regulatory mechanisms underlying these microbiota-dependent adaptations remain unclear. Hepatocyte nuclear factor 4 alpha (HNF4A) is a nuclear receptor transcription factor expressed in intestinal epithelial cells (IECs) that regulates intestinal homeostasis and lipid metabolism, as deletion of intestinal HNF4A decreases lipid absorption and transcription of lipid metabolism genes. We recently discovered that microbiota colonization of the zebrafish and mouse gut leads to suppression of HNF4A-activated genes and genome-wide reduction of HNF4A DNA occupancy in IECs, respectively. This suggests microbiota suppress HNF4A to potentially alter intestinal lipid metabolic processes; however, we do not know the mechanism by which HNF4A is suppressed by microbiota. To explore potential routes of microbial suppression of HNF4A, we conducted an unbiased coimmunoprecipitation-mass spectrometry analysis comparing HNF4A interacting partners in IECs from germ-free and conventionalized (germ-free mice colonized with feces from SPF mice for 2 weeks) mice. We identified many previously reported interacting partners in both conditions, such as HNF4G, SMAD4, FOXO1, TBP, and TFIID components, establishing the validity of our methods. There were significantly more enriched and unique HNF4A interacting partners in germ-free mice, including proteins involved in transcription initiation (TAF8, CHD7, NR1D2, and TCF7L2) and mRNA processing (TOE1, TENT2, and APOBEC1), consistent with HNF4A being more transcriptionally active in germ-free mice. In conventionalized mice, HNF4A uniquely interacted with PRKACA, the catalytic subunit of PKA, and IKBKE, a non-canonical activator of NF-κB signaling. PKA is a cAMP activated kinase known to suppress HNF4A through phosphorylating HNF4A. IKBKE has never been reported to interact with HNF4A, but its activity is stimulated by viral and microbial metabolites, positioning it as a potential mediator of microbial signals. Our continuing work aims to determine if microbiota stimulate PKA and/or IKBKE to directly suppress HNF4A activity, and the microbial species/metabolites that activate these pathways. Identification of these interactions and pathways may provide new targets for regulating intestinal metabolism, host-microbiota interactions, energy balance, and obesity.
THE EFFECT OF HYDROGEL MATERIALS ON LIPID METABOLIC CAPACITY OF THREE-DIMENSIONAL PRIMARY HUMAN HEPATOCYTE CULTURES

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INTRODUCTION
The extracellular matrix (ECM) of the liver is a key component of the organ’s function. Its composition and mechanical properties have been shown to influence cell-matrix interactions and hepatocyte viability and functionality in both in vitro and in vivo settings. In three-dimensional (3-D) hepatic cultures, primary human hepatocytes (PHH) replicate in vivo-like drug metabolic functionality, but there is limited knowledge about their fatty acid and lipid metabolic functionality. In this study, we aimed to evaluate the impact of the ECM on lipid metabolism within a 3-D hepatic culture model. We hypothesized that hydrogel materials, representing the ECM, would significantly affect de novo lipogenesis (DNL) and lipid esterification (LE).

METHODOLOGY
PHHs (200,000 cells/construct, Lonza) were cultured in three commonly used hydrogels: (1) GelMa (gelatin-based) with RGD peptides and crosslinked with Lithium Phenyl-2,4,6-Trimethylbenzoylphosphinate (GelMa+RGD), (2) HyStem (hyaluronic acid, gelatin, and polyethylene glycol) with thiolated fibronectin and crosslinked with Irgacure (HyStem+Fib), and (3) maleimide-modified hyaluronic acid (malHA) with RGD and crosslinked with 5 kDa dithiol polyethylene glycol (malHA+RGD). PHHs were maintained for a week. Viability was assessed via ATP quantification and LIVE/DEAD imaging with calcein and ethidium homodimer. PHH constructs were exposed to lipoprotein deficient media overnight, then exposed to [3H]-oleate (for LE quantification) and [14C]-acetate (for DNL assessment) at doses of 1 and 10 μCi respectively for 90 minutes. Lipids were extracted using 3:2 hexane:isopropanol and sorted into phospholipid (PL), triglyceride (TG), free cholesterol (FC) and cholesteryl ester (CE) fractions using thin-layer chromatography (TLC). A scintillation counter was used to quantify the presence of isotopes in each species, and counts were normalized to total DNA. Relevant lipid metabolic gene expressions—including FASN, ACC1, DGAT2, and Lipin2—were quantified through qPCR. ANOVA was used to assess differences in hepatic function between hydrogel material groups.

RESULTS
PHH viability at day 6 was significantly higher in GelMa+RGD (p=0.018) and malHA+RGD (p=0.0015) constructs compared to Hystem+Fib, as indicated by higher ATP, albumin, and urea levels. However, no difference in viability was observed between PHHs culture in GelMA+RGD or malHA+RGD. PHHs cultured in GelMa+RGD and malHA+RGD appeared to form larger clusters on day 6, compared to those in HyStem+Fib. Hydrogel material had a significant effect on both DNL (p<0.0001) (Fig. 1a) and LE (p<0.0001) (Fig. 1b). PHHs cultured in malHA+RGD hydrogels produced significantly higher levels of PL...
and TG compared to both GelMa+RGD and HyStem+Fib. Expression levels of FASN and DGAT2 tended to be highest in malHA+RGD constructs, though not statistically significant.

**CONCLUSIONS**

We successfully demonstrated that PHHs cultured in 3D hydrogels were capable of DNL and LE within 1 week of culture. The hydrogel material significantly impacted lipid metabolic functionality, with malHA+RGD hydrogels exhibiting the best environment for lipid metabolism. This increase in functional metabolic capacity was not mirrored in other hepatic functional metrics such as urea or albumin production. Interestingly, malHA+RGD and GelMa+RGD hydrogels behaved comparably in all other measures. We postulate that this effect of malHA+RGD on lipid metabolism may be due to cell-ECM interactions between the hyaluronic acid and cell surface markers or possibly maleimide signaling. Future studies are needed to explore the mechanisms behind this enhanced lipid metabolic functionality.

**ACKNOWLEDGMENTS**

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![Figure 1](image-url) – malHA+RGD hydrogels display the highest (a) DNL and (b) LE functionality.
**Poster 22:**

**Pyruvate dehydrogenase kinase supports macrophage NLRP3 inflammasome activation during acute inflammation**

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**Summary**
Activating macrophage NLRP3 inflammasome can promote excessive inflammation, leading to severe cell and tissue damage and organ dysfunction. Here, we show that pharmacological or genetic inhibition of mitochondrial pyruvate dehydrogenase kinase (PDHK) significantly attenuates NLRP3 inflammasome activation in murine and human macrophages. PDHK inhibition lowers caspase-1 cleavage and IL-1β secretion in septic mice. PDHK inhibition reverses NLRP3 inflammasome-induced metabolic defect and enhances autophagic flux. Moreover, PDHK inhibition favors mitochondrial fusion over fission, attenuates mitochondrial ROS production, and preserves cristae ultrastructure. Unexpectedly, the suppressive effect of PDHK inhibition on the NLRP3 inflammasome is independent of pyruvate metabolism, pyruvate dehydrogenase, autophagy, or ROS production. In conclusion, our study suggests a non-canonical role for PDHK in supporting mitochondrial dysfunction and NLRP3 inflammasome activation during acute inflammation, independent of its canonical role as a pyruvate dehydrogenase regulator.
Non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2D) commonly exist together. The prevalence of NAFLD is 45-75% in T2D patients. The dynamic association between increased free-fatty acid flux into the liver and hepatic insulin resistance underlies the impaired activation of insulin signaling that defines T2D at cellular level. Neutral sphingomyelinase 2 (nSMase-2) is a membrane-associate enzyme that hydrolyses sphingomyelin to ceramide at the inner leaflet of the plasma membrane and this activity is important for membrane trafficking, receptor clustering and signal transduction. Earlier work in our lab has shown that diet-induced hepatosteatosis is associated with elevated nSMase-2 at transcript, protein and activity level. Moreover, treatment of primary hepatocytes with palmitic acid induces the translocation of the enzyme from the Golgi to the plasma membrane. Thus, we hypothesize that stimulated hepatic nSMase-2 activity contributes to ceramide accumulation leading to hepatic insulin resistance. To investigate this, we generated liver-specific nSMase-2 knockout mouse, termed NASKOh mice (Neutral Sphingomyelinase KnockOut in hepatocytes) using the Cre-LoxP strategy. To recapitulate features of diet-induced hepatosteatosis in a mouse model, we challenged the mice to a 60% fat diet (HFD) for 12 weeks. All mice in HFD, independently of genotype, gained similar weight and adiposity, and developed hepatic steatosis. Induction and accumulation of ceramide species in response to obesity-associated hepatosteatosis were limited to C14:0, C16:0, C18:0, C18:1, C20:0 and C20:1-ceramide, while C24:0 and C24:1 ceramide decreased. Liver nSMase-2 deficiency abrogated the increases in ceramide species, suggesting that nSMase-2 as the main contributor for the accumulation of ceramide in diet-induced steatosis. Glucose homeostasis analysis revealed an improved fasted glucose in lean and obese NASKOh mice, accompanied with lower fasting serum insulin compared to their WT counterparts. Furthermore, whole-body glucose clearance following an insulin injection was enhanced in lean and obese NASKOh mice. To investigate whether ceramide generated by nSMase-2 activity influenced hepatic insulin signaling, insulin was administered intraperitoneally 20 minutes before sacrifice and activation of AKT was determined in liver tissue homogenates. As expected, phosphorylation of AKT was blunted in WT obese mice, but it was enhanced in lean and obese NASKOh mice. In summary, these studies describe nSMase-2 activity as a novel player in hepatic insulin signaling.
The obesity epidemic is a serious global public health crisis that is projected to continue to expand in the coming years. Gaining an understanding of the development and progression of obesity is imperative to combatting its spread. Adenosine, a nucleoside released from cells during metabolic stress or inflammation, may play an important role in this process. Our lab has identified a direct relationship between transcription of adenosine 2B (A2BR) and feeding after overnight fasting. This transition alters the metabolism of adipocytes and, ultimately, the whole organism. There is an increase in A2BR transcription after feeding in both 3T3-L1 adipocytes and mouse adipose tissue, however this effect cannot be recapitulated with simple insulin supplementation. Experiments with 3T3L1 cells demonstrated that charcoal-stripped or trypsin-treated serum fails to induce A2BR transcription. Mechanistically, we have discovered that the pharmacological inhibition of several components in the TLR4-initiated NF-kB pathway halts A2BR transcription, indicating the importance of TLR4. We hypothesize that feeding triggers the release of an unknown lipid or protein into serum which activates the NF-kB pathway via TLR4, and subsequently causes an increase in A2BR transcription in adipocytes. Acute stimulation of A2BR causes glucose excursion and insulin insensitivity, and we have found that obese mice display elevated A2BR levels. These observations suggest that dysregulation of A2BR signaling may be a potential contributor to the development of obesity-induced insulin resistance.
**Poster 25:**

**Carnitine Palmitoyltransferase 1a Modulates Sexual Dimorphic NAFLD in Mice**

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**Graduate Student**

**Background:** Nonalcoholic fatty liver disease (NAFLD) affects almost 1 billion people worldwide and is associated with cardiometabolic risk factors such as obesity and dyslipidemia. While NAFLD is largely considered a sexual dimorphic disease with generally higher prevalence in men, women exhibit a greater risk of progressing to more severe NAFLD. Studies have linked variants and methylation status of carnitine palmitoyltransferase 1a (CPT1a) to changes in body composition and circulating triglyceride levels. Here, we demonstrate that hepatocyte-specific deletion of \(^{Cpt1a}\) in female mice promotes more profound panlobular microvesicular steatosis and associated liver injury than in male mice.

**Methods:** Eight-week old male and female \(^{Cpt1a}\)∆Alb and littermate controls (\(^{Cpt1a}\)F/F) were placed on a low-fat or high-fat diet (HFD; 60% kcal fat) for 15 weeks. Body weights were measured weekly, and body composition was measured by MRI at the beginning and end of the study. Glucose and insulin tolerance tests were completed after 10 and 12 weeks of diet feeding, respectively. Mice were necropsied after a 16 hour fast, and tissues and serum were collected and analyzed for lipidomics, bulk RNA sequencing, histology, and protein expression by immunoblotting.

**Results:** Male and female \(^{Cpt1a}\)∆Alb mice did not exhibit changes in overall body weight or adiposity but displayed improved insulin sensitivity as assessed by HOMA-IR. Upon HFD-feeding, male \(^{Cpt1a}\)∆Alb mice did not exhibit changes in hepatic triglycerides or cholesterol levels as compared to control \(^{Cpt1a}\)F/F mice. However, female \(^{Cpt1a}\)∆Alb mice displayed a significant increase in hepatic triglycerides and cholesterol levels, while female control \(^{Cpt1a}\)F/F mice were largely protected against HFD-induced hepatic lipid accumulation. Histologically, both male and female \(^{Cpt1a}\)∆Alb mice displayed significant, diffuse, panlobular microvesicular steatosis as compared to their respective controls. Bulk RNA-sequencing analysis revealed that both male and female \(^{Cpt1a}\)∆Alb mice increased a selective group of PPARα-target genes that associated with increased lipid droplet storage/fusion (Plin 2, 5; Cidec) and impaired lipid droplet hydrolysis (G0s2). Consistent with gene expression, protein levels of PLIN 2 and 5 were significantly elevated in \(^{Cpt1a}\)∆Alb mice and associated with impaired triglyceride hydrolysis activity in these mice.

**Conclusions:** Liver specific deletion of CPT1a does not influence body weight or adiposity. Both male and female \(^{Cpt1a}\)∆Alb mice exhibit microvesicular steatosis; however, female \(^{Cpt1a}\)∆Alb exhibit exacerbated NAFLD given female \(^{Cpt1a}\)F/F control mice are largely protected from HFD-induced hepatic lipid
accumulation. The upregulation of specific PPARα-target genes yields mechanistic insight into the microsteatosis observed in $Cpt1a^{\Delta\text{Alb}}$ mice.
**Poster 26:**

**Hepatocyte-specific angiotensinogen deficiency prevents Western Diet-induced fatty liver in mice**

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**Graduate Student**

**Background:**
Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease and its onset is characterized by steatosis. We have shown consistently that hepatocyte-specific deficiency of angiotensinogen (AGT), the unique substrate of the renin-angiotensin system, alleviates Western Diet (WD)-induced liver steatosis in mice. However, the mechanism by which AGT contributes to WD-induced fatty liver is unknown.

**Methods and Results:**
We first performed sequential bulk RNA sequencing on mouse livers to determine the impact of WD on the hepatic transcriptome. Low-density lipoprotein receptor (LDLR)⁻/⁻ mice were fed WD (42% of calories from fat) for 5, 14, or 42 days and evaluated against mice fed normal laboratory diet. WD feeding induced many transcriptomic changes in the liver as early as 5 days. Of the 31,116 genes detected, 6,001 were differentially expressed (FDR < 0.05). Most of the most upregulated genes were induced by WD feeding at 5 days and persisted through 14 and 42 days of WD feeding. Gene Ontology (GO) analysis was performed for differentially expressed genes (DGEs) at each interval. Nine of the top 10 upregulated GO pathways are present at all intervals. The top pathway at each interval was positive regulation of cytokine production. All intervals had 2 of the top 10 downregulated GO pathways: steroid and fatty acid metabolic processes. To evaluate the contribution of AGT to WD-induced steatosis, liver transcriptomes from hepatocyte-specific AGT deficient (hepAGT⁻/⁻) mice and their wild type (hepAGT⁺/⁺) littermates were compared after 14 days of WD feeding, an interval that represents the initiation of liver steatosis. All mice were LDLR⁻/⁻ background. There were 132 DGEs between genotypes and GO analysis identified “steroid metabolic process” and “DNA packaging” as the top annotation in up- and downregulated DGEs, respectively.

**Conclusions:**
Angiotensinogen may contribute to WD-induced liver steatosis via downregulation of the steroid metabolic process. Future studies will evaluate the impact of these pathways through *in vivo* experiments.
Poster 27:

Development of NAPE-PLD activators as a potential treatment strategy for treatment of metabolic diseases.


Vanderbilt University and Vanderbilt University Medical Center, Nashville, TN.

Faculty

Our lab seeks to develop effective treatments for metabolic diseases including obesity, type 2 diabetes, non-alcoholic fatty liver disease, and atherosclerosis. Oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) are bioactive lipids that exert pleiotropic protective effects against metabolic diseases. OEA promotes satiety, fatty acid oxidation, and glucose-stimulated insulin secretion. PEA also promotes satiety, exerts significant anti-inflammatory effects including inhibiting mast cell activation, and enhances the efferocytotic capacity of macrophages. We hypothesize that raising peripheral levels of OEA and PEA will reduce obesity and inflammation and enhance resolution and fat oxidation, thereby leading to reduce metabolic disease. OEA and PEA are generated by the hydrolysis of appropriate N-acyl-phosphatidylethanolamines (NAPEs) by NAPE-hydrolyzing phospholipase D (NAPE-PLD). Expression of NAPE-PLD appears to be reduced by high-fat diets and in the development of metabolic disease. We hypothesize that enhancing the remaining NAPE-PLD activity in peripheral tissues using positive allosteric modulators (activators) of NAPE-PLD will be a useful treatment for metabolic diseases. To identify potential NAPE-PLD activators, we screened a chemical library of 40K compounds using a fluorogenic NAPE and recombinant mouse Nape-pld. We identified a potential activator scaffold and performed pilot structure activity relationship (SAR) studies to establish the chemical characteristic needed for efficacy. The most potent activators from these pilot SAR studies, VU533 and VU534, enhanced the activity of recombinant NAPE-PLD 2-fold with a potency of 0.3 μM. HepG2 hepatocytes and RAW264.7 macrophages treated with 10 μM VU533 or VU534 showed significantly enhanced NAPE-PLD activity. Preliminary studies with bone marrow derived macrophages (BMDM) showed that treatment with 10 μM VU533 and VU534 enhanced their efferocytosis capacity, while treatment with a NAPE-PLD inhibitor reduced efferocytosis capacity. Preliminary studies also showed that Nape-pld−/− BMDM had reduced efferocytosis capacity. Future studies are needed to determine the effects of these NAPE-PLD activators in other cellular assays and mouse models of metabolic diseases.
**Poster 28:**

**Effects of Exercise Training on ANGPTL3/8 and 4/8 and their Associations with Lipid and Cardiometabolic Traits**

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**Faculty**

**Background.** Angiopoietin like protein (ANGPTL) complexes 3/8 and 4/8 are established inhibitors of lipoprotein lipase (LPL). Acute exercise induces ANGPTL4, while exercise training increases LPL activity. However, the effects of chronic exercise on ANGPTL 3/8 and 4/8 complexes are unknown.

**Purpose.** The purpose of this study was to examine the effects of exercise training on ANGPTL3/8 and 4/8 levels and the relationship between exercise training-induced changes in the complexes and concomitant changes in lipid and cardiometabolic traits.

**Methods.** Measurements were taken before and after 20 weeks of standardized, endurance exercise training in Black and White adults of the HERITAGE Family Study (n=642). MesoScale Discovery electrochemiluminescence immunoassays were used to measure ANGPTL3/8 and ANGPTL4/8 complexes in serum. For the ANGPTL3/8 assay, the capture antibody recognized ANGPTL8, and the detection antibody recognized ANGPTL3. For the ANGPTL4/8 assay, the capture antibody recognized ANGPTL4, and the detection antibody recognized ANGPTL8.

**Results.** Both ANGPTL 3/8 and 4/8 decreased with exercise training, with only the decrease in ANGPTL 3/8 statistically significant. Significant sex differences in the training response of ANGPTL4/8 were observed, with men showing a significant decrease, while women showed no change. Baseline quintiles of both ANGPTL3/8 and 4/8 showed inverse, linear relationships with their respective exercise-induced changes, with the lowest quintiles (Q1 & 2) experiencing mean increases with training, while the highest quintiles (Q3-Q5) showed progressively larger mean decreases with training (p<0.0001 for trend). In multivariable regression models the strongest predictors of change in ANGPTL3/8 were baseline level (partial R²=0.14) and concomitant changes in total cholesterol, glucose effectiveness, fasting glucose, very large TG-rich lipoproteins, LPL and hepatic lipase activities, and acute insulin response to glucose (model R²=0.25), while the strongest predictors of change in ANGPTL4/8 were baseline level (partial R²=0.155) and concomitant changes in waist circumference (partial R²=0.016) and TG (partial R²=0.012).

**Conclusions.** ANGPTL3/8 and 4/8 both decreased with exercise training, with the largest reductions occurring in individuals that started with the highest levels. ANGPTL3/8 and 4/8 changed concomitantly with many lipid and insulin traits, while change in ANGPTL4/8 was also associated with changes in body...
composition. Our findings reflect the potential of exercise-based treatments for the ANGTP3L3/8 and 4/8 complexes, although further study is needed.
**Poster 29:**

**Shedding light on the dark yolk phenotype: identifying novel regulators of lipoprotein metabolism**

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**Post Doc**

In digestive tissues such as the liver and intestine, lipids can be stored or secreted into circulation for distribution to peripheral tissues. While elevated circulating lipids are a risk factor for atherosclerotic disease, increased lipid storage is also problematic, and underlies fatty liver disease and is a side-effect of lipid-lowering drugs. Lipid fate hinges in the ER where lipid is either packaged into ApoB-containing lipoproteins (B-lps) for secretion or exported from the ER into cytosolic lipid droplets. While B-lp biogenesis plays a key role in governing lipid flux, we have an incomplete understanding of the factors controlling B-lp capacity (size) and number. These knowledge gaps have been difficult to investigate due to the limitations of cell culture models for studying multi-organ phenomena and the relative inaccessibility of mammalian whole-animal models to visualize lipoprotein dynamics at the subcellular level. The zebrafish presents a unique solution to these challenges – in the embryonic and larval stages, the zebrafish robustly produces B-lps using genetically conserved pathways and is optically clear. Moreover, zebrafish with deficiencies in B-lp production display a distinct phenotype - “dark yolk” - due to abnormal lipid accumulation which is easily visualized using low magnification microscopy. We have leveraged this unique and recently appreciated phenotype to identify additional genes involved in lipoprotein processing using a forward genetic screening approach. While screening is ongoing, we have already identified over 20 dark yolk mutants. This screening strategy is robust: of the identified mutants, 4 are at previously identified dark yolk loci. Novel mutants exhibit a range of phenotypes, varying in onset and severity. Secondary screening assays reveal that many of the mutants produce abnormal lipoprotein numbers and sizes. A whole-genome sequencing approach has been developed to efficiently map and identify gene candidates for follow up and has been applied to selected mutants. The unique features of the zebrafish model have allowed this large scale unbiased forward genetic screening approach which will help broaden our understanding of lipoprotein biology by identifying novel genetic regulators.
Characterization of the mammalian Microtubule Associated Serine/Threonine Kinase 2

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Graduate Student

The AGC kinase effectors of the mTOR pathway are not only key intermediates of anabolic signaling, but also represent potential therapeutic targets for cancer and metabolic diseases. Mammalian Microtubule Associated Serine-Threonine Kinase 2 (Mast2) is an AGC kinase suggested to function in cytoskeletal organization, cell size, and growth factor signaling. Data from our laboratory and others suggests that Mast2 is downstream of mTORC1, however, very little is known about Mast2 biochemistry. To develop its pharmacological profile, we have interrogated the biochemical activity, regulation, and putative substrates of Mast2. Purified murine Mast2 readily phosphorylates myelin basic protein (MBP), as well and itself in vitro. Using a variety of Mast2 deletion and truncation mutants we have dissected possible Mast2 regulatory regions including the PDZ and Domain of Unknown Function (DUF1908) domains. Mast2 itself is also highly phosphorylated, and we have utilized point mutations to identify potential mTORC1 dependent phosphoregulatory sites. Stable γ-18O-ATP isotope labelled kinase assay linked phospho-proteomics (siKALIP) has revealed potential mammalian Mast2 substrates. Our results characterize essential residues and domains of Mast2 kinase, provide support that it is a downstream target of mTORC1, and begin to identify endogenous Mast2 substrates that will better describe Mast2's cellular function.
**Poster 31:**

**Title:** Characterization of Sitosterolemia-causing mutations in the cytosolic domains of ABCG5 and ABCG8

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**Undergraduate**

**Background:** Sitosterolemia is a rare, recessive form of familial hypercholesterolemia and is caused by mutations in *ABCG5* and *ABCG8*. ABCG5 and ABCG8 are glycoproteins that form an obligate heterodimer (G5G8) within the endoplasmic reticulum prior to trafficking to the cell surface where they promote biliary secretion of sterols. Missense mutations in clinically confirmed cases of Sitosterolemia are of particular interest for their potential to reveal structure-function relationships in ABCG5 ABCG8 and other ABC transporters. Mutants may then be classified based on the underlying molecular defect resulting in Sitosterolemia.

**Methods & Results:** 316 patients were enrolled in a study to determine the utility of whole genome sequencing at the Englander Institute for Precision Medicine at the Weill Cornell Medical Center. Inclusion criteria included pre-diabetes, low HDL, NAFLD, and diabetes mellitus type 2. Patients were consented for whole genome sequencing at the New York Genome Center and plasma analysis of measures related to inflammation, glycemic control and blood lipids at Boston Heart Diagnostics. Analysis of plasma lipids indicated elevated plasma phytosterols consistent with a diagnosis of Sitosterolemia in three subjects. Analysis of the coding region for *ABCG5* and *ABCG8* revealed three variants classified as Likely Pathogenic. Variants (ABCG8_G216D, ABCG5_R446Q, and ABCG5_Q392P) were generated by long-range PCR site-directed mutagenesis and confirmed by Sanger sequencing. Plasmids encoding native and variant ABCG5 and ABCG8 were co-transfected into human Huh7 hepatocytes and cell lysates analyzed by SDS-PAGE and immunoblot analysis. Maturation of G5G8 was assessed by electrophoretic mobility of ABCG5 ABCG8. Each variant supported maturation of G5G8. Two mutants in ABCG5 enhanced maturation G5G8 (R446Q, Q392P). The subcellular distribution of each variant was then assessed by indirect immunofluorescence microscopy. Whereas native G5G8 was localized to the cell surface, ABCG5_R446Q and ABCG8_G216D was distributed within a yet to be identified intracellular compartment.

**Conclusions:** Novel, likely pathogenic alleles in *ABCG5* and *ABCG8* associated with the clinical presentation of Sitosterolemia do not interfere and may enhance maturation of G5G8. However, two appear to disrupt trafficking of the G5G8 transporter to the cell surface.
Poster 32: 

Obesity impairs polyunsaturated fatty acid-derived metabolism related to inflammation initiation and resolution

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Graduate Student

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Obesity dysregulates inflammation upon lung injury; however, the mechanisms by which obesity primes the lungs prior to external injury are not well studied. Herein, we tested the hypothesis that obesity dysregulates pulmonary polyunsaturated fatty acid (PUFA) metabolism that is central to inflammation initiation and resolution. We first show that a high-fat diet (HFD) administered to C57BL/6J mice increased the concentration of PUFA-derived oxylipins (particularly prostaglandins and hydroxyeicosatetraenoic acids), independent of an increase in total pulmonary PUFAs, prior to onset of pulmonary inflammation. Experiments with a genetic model of obesity (ob/ob) generally recapitulated the effects of the HFD on the pulmonary oxylipin signature. Subsequent pulmonary next-generation RNA sequencing identified complex transcriptional regulation with the HFD. The HFD changed biological processes relating to lipid metabolism, oxidative stress, and immunity. Furthermore, computational integration of lipidomic with transcriptomic data revealed novel HFD-driven networks within glycerophospholipid metabolism and B cell receptor signaling pathways with specific PUFA-derived oxylipins. Finally, we confirmed the hypothesis that pulmonary oxylipins and inflammatory markers were generally increased with a HFD using an ozone-induced acute lung injury model. Collectively, these data suggest that obesity predisposes the lungs to respond poorly to inflammatory challenges by dysregulating pulmonary PUFA metabolism.
High Linoleic Acid Drives Increase in Oxylipin Products and Impairs Early Differentiation in MC3T3-E1 Osteoblast Precursor Cells

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Background: Osteoporosis is a serious bone disease, resulting from low bone mineral density (BMD), which significantly increases the risk of a bone fracture. Untargeted high-resolution metabolomics analyses by our group have shown that plasma linoleic acid (LA) and its oxidized products, hydroperoxyoctadecadienoic acid (HPODE), hydroxyoctadecadienoic acid (HODE), and epoxyoctadecenoic acid (EpOME), were inversely associated with BMD Z-score in an US adult cohort. LA and LA-derived oxylipins have been shown to impair differentiation of bone-forming cells (osteoblasts) and are associated with pro-inflammatory processes in clinical studies. Here, we examined the effect of increasing LA doses on cellular oxylipin concentrations and early differentiation in osteoblast precursor cells to determine whether high LA is detrimental to osteoblast function.

Methods: MC3T3-E1 osteoblast precursor cells were treated with 0 µM (control), 1 µM, or 50 µM LA, cultured in osteogenic differentiation media supplemented with 50 µM L-ascorbic acid and 2 mM β-glycerophosphate. To assess the effect of LA on early differentiation, cells were stained for alkaline phosphatase activity at 7 days post-treatment. In another experiment, cells were collected after 24 hours of LA treatment and lipids were extracted to determine the effect of LA on cellular oxylipin concentrations. Oxylipin species were detected by liquid chromatography/mass spectrometry and validated with authentic standards. Differences in oxylipin concentration by LA treatment were determined using one-way ANOVA with FDR correction.

Results: LA treatment resulted in a dose-dependent decrease in alkaline phosphatase activity, suggesting a decrease in early differentiation. LA at 50 µM significantly increased concentrations of its oxylipin products: 9(S)-HODE and 13(S)-HODE ($P < 0.001$). Their respective immediate precursor metabolites, 9(S)-HpODE and 13(S)-HpODE, concentrations were not significantly changed with LA treatment ($P > 0.05$). An LA oxylipin generated from cytochrome P450, 12(13)-EpOME, was also significantly increased by LA treatment ($P < 0.001$).

Conclusion: In osteoblast precursor cells, we show that LA increases specific cellular oxylipin concentrations, some of which are inversely associated with adult BMD in previous observational clinical studies, and impaired early osteoblast differentiation in primary cells. Increased cellular LA-derived oxylipins could play a role in the impairment in early differentiation. Understanding the mechanism of how LA and/or its oxidized metabolites blunt osteoblast differentiation and bone formation may allow for new strategies to prevent bone loss and reduce fracture.
**Selected Abstract Presentation**

**DGAT2 Inhibition Blocks SREBP-1 Activation and Improves Hepatic Steatosis by Increasing Phosphatidylethanolamine Content in the Endoplasmic Reticulum**

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**Faculty**

Diacylglycerol acyltransferase (DGAT) -2 catalyzes the final step of triglyceride (TG) synthesis. Deletion of DGAT2 reduces liver TG content in mice and inhibitors of DGAT2 are under investigation for the treatment of non-alcoholic fatty liver disease (NAFLD) in humans. Here, we show that in addition to blocking the final step of TG synthesis, DGAT2 inhibition also suppresses SREBP-1 activation, resulting in reduced hepatic fatty acid synthesis and TG secretion. Additional studies reveal DGAT2 inhibition does not alter the cholesterol/sterol or phospholipid polyunsaturated fatty acid contents of the ER. However, phosphatidylethanolamine (PE) levels were significantly increased in the ER of livers of animals treated with a DGAT2 inhibitor. In vitro, enrichment of PE in ER inhibits SREBP-1 activation, suggesting that PE content in the ER acts as a regulator for SREBP-1 activation. Combined, our studies reveal a novel mechanism by which DGAT2 inhibition regulates SREBP-1 activation and fatty acid synthesis in liver.
AAV8-mediated expression of scFv-E06 to target oxidized phosphatidylcholines is an effective therapeutic intervention to prevent progression to hepatic fibrosis

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**Graduate Student**

**Abstract:** Oxidized phosphatidylcholines (OxPC) have been implicated in chronic tissue damage including non-alcoholic fatty liver disease (NAFLD). We hypothesized that OxPC species drive development and progression of diet induced NAFLD. To test this hypothesis we expressed a single chain variable fragment of E06 (scFv-E06), a natural IgM antibody that recognizes OxPCs, in a cre-dependent manner in the liver of Speer6-ps1Tg(Alb-cre)21Mgn/J (Alb-cre) using an adeno-associated virus serotype 8. We demonstrated that hepatic expression of scFv-E06 prior to the start of FPC diet prevented development of diet induced hepatic steatosis. Expression of scFv-E06 after the development of hepatic steatosis prevented progression of NAFLD from hepatic steatosis to hepatic fibrosis. To identify what OxPC species are present in settings of hepatic steatosis and fibrosis in the plasma and liver we developed a mass-spectrometry based oxophospho-lipidomics method. We identified OxPC species present in both disease settings, determined potential pathologic OxPC species that were reduced by scFv-E06, and showed that these OxPC species dysregulated mitochondrial metabolism and gene expression in hepatocytes and hepatic stellate cells *in vitro*. Taken together, we demonstrated that OxPC species independently affect development of hepatic steatosis and progression to hepatic fibrosis and provided the first evidence that AAV8-mediated expression of scFv-E06 is an effective therapeutic intervention.
**Poster 34:**

**Title:** Classification and Effect of Correctors on Sitosterolemia-Associated Cytosolic Mutants in ABCG8

**Authors:** Brittney Poole\(^1\), MS, Kori S. Williams\(^2\), MPH, Isha Chauhan\(^3\), Rachel Hutchison\(^4\), Mikala Zelows\(^5\), Dr. Jyh-Yeuan Lee\(^6\), PhD, Marcus DaSilva Goncalves\(^7\), MD, PhD, Gregory A. Graf\(^5,8\), PhD

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**Graduate Student**

**Objective:** To classify mutants of ABCG8 identified in subjects with clinically confirmed Sitosterolemia, a rare form of Familial Hypercholesterolemia distinguished by the accumulation of phytosterols in plasma and tissues, and determine the effects of correctors and regulators of proteostasis on maturation-defective (Class II) mutants of ABCG8.

**Methods:** Disease-causing missense mutants within the cytosolic domain of ABCG8 were generated through long-range PCR site-directed mutagenesis. Normal and mutant proteins were expressed in human Huh7 hepatocytes. Cellular proteins were prepared and analyzed by SDS-PAGE and immunoblot analysis. Maturation of G5G8 was assessed by reduced electrophoretic mobility and the appearance of the higher molecular weight, mature forms of each glycoprotein. The impact of correctors, alone or in combination on maturation of Class II mutants was determined. Regulators of Proteostasis have also been shown to rescue misfolded proteins. An initial assessment of 12 compounds on maturation of the native G5G8 complex was also examined with our bioassay.

**Results:** Approximately 44% of cytosolic, Sitosterolemia-associated mutants in ABCG8 are maturation incompetent (Class II). Of those which matured beyond the ER, 60% were unable to traffic to the cell membrane. Correctors, alone or in combination, failed to restore maturation of Class II mutants under conditions suitable for restoration of maturation of other Class II ABC transporter mutants. Four analogs of Roscovitine that modulate proteostasis elevated immature and mature native G5G8.

**Conclusion:** HuH-7 cells are an efficiently transfected hepatocyte cell line that provides a system to assess variants in ABCG5 and ABCG8 for maturation and trafficking of G5G8. Correctors failed to restore maturation of cytosolic mutants of ABCG8 under the tested conditions. Analogs of Roscovitine enhance the abundance of native G5G8 and may enhance G5G8 activity in as well as restore maturation of some Class II mutants of ABCG8.
Title: Thermoneutral housing and high fat diet feeding regulate adipogenic differentiation by altering histone deacetylase 9 expression in adipose tissue

Authors: Brandee Goo¹, Abdalrahman Zarzour¹, Samah Ahmadieh¹,², David Kim¹, Praneet Veerapaneni¹, Mourad Ogbi¹, Yun Lei³, Xin-Yun Lu³, Ha Won Kim¹ and Neal L. Weintraub¹,²

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Background: Impaired adipogenic differentiation can contribute to metabolic disease in obesity. We previously reported that when housed at thermoneutral (TN) temperature, mice fed a high fat diet (HFD) exhibit impaired adipogenic differentiation that can be ameliorated by histone deacetylase 9 (HDAC9) gene deletion. However, the impact of HFD on adipogenic differentiation is variable in reported studies. Here, we hypothesize that housing temperature alters the adipogenic response to HFD feeding by altering HDAC9 expression.

Methods and Results: As reported previously, mice housed at TN temperature (28-30°C) gain more weight compared to mice housed at ambient temperature (21-22°C) despite reduced food consumption. qPCR analysis of mouse inguinal adipose tissue showed that HFD increased expression of HDAC9 more in mice housed at TN temperature (12-fold) compared to mice housed at ambient temperature (2-fold). Additionally, qPCR analysis of adipogenic genes (C/ebpα, Pparγ, Fabp4) in inguinal adipose tissue showed that HFD impaired adipogenesis in mice housed at thermoneutral temperature to a greater extent than mice housed at ambient temperature. Interestingly, qPCR analysis showed that acute ambient temperature cold exposure selectively reduced HDac9 expression in mouse adipose tissues. Interestingly, intraperitoneal injection of β3-adrenergic receptor agonist, CL316243, significantly reduced HDac9 expression in mouse adipose tissues. However, non-selective β-adrenergic receptor blocker, propranolol, was unable to prevent downregulate of HDAC9 in mice exposed to ambient temperature, suggesting that acute ambient temperature exposure reduces HDac9 expression in a sympathetic β-adrenergic receptor independent manner. Interestingly, western blot analysis of human inguinal adipose tissue showed that HDAC9 expression is positively correlated with body mass index (r²=0.40).

Conclusion: HDAC9 is a temperature sensitive gene in adipose tissue that regulates adipogenic differentiation. HDAC9 may be a relevant target to human obesity-related metabolic disease.

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Poster 36:

APOE Genotype Alters the Lipid Droplet Proteome and Modulates Droplet Dynamics

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Graduate Student

Background: The microglial immune response is a significant contributor to Alzheimer’s disease (AD) pathophysiology and neurodegeneration. Aging microglia accumulate lipid droplets (LDs), have high levels of reactive oxygen species, secrete pro-inflammatory cytokines, and are defective in phagocytosis. The E4 allele of Apolipoprotein E (APOE) is the strongest genetic risk factor for late-onset AD, and is associated with heightened neuroinflammation and increased LD formation. We hypothesize E4 microglia have increased LD formation under basal conditions and a higher capacity to form LDs under stress, resulting in greater pro-inflammatory cytokine production. We characterized LD development in microglia in the context of APOE genotype and analyzed LD surface proteins and lipid content from control and lipopolysaccharide (LPS) stimulated ApoE3 and ApoE4 mice.

Methods: Primary microglia were isolated from mice expressing human ApoE3 and ApoE4. Microglia were exposed to 250uM oleic acid (OA), 10ug/mL LPS, OA+LPS, dead N2A cells, or dead N2As+LPS. ApoE3 and ApoE4 expressing mice were injected with saline (control) or LPS (5mg/kg) and perfused at 24h. Livers were extracted, the LD enriched supernatant fraction was collected after centrifugation, and proteomic and lipidomic analyses were performed.

Results: Primary microglia from ApoE4 mice accumulated more LDs at baseline, with exogenous OA, LPS stimulation, and N2As as a percentage of E3 control across multiple experiments (E3 v E4 p values: baseline, 0.0317; LPS, 0.0032; OA, 0.0277; N2A, 0.0192). Western blots on LD fractions confirm LD enrichment by surface protein, PLIN2, along with increased expression of PLIN2 (i.e. more LDs) in E4 LPS mice. Proteomics reveal LD fractions from E4 mice are enriched for proteins involved in innate immunity, while E3 LDs are enriched for proteins involved in lipid β-oxidation.

Conclusion: E4 microglia accumulate more LDs compared to E3 microglia under all conditions tested. The proteomic profile of E4 liver LDs support the hypothesis that E4 expression increases inflammation under basal conditions, and upon stimulation, causes a more robust response. Increased LD formation is present in non-aged, non-diseased E4 cells, suggesting preclinical dysfunction associated with the highest risk APOE genotype. A better understanding of LD dynamics within these cells and their functional implications can provide targets to improve E4-related outcomes.
Selected Abstracts Presentation

**Title:** Glycerate Production from Enteric Fructose Metabolism is Elevated by Dietary Fat, Inducing Glucose Intolerance Through β-cell Damage

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**Abstract:** Dietary fructose, especially in the context of a high-fat western diet, has been linked to type 2 diabetes. Although the effect of fructose on liver metabolism has been extensively studied, a significant portion of the fructose is first metabolized in the small intestine. We recently reported that dietary fat enhances enteric fructose metabolism, increasing the circulating glycerate pool. High systemic glycerate levels reduce pancreatic islet sizes and β-cell content, inducing glucose intolerance. Our findings provide a new link between dietary fructose and glucose intolerance that is modulated by dietary fat.
Eicosapentaenoic acid lowers IL-6 secretion from pulmonary alveolar macrophages through a potential mechanism mediated by downstream hydroxylated oxylipins

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Graduate Student

Chronic inflammation is recognized as playing a pivotal role in the pathogenesis of many diseases, made increasingly evident by the COVID-19 pandemic. The resolution of inflammation is an active process governed by several classes of oxylipins, synthesized primarily from omega-3 (n-3) and select omega-6 (n-6) polyunsaturated fatty acids (PUFAs). Notably, these oxylipins play a pivotal role in protecting lung epithelia, exposed to airborne pathogens and allergens, from chronic inflammation that can result in tissue damage and pulmonary fibrosis. The relationship between oxylipins and inflammation resolution is emerging, however, the role of n-3 PUFAs and their downstream metabolites on the pulmonary macrophage mediated response remains poorly studied. Herein, we tested the hypothesis that long chain n-3 PUFAs could potentially control alveolar macrophage inflammatory cytokine secretion using mouse alveolar macrophages (MH-S) in response to lipopolysaccharide (LPS) induced inflammation. Relative to controls, pre-treatment of MH-S cells with eicosapentaenoic acid (EPA) but not docosahexaenoic acid (DHA) decreased pro-inflammatory cytokine IL-6 secretion but had no effect on TNFa levels. LC-MS/MS analyses revealed select EPA-derived oxylipins (i.e., 5-HEPE, 18-HEPE, 17(18)-EpETE) were dramatically elevated upon EPA treatment. Current in vitro and in vivo studies are teasing apart the EPA-oxylipin pathway using enzyme inhibitors and add-back studies on macrophage IL-6 secretion. Collectively, these results suggest activation of EPA-derived oxylipins may be a lead for improving macrophage mediated IL-6 secretion in pulmonary tissue.

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