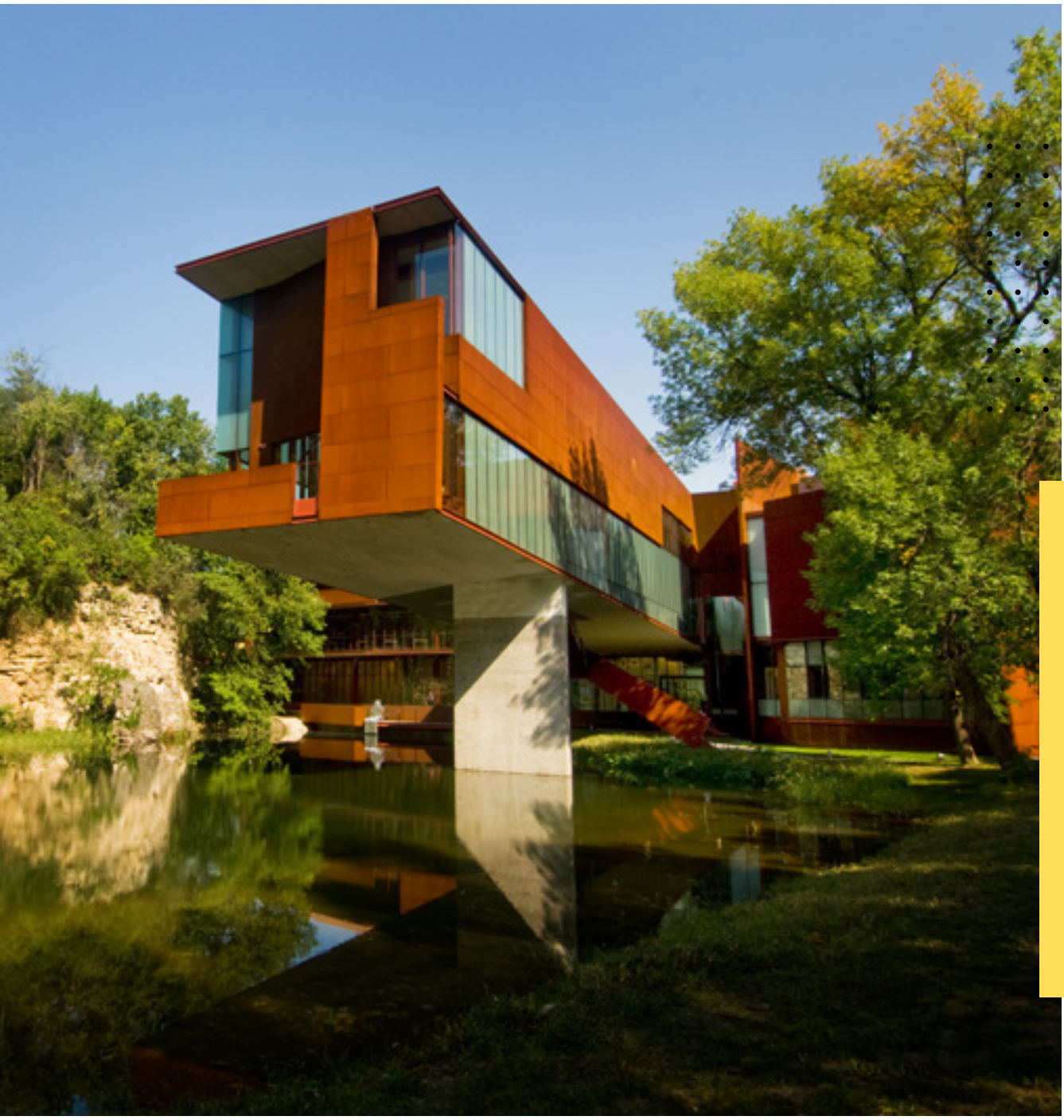
BIG ACADEMIC ALLIANCE



5TH BIG TEN ACADEMIC ALLIANCE LIPID SYMPOSIUM

University of Iowa, Iowa City, IA

October 27, 2023

SYMPOSIUM SCHEDULE

FRIDAY, OCTOBER 27, 2023

7:30 - 8:30 **Check in and Poster Setup**

8:20 - 8:30 **Introduction and Welcome**
Brandon Davies, Associate Professor, University of Iowa
Bhagriath Chaurasia, Assistant Professor, University of Iowa

SESSION 1:

8:30 - 9:00 **Gregory Shearer, PhD**
Professor, Pennsylvania State University
An end to the age of univariate, monotonic analyses on fatty acids and their oxylipins

9:00 - 9:30 **Jeffery Klauda, PhD**
Professor, University of Maryland
Modelling Natural Cell Membranes and their Lipid Asymmetry

9:30 - 10:00 **Andres Contreras, DVM, PhD**
Associate Professor, Michigan State University
Oxylipins, Modulators of Inflammation during Metabolic Stress

10:00 - 10:30 **Coffee Break**

SESSION 2:

10:30 - 11:00 **Kristy Townsend, PhD**
Associate Professor, The Ohio State University
Nerves and Lipids: A crucial interaction for metabolic health

SYMPOSIUM SCHEDULE

11:00 - 11:30 **Christina Camell, PhD**
Assistant Professor, University of Minnesota
Adipose tissue immune cells regulate bacteria-stimulated lipolysis during aging

11:30 - 12:00 **Shihuan Kuang, PhD**
Professor, Purdue University
Mitochondrial remodeling during cold-induced thermogenesis in brown fat

12:00 - 12:30 **Pick up Box Lunch**

12:30 - 1:15 **Poster Session (Odd # Posters)**

1:15 - 2:00 **Poster Session (Even # Posters)**

SESSION 3:

2:00 - 2:30 **Laura Lackner, PhD**
Associate Professor, Northwestern University
A Mitochondria-ER-PM contact site regulates the distribution of phosphatidylinositol-4-phosphate on the plasma membrane

2:30 - 3:00 **Ormond MacDougald, PhD**
Professor, University of Michigan
Lipolysis of bone marrow adipocytes is required to fuel bone and the marrow niche during energy deficits.

3:00 - 3:30 **Yumi Imai, MD**
Professor, University of Iowa
How to protect pancreatic beta cells under nutritional stress: from a viewpoint of lipid droplets

3:30 **Final Comments and Adjournment**

BTAAL SYMPOSIUM HOSTS & ORGANIZERS

2016 - Rutgers University
Dr. George Carman

2018 - Purdue University
Dr. Kimberly Buhman

2019 - University of Minnesota
Dr. Douglas Mashek & Dr. David Bernlohr

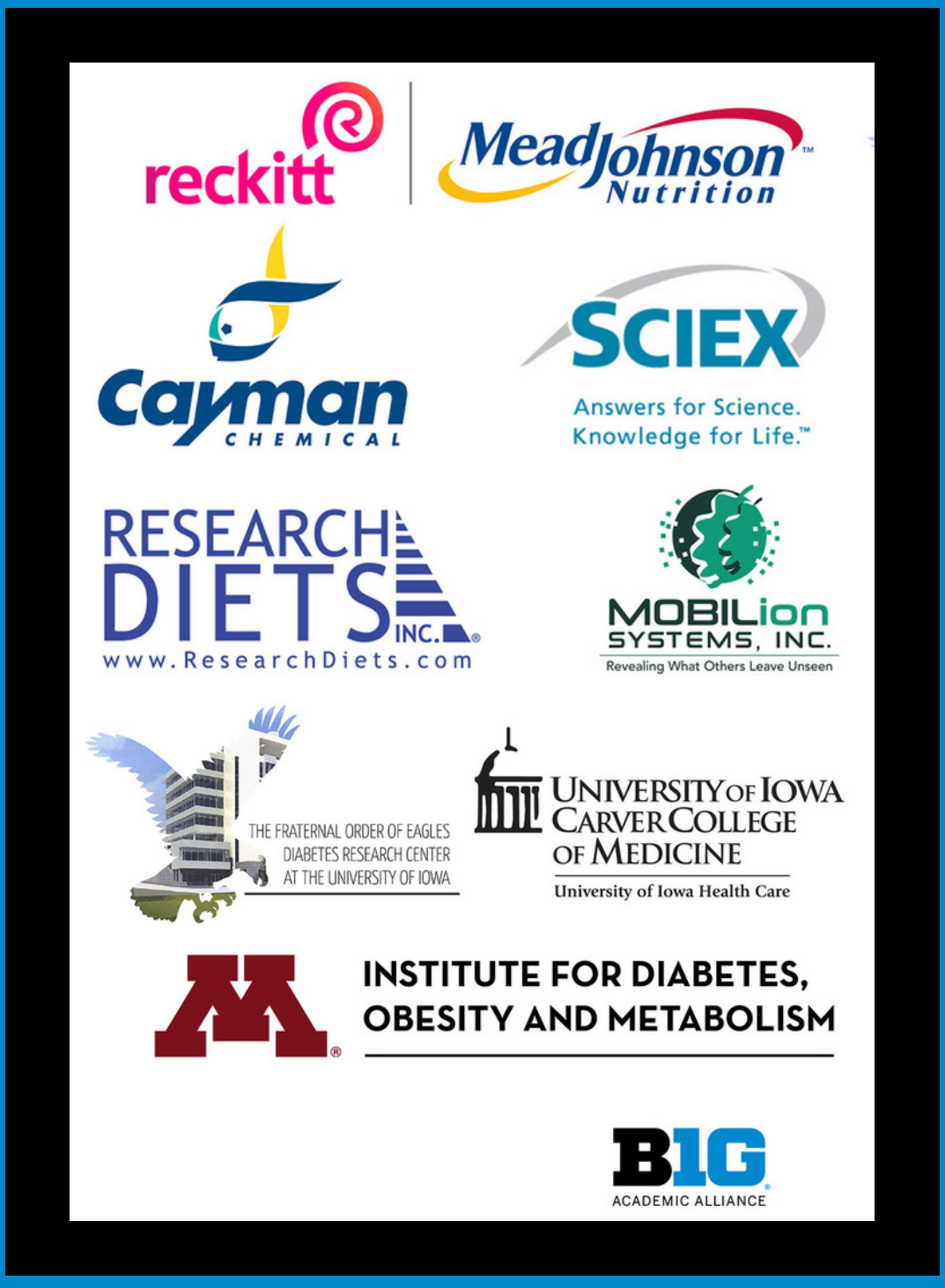
2022 - University of Illinois Urbana-Champaign
Dr. Sayeepriyadarshini Anakk

2023 - University of Iowa

Dr. Brandon Davies
brandon-davies@uiowa.edu

Dr. Bhagirath Chaurasia
bhagirath-chaurasia@uiowa.edu

2025 - The Ohio State University
Dr. Kristy Townsend & Dr. Kristin Stanford



Thank you to the sponsors of the 5th BTAAL Symposium!

1.

Plasma levels of endocannabinoids and related lipids before and after mTBI

Emily Richter, Clare Johnson, Praveen Kulkarni, Craig Ferris, and Heather Bradshaw

Indiana University

Introduction: Mild Traumatic Brain Injury (mTBI), or concussion, can be attributed to a majority of the head-injury related emergency room visits every year and are caused by a hard hit to the head, which can result in symptoms of cognitive impairment. Usually these symptoms will resolve within a matter of weeks with correct care, but can remain longer especially if multiple head hits occurred. Here we sought to elucidate some of the effects at a molecular level from one or multiple head hits by examining the levels of endogenous cannabinoids and related lipids in plasma post-injury. Specifically, we hypothesized that endocannabinoids, such as N-acylethanolamines (NAEs) and 2-acyl-sn-glycerols (2-AGs), as well as other major lipid families such as Free Fatty acids (FFAs) and N-acyl glycines would rapidly show differences in their concentration after at least one injury when compared to before.

Methods: Adult male Sprague Dawley rats underwent mTBI performed by a closed-head momentum exchange model to produce one (n=7), two (n=7) or three (n=6) mild head injuries (mTBI) in young adult male rats compared to non-injured, age and weight-matched controls (n=8). Plasma was collected prior to the first hit and 15 minutes after the 1st, 2nd, and 3rd hits. To extract lipids, samples were incubated in methanol + 5µL of 1µM internal standard (d8-AEA) for 2h in the dark on ice, then homogenized via sonication, centrifuged at 19,000G, 20˚ C for 20 min, and the lipid-containing supernatant decanted into water to make a ~25% organic solution. Lipids were partially purified from this solution using C18 solid phase extraction columns, eluting into 1-1.5mL of 65%, 75%, and 100% methanol. HPLC/MS/MS was then used to screen for ~87 lipids and calculate mol/gram concentrations for each tissue type. One-way ANOVAs with Fisher's LSD post-hoc tests were used to determine significant differences in lipid levels after one, two, and three head hits when compared to controls. A heatmap showing these changes was made.

Results: Results show that after one head hit, compounds in the NAE, FFA, and N-acyl glycine families increased, while compounds in the 2AG family both increased and decreased. Specifically, all of the compounds in the FFA family were significantly increased by at least three orders of magnitude after one head hit, while all but one compound in the N-acyl glycine family increased by at least two orders of magnitude. Interestingly, the NAE and 2AG families only had one of six and one of four compounds show an increase of two and four orders of magnitude, respectively. The only compound to show a significant decrease was in the 2AG family, which was 2-oleoyl-sn-glycerol by four orders of magnitude.

Conclusions: These findings provide a basis for investigating rapid plasma lipid level change post single heat hit injury in adult male mice. Future studies will investigate the effects of non-head hits (e.g. shoulder hits) and genetic sex.

2.

Uncovering Sensory Nerve Functions in White Adipose Tissue

Gargi Mishra

Ohio State University

White adipose tissue (WAT) is the primary energy storage site in the body, and is densely innervated by sensory and sympathetic nerves that enable bi-directional communication with the central nervous system. The sympathetic neurotransmitter norepinephrine is the best-studied nerve product in WAT, and is known to stimulate lipolysis, browning (development of brown adipocytes in WAT), thermogenesis, and other metabolically favorable processes. Conversely, WAT sensory nerves secrete neuropeptides like calcitonin gene related peptide (CGRP), a vasodilatory neuropeptide that also impacts lipolysis, but CGRP functions and mechanisms remain vastly understudied, especially in adipose tissues. Circulating CGRP levels are known to be increased in obese humans, but given the beneficial metabolic effects of CGRP, this may be a case of CGRP dysregulation or resistance. Thus, a more comprehensive investigation into CGRP's roles in adipose tissue and the importance of the sensory nerve supply for WAT function and obesity is warranted. First, we identified CGRP distribution in sensory nerves innervating mouse WAT by whole-mount imaging the inguinal WAT depot from sensory nerve reporter (Nav1.8Cre x tdTomato) marked with CGRP. We then observed significant increase in WAT CGRP levels by ELISA 30 minutes after directly delivering 13-HODE, a naturally occurring lipid-based sensory nerve receptor transient potential vanilloid channel agonist, into WAT. However, CGRP levels returned to baseline by 120 minutes after treatment, suggesting CGRP release may be an acute response to incoming stimuli and a consequence of interoceptive response by sensory nerves. Moreover, in vitro treatment of pre-adipocytes with recombinant CGRP for 60 minutes upregulated lipolytic pathways, as measured by phosphorylated hormone sensitive lipase levels (pHSL/HSL) western blot. Hence, sensory nerve derived CGRP may directly promote lipolysis in adipocytes. Moreover, using CGRP ELISA, we have observed CGRP levels in WAT are altered with changing energy balance states, as well as by acute versus chronic changes to altered energy balance states. Specifically, with obesity, in both diet-induced and genetic ob/ob mice, we see increased WAT CGRP levels, which fits the reports in humans and may be due to chronically elevated tissue lipids. Collectively, CGRP is a novel neuropeptide in adipose that may be relevant for lipolytic stimulation, changes in energy balance statues, and likely contributes to metabolic health.

3.

Tracking Lipid Bodies in Macrophages Infected with Leishmania infantum

HD Mennenga, GA Schaefer, K Lu, RD Lockard, VA Soupene, ME Wilson and **NE Rodríguez**

University of Northern Iowa

Worldwide, 12 million people are infected with Leishmania spp. Leishmania are vector-borne protozoa that parasitize and replicate in macrophages causing various disease manifestations including cutaneous, mucocutaneous, and visceral which could be fatal. The subject of our studies is L. infantum which causes visceral leishmaniasis in South America. Epidemiological data shows that despite similar exposure, there is a predominance of male patients. Similarly, animal studies show exacerbated L. infantum infection in males, supporting a biological predisposition for susceptibility. Recently, we showed higher survival of L. infantum in macrophages from male versus female mice. However, the mechanisms underlying these sex-based differences are not fully elucidated. Our group has also shown that L. infantum infection increases lipid bodies (LBs) in mice macrophages. LBs are organelles comprised of neutral lipids which are modulated by various stimuli and can regulate the immune response through the production of eicosanoids. Additionally, intracellular pathogens could use LBs as a source of nutrients or structural components. As such, LBs have the potential to alter infection outcome through various mechanisms. Herein, we used confocal microscopy to examine LBs in L. infantum-infected macrophages of male versus female C57BL/6 mice. Compared to non-infected controls, 48h of infection increased LBs from 89 to 317 in female-derived macrophages. In macrophages from males the number of LBs increased from 158 to 527 (200 cells/experiment, N=3). In addition, LBs reached a larger size in macrophages from males. The preliminary data suggest that LBs are involved in the sex-dependent differences observed in L. infantum-infected C57BL/6 mice macrophages. Initial studies with human macrophages also showed LB accumulation after L. infantum infection. We hypothesize that increased LB expression in macrophages from males facilitates parasite survival and provides a basis for the increased male susceptibility observed in L. infantum infection.

4.

The physiological impact of EL inhibition by ANGPTL3 on HDL

Kelli Davie, Markus Heine, Franz Rinninger, Joerg Heeren, Sean Davies, and Brandon Davies

University of Iowa

Diabetes is often accompanied by low levels of circulating high density lipoproteins (HDL) and high levels of plasma triglycerides (TG), risk factors for cardiac disease and atherosclerosis. Angiopoietin-like 3 (ANGPTL3), regulates both plasma TG and HDL levels through inhibition of lipoprotein lipase (LPL), which hydrolyzes the triglycerides of TG-rich lipoproteins, and endothelial lipase (EL), which hydrolyzes the phospholipids of HDL. Human deficient in ANGPTL3 have decreased risk for both coronary artery disease and Type 2 Diabetes. Physiologically, it is not understood when and where ANGPTL3 inhibits EL in vivo, nor is it know how this inhibition affects HDL functionality. This project aims to answer the physiological role of ANGPTL3-mediated EL inhibition. Tissues (heart, liver, kidney, spleen, gWAT, sWAT, BAT, lung, skeletal muscle, and gonads) from fed and fasted wild-type and Angptl3-/- mice were collected and processed to measure phospholipase activity and gene expression of Lipg (EL) and Angptl3. Dual-labelled HDL (3H and 125I) was injected into wild-type and Angptl3-/- mice to measure clearance from plasma and uptake into tissues. In fed and fasted mouse tissues, phospholipase activity remains equal or trends to have increased activity in Angptl3-/- mice. Lipg gene expression is highest in liver, lung, and gonads. In fed mice, expression of Lipg significantly decreases in Angptl3-/- liver and gonads. Measuring uptake of dual-labelled HDL, Angptl3-/- mice had increased plasma lipid clearance compared to wild-type controls. Angptl3-/- mice had increased HDL-cholesterol delivery to the liver but no difference in whole particle delivery compared to wild-type mice. The highest level of phospholipase activity and Lipg gene expression in mouse tissues were located in the liver, lung, and gonads. Interestingly, the phospholipase activity in both fed and fasted tissues remained similar in the absence of Angptl3. We hypothesize that the Lipg downregulation in Angptl3-/- mice in the liver and gonads could be compensatory for the more active EL when Angptl3 is not inhibiting, indicated by relatively unchanged activity levels. In the absence of Angptl3, HDL-C is selectively cleared to the liver perhaps indicative of increased Reverse Cholesterol Transport which is facilitated by the activity of EL on HDL.

5.

A comparative study on the identification of plasma lipids using collision-induced dissociation and electron-activated dissociation

Randy Arnold, Kiran Maan, Dipankar Malakar, Cagakan Ozbalci

SciEx

Lipidomics profiling is widely being used for untargeted and targeted analysis. The diversity of lipids makes comprehensive profiling and identification of lipid species challenging. There is an ongoing need for accurate lipid identification. For fragmentation, collision-induced dissociation (CID) and electron-activated dissociation (EAD) are currently available. EAD, as addition to standard CID, delivers unique fragment ions that are required for deep lipid characterization. Good numbers of hits were obtained using SimLipid and MS-DIAL in both CID and EAD fragmentation modes with some unique identification. Utilization of both CID and EAD for better identification and characterization along with use of multiple software (MS-DIAL and SimLipid) is demonstrated.

6.

Novel Role of Intestinal Fatty Acid Binding Proteins in Systemic Energy Homeostasis

Kalhara Menikdiwela, Yin Xiu Zhou, Xuanyu Zhou, Rohan Patel, Judith Storch

Rutgers University

Background: Fatty acid binding protein 1 (FABP1) and FABP2 are abundantly expressed in the proximal small intestine and are thought to play an important role in intracellular lipid transport. We have shown that they also differentially regulate systemic energy metabolism. High fat (HF)-fed whole body FAPB1 null mice (FABP1 -/-) were obese yet demonstrated a metabolically healthy phenotype with improved glucose tolerance and better exercise capacity. Additionally, intestine-specific ablation of FABP1 (FABP1int-/-) resulted in a similar phenotype, with improved exercise capacity. On the other hand, HF-fed whole body FABP2 knockout mice (FABP2-/-) were leaner than Wt mice and demonstrated an improved glucose clearance ability. It has also been found that plasma incretin levels are higher in both FABP1 and FABP2 null mice. These higher levels of plasma glucagon inhibitory polypeptide (GIP) and/or glucagon-like peptide 1 (GLP-1) may be responsible, at least in part, for the improved glucose metabolism and overall energy homeostasis in the FABP knockout mice. Interestingly, it has been shown that both FABP1 and FABP2 are expressed not only in enterocytes, as has been known for decades, but also in enteroendocrine cells (EEC).

Aim: Thus, we hypothesize that intestinal FABPs, particularly within EECs, regulate peripheral tissue homeostasis and systemic energy balance.
Method: To better understand the role of FABPs in EECs, we recently generated an intestinal EEC-specific FABP1 KO (FABP1Int-EEC-/-) mice line using mice where Cre is driven by the NeuroD1 promoter crossed with mice with loxP sites flanking the FABP1 gene (FABP1fl/fl). The ablation of FABP1 specifically in EECs was confirmed by immunohistochemistry and immunofluorescence-based co-localization approaches. The initial cohort of FABP1Int-EEC-/- and WT littermate control (FABP1fl/fl) mice (both male and female) are being fed a high fat (HF) diet for 12 weeks.

Results: No significant difference in body weight was observed among the male or female mice at the start of the dietary intervention. However, FABP1Int-EEC-/- male mice demonstrated an increased body weight starting at week 3, compared to Wt mice; no such body weight changes were observed in female mice. Although FABP1Int-EEC-/- male mice showed significantly higher weight gain and fat mass, they displayed improved blood glucose clearance and an improved exercise capacity compared to control mice. Again, no changes were seen in female mice.

Conclusion: These studies suggest that in males, in addition to its role in enterocyte lipid transport and metabolism, the FABP1 expressed in EECs is involved in the regulation of peripheral tissue homeostasis and systemic energy balance.

7.

Prostaglandins and lipid droplet-associated proteins act together and separately to promote actin remodeling during Drosophila oogenesis

Michelle S. Giedt, Jonathon M. Thomalla, Roger P. White, Michael A. Welte, Tina L. Tootle

University of Iowa

Prostaglandins (PGs) are lipid signaling molecules that act over a short range to regulate a myriad of cellular processes, including actin remodeling necessary for developmental events. While it is known how PGs are produced and signal, the mechanisms regulating their production and their specific downstream targets remain poorly understood. To address this gap in knowledge, we exploit the robust genetic and developmental system of Drosophila oogenesis or follicle development. Loss of Pxt, the cyclooxygenase (COX) enzyme required for PG production, causes severe disruption of actin remodeling, resulting in female sterility. We recently found that ATGL, a lipid droplet (LD) associated triglyceride lipase, regulates the release of arachidonic acid, the Pxt substrate required for PG production, and this ATGL function is required for PG-dependent actin remodeling. This finding raised the question of whether other LD-associated proteins also control PG signaling and/or actin remodeling. Interestingly, we find that loss of Perilipin 2 (PLIN2) also perturbs actin, but does so via an undefined PG-independent pathway. In contrast, the LD-protein Jabba, a protein anchor, works in a PG-dependent pathway to promote actin remodeling. However, this pathway is independent of the ATGL-PG pathway we previously described, raising the question of what the relationship is between Jabba, PGs, and actin remodeling. Current efforts are focused on determining whether Jabba acts upstream of PGs or vice versa. Preliminary evidence suggests that PGs may act upstream of Jabba, as loss of Pxt alters the levels of the different isoforms of Jabba. Future studies will focus on uncovering the specific mechanism whereby this pathway regulates actin dynamics. Ultimately, this work provides the first evidence that LDs and PGs act together to control actin remodeling. Similar pathways connecting LDs, PGs, and actin are likely utilized in other development contexts, and misregulated in diseases such as metabolic syndrome and cancer.

8.

Phosphatidate phosphatase Pah1 contains a novel RP domain that regulates its phosphorylation and function in yeast lipid synthesis

Geordan J. Stukey, Gil-Soo Han, and George M. Carman

Rutgers University

The Saccharomyces cerevisiae PAH1-encoded phosphatidate (PA) phosphatase, which catalyzes the Mg2+-dependent dephosphorylation of PA to produce diacylglycerol, is one of the most highly regulated enzymes in lipid metabolism. The enzyme controls whether cells utilize PA to produce membrane phospholipids or the major storage lipid triacylglycerol. PA levels, which are regulated by the enzyme reaction, also control the expression of UASINO-containing phospholipid synthesis genes via the Henry (Opi1/Ino2-Ino4) regulatory circuit. Pah1 function is largely controlled by its cellular location, which is mediated by phosphorylation and dephosphorylation. Multiple phosphorylations sequester Pah1 in the cytosol and protect it from 20S proteasome-mediated degradation. The endoplasmic reticulum-associated Nem1-Spo7 phosphatase complex recruits and dephosphorylates Pah1 allowing the enzyme to associate with and dephosphorylate its membrane-bound substrate PA. Pah1 contains domains/regions that include the N-LIP and haloacid dehalogenase-like catalytic domains, N-terminal amphipathic helix for membrane binding, C-terminal acidic tail for Nem1-Spo7 interaction, and a conserved tryptophan within the WRDPLVDID domain required for enzyme function. Through bioinformatics, molecular genetics, and biochemical approaches, we identified a novel RP (regulation of phosphorylation) domain that regulates the phosphorylation state of Pah1. We showed that the DRP mutation results in a 57% reduction in the endogenous phosphorylation of the enzyme (primarily at Ser-511, Ser-602, and Ser-773/Ser-774), an increase in membrane association and PA phosphatase activity, but reduced cellular abundance. This work not only identifies a novel regulatory domain within Pah1 but emphasizes the importance of the phosphorylation-based regulation of Pah1 abundance, location, and function in yeast lipid synthesis.

9.

Impact of Vitamin D and Pyruvate Carboxylase on the Lipid Droplet Proteome of Metastatic Breast Cancer Cells

Yazhen Song, Chaylen Andolino, Dorothy Teegarden

Purdue University

Metastatic breast cancer is a leading cause of mortality among women worldwide. Elevated lipid accumulation stored in cytoplasmic lipid droplets (CLD) in metastatic cells is associated with poorer patient outcomes. Our previous studies demonstrate that the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)2D), downregulates pyruvate carboxylase (PC) expression in metastatic breast cancer cells, resulting in reduced lipid accumulation. The purpose of this study was to investigate the impact of 1,25(OH)2D treatment or reduction in PC expression (shPC) on the proteome of CLD's of human metastatic breast cancer cells (MCF10CA1a). CLDs were isolated and proteins processed for proteomic analysis. In the CLD fractions, 1562 proteins were common to both MCF10CA1a and 1,25(OH)2D treated cells, and 160 or 550 more enriched in the MCF10CA1a or 1,25(OH)2D treated cells, respectively, compared to the other condition. Also, in the CLD fraction, 380 are common to both MCF10CA1a and shPC, and 65 or 1268 were more enriched in the shPC or MCF10CA1a compared to the other cell condition, respectively. There were 6 proteins (KLF5, PTMA, TTN, HILPDA, GFAP, WBP2) more enriched in the CLD fraction of the metastatic MCF10CA1a cells involved in ferroptosis, an iron-dependent lipid peroxidation process that induces cell death, and thus CLD association of these proteins may reduce metastatic breast cancer cell death. These findings provide a future direction to investigate the role of proteins on CLDs in the progression of breast cancer and potential development of strategies to reduce the burden of metastatic breast cancer.

10.

HEPATIC IRE1 PROTECTS AGAINST SEPTIC CARDIAC FAILURE

Mark Li1,2,3, Roger R. Berton4, Qingwen Qian1,2,3, Juan J. Rodriguez1,2,3, J. Alan Maschek5, Biyi Chen2,6, Elizabeth Barroso1,2,3, Adam J. Rauckhorst2,3,7, Thomas S. Griffith8, Eric B. Taylor2,3,7, James E. Cox5, Vladimir P. Badovinac4, Gökhan S. Hotamisligil9, Long-Sheng Song2,6, Ling Yang1,2,3*

1Department of Anatomy and Cell Biology; 2Fraternal Order of Eagles Diabetes Research Center; 3Pappajohn Biomedical Institute; 4Department of Pathology and Interdisciplinary Program in Immunology; 6Department of Internal Medicine; 7Department of Molecular Physiology and Biophysics; University of Iowa Carver College of Medicine, Iowa City, IA 52242 5Department of Biochemistry, Department of Nutrition and Integrative Physiology and Metabolomics Core Research Facility, University of Utah, Salt Lake City, UT 84112 8Department of Urology, University of Minnesota, MN 55455 9Department of Molecular Metabolism, Harvard T.H. School of Public Health, Boston, MA 02115

Metabolic reprogramming in response to infection plays a critical role for septic survival. During a septic episode, the heart heavily relies on hepatic lipid particles to prevent heart damage and failure. However, the causal mechanisms of liver-mediated metabolic regulation of septic heart remain largely unknown. Inositol-Requiring Enzyme 1 (IRE1) is the most conserved unfolded protein response (UPR) regulator that governs homeostasis of the endoplasmic reticulum (ER), the main cellular site for lipid synthesis and processing in the hepatocyte. In this study, we performed RNAseq, lipidomic and metabolomic analyses in mice with hepatic IRE1-deletion and controls in the presence of septic challenge. We show that hepatocyte IRE1 is indispensable for protecting against septic mortality in two different rodent models of experimental sepsis, namely cecal ligation and puncture (CLP) and LPS. We found that the protective effect of hepatic IRE1 was not attributed to the inflammatory response since hepatic IRE1 deletion did not alter hepatic or systemic cytokine response. However, loss of IRE1 in the liver significantly augmented septic cardiac dysfunction in part due to a skewed immune-metabolic balance. Lipidomic and metabolomic analyses further revealed that loss of IRE1 in the liver compromised adaptive intrahepatic and circulating lipid reprogramming, including VLDL, in response to septic challenge. Furthermore, we identified that the protective effects against septic mortality are mediated by a non-canonical IRE1-dependent mechanism. Together, our study provides the first insight into how a disruption of hepatic ER-mediated lipid metabolic regulation promotes sepsis-associated cardiac immuno-metabolic imbalance. Knowledge gained from this study might speed development of novel therapeutic targets for hepatic dyslipidemia in sepsis-induced organ failure.

11.

Disruption of Pituitary-Hepatic UPR Crosstalk Promotes NAFLD Progression

Qingwen Qian1, Mark Li1, Zeyuan Zhang1, Shannon Davis2, Kamal Rahmouni1, Andrew W. Norris1, Huojun Cao3, Wen-xing Ding4, **Ling Yang1***

1Fraternal Order of Eagles Diabetes Research Center, University of Iowa Carver College of Medicine 2College of Arts and Sciences, University of South Carolina 3University of Iowa College of Dentistry 4The University of Kansas Medical Center

Obesity is the major risk factor for nonalcoholic fatty liver disease (NAFLD), for which effective cures are lacking. Despite the notion that obesity is associated with aberrant levels and action of pituitary hormones that are essential for maintaining hepatic metabolic and inflammatory states, the intrinsic pituitary endocrine abnormalities and their hepatic consequences are incompletely defined. By characterizing the impact of diet-induced obesity (DIO) on the pituitary whole tissue and single cell transcriptome, we demonstrated that obesity disrupts pituitary endoplasmic reticulum (ER) homeostasis by suppressing the inositol-requiring enzyme-alpha (IRE1 alpha)-mediated adaptive unfolded protein response (UPR). We further showed that defective pituitary UPR by IRE1 alpha-deletion in the anterior pituitary augmented obesity-associated systemic metabolic abnormalities, particularly the NAFLD-associated pathologies. Conversely, enhancing the adaptive UPR in the anterior pituitary gland, by genetic gain-of-function of spliced X-box binding protein 1 (sXBP1), ameliorated the hepatic metabolic defects observed in mice with pituitary IRE1 alpha deletion. Intriguingly, disruption of the UPR in the pituitary gland resulted in impaired hepatic UPR, which was in part due to a defective thyroid hormone receptor (THR)-mediated activation of hepatic Xbp1. In contrast, activation of the hepatic THR signaling by a liver-specific THR agonist (MGL-3196) improved hepatic metabolic and ER homeostasis in anterior pituitary-IRE1 alpha deficient mice. Together, our study provides the first insight into disruption of pituitary endocrine signaling-mediated inter-organ UPR communication drives NAFLD progression. Unraveling these connections might uncover new therapeutic targets for NAFLD.

12.

Ceramide-FGF13 axis regulate glucose and energy homeostasis

Jamal Naderi1#, Amanda Kelsey Johnson1#, Bhawna Chandravanshi1#, Alec Ksiazek1#, Himani Thakkar1, Ajay Anand1, Vinnyfred Vincent1, Aaron Tran1, Pratibha Singh1, Ayushi Sood1, Aasthika Das1, Chad Lamar Talbot1, Isabella A. Distefano7, J. Alan Maschek3, James Cox2, Ying Li3, Scott A. Summers2,3, Donald J. Atkinson3, Tursun Turapov3, Jason A Ratcliff4, Javis Fung5, Asim Shabbir5, M. Shabeer Yassin6, Sue-Anne Toh Ee Shiow6, William L. Holland2,3, Geoffrey S. Pitt7, Bhagirath Chaurasia1*

1Division of Endocrinology, Department of Internal medicine, Fraternal Order of Eagles Diabetes Research Center, University of Iowa, Iowa City, Iowa 2Department of Biochemistry, University of Utah, Salt Lake City, Utah 3Department of Nutrition and Integrative Physiology and the Diabetes and Metabolism Research Center, University of Utah, Salt Lake City, Utah 4Iowa Institute of Human Genetics, University of Iowa, Iowa City, Iowa 5Department of Surgery, University Surgical Cluster, National University Health System, Singapore 6Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 7Cardiovascular Research Institute, Weill Cornell Medicine, New York, NY

Ceramide accumulation impairs nutrient sensing in adipocytes, leading to deterioration in energy and glucose homeostasis. Using a comparative transcriptomic screen, we identified fibroblast growth factor 13 (FGF13) as a ceramide-regulated factor that impairs adipocyte nutrient sensing. We demonstrate that obesity robustly induces FGF13 expression in adipose tissue in mice and humans and is positively associated with glycemic indices of type 2 diabetes. Pharmacological or genetic inhibition of ceramide biosynthesis reduces FGF13 expression. Using mice with loss and gain-of-function of FGF13, we demonstrate that FGF13 is both necessary and sufficient to impair glucose and energy homeostasis independent of ceramides. Mechanistically, FGF13 exerts these effects by inhibiting caveolae abundance which impairs glucose utilization and thermogenesis. These studies suggest that FGF13 inhibition could be harnessed to prevent and treat metabolic diseases.

13.

The role of lipid metabolism in DNA homeostasis

Mahima Devarajan1, Douglas G. Mashek1,2

1 Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis MN
2 Division of Diabetes, Endocrinology, and Metabolism, Department of Medicine, University of Minnesota, Minneapolis MN

It is well-established that DNA damage plays a contributory role in aging. Fasting and caloric restriction are arguably the most well-established interventions that extend lifespan across organisms, a mechanism that is supported by evidence that fasting can enhance genomic stability. In a fasting state, activation of lipolysis leads to the breakdown of lipid droplets (LDs), and lipolytic enzymes such as adipose triglyceride lipase (ATGL) provide a source of energy as well as contribute to multiple signaling cascades. Given the contributory role of LDs and ATGL in the fasting response and the link between fasting and genomic stability, our objective is to elucidate the mechanisms by which LD metabolism regulates DNA homeostasis.

We have shown in multiple cell types that LDs accumulate in response to direct DNA damage and replicative stress. Further, we show that LD-rich cells acquire less nuclear damage than cells with few or no LDs. Consistent with this, we show that LD ablation prior to senescence induction increases DNA damage and subsequently increases senescence, further suggesting a protective role for LDs in DNA damage and senescence. Importantly, we show that overexpression of ATGL (increased lipolysis) reduces DNA damage, reduces the DNA damage response, and enhances DNA repair in response to both etoposide and ionizing radiation, suggesting that the protective role LDs play is due to enhanced lipolysis. Overall, these studies reveal a novel role for LDs and LD proteins in DNA damage and repair, thus unveiling a novel mechanism by which altered metabolism contributes to genomic stability and aging.

14.

Assessing the interactions between lipoprotein lipase and cell-surface receptors

Shwetha K. Shetty, Gabriel Ewert, Ethan Hahlbeck, Hannah Johnson, and Brandon S.J. Davies

University of Iowa

LPL is the primary enzyme responsible for the hydrolysis of circulating triglycerides from triglyceride-rich lipoproteins (e.g. VLDL and chylomicrons), liberating fatty acids for the tissue uptake. In addition to hydrolysis of triglycerides, LPL is also involved in whole lipoprotein clearance by non-catalytically bridging lipoproteins to cell surface receptors. In vivo and in cell culture, LPL can be cleaved by proprotein convertases, separating the N-terminal domain, which contains the catalytic domain, from the C-terminal domain, which is responsible for binding lipoproteins and cell-surface receptors. This cleavage abolishes the enzymatic activity of LPL, but it is unknown how cleavage alters its bridging function. The purpose of our study is to investigate the ability of the C-terminal domain of LPL to bind various cell surface receptors known to bind LPL, including GPIHBP1, VLDLR, LDLR, LRP1, and/or HSPGs, and to mediate bridging of lipoproteins. We also aim to identify LPL mutations that disrupt binding of LPL to VLDLR, LDLR, LRP1, and/or HSPGs, but retain catalytic function and the ability to bind GPIHBP1. Such mutations could be used in vivo to test the consequences of disrupting LPL bridging without disrupting LPL-mediated lipolysis. Using a split-luciferase NanoBiT assay and immunofluorescence to measure the interactions between LPL and cell surface receptors, we found that the cleaved C-terminal domain of LPL could bind both GPIHBP1-, LDLR, and VLDLR expressing endothelial cells. We also began characterizing the ability of various LPL mutants to bind GPIHBP1, VLDLR, and LDLR.

15.

Defining the Role of Adipose Triglyceride Lipase in Drosophila Border Cell Migration

Israel Wipf

University of Iowa, Departments of Biology and Anatomy & Cell Biology

Lipid droplets (LDs) are dynamic cellular organelles responsible for mediating key steps in lipid metabolism and signaling. LD dysregulation and accumulation is thought to promote cancer invasion and metastasis, but the precise role of LDs in collective cell migration is unknown. One possibility is that LDs regulate cell migration by modulating cytoskeletal dynamics. Recently, we have identified the conserved LD lipase, Adipose Triglyceride Lipase (ATGL), as a novel regulator of actin remodeling during Drosophila oogenesis. Homozygous loss of ATGL results in severe actin defects, impacting both actin bundle formation and cortical actin integrity. Genetic interaction assays indicate that ATGL's control over actin remodeling is dependent on prostaglandin (PG) signaling, with ATGL acting upstream of Pxt, the cyclooxygenase-like enzyme in Drosophila responsible for PG synthesis. Intriguingly, prior work in our lab has demonstrated that PGs are critical orchestrators of cell migration during Drosophila oogenesis. This, together with ATGL's PG-dependent control over actin remodeling, leads us to hypothesize that LDs and ATGL might also contribute to PG-dependent cell migration. To test this hypothesis, we use border cell migration during Stage 9 of Drosophila oogenesis, an in vivo model of collective, invasive cell migration. We analyzed the migration of border cells in wildtype vs ATGL null flies and found that loss of ATGL results in both delayed migration and failed delamination. These findings suggest a novel role for both ATGL and LDs in facilitating on-time border cell migration. Interestingly, a further genetic interaction assay indicated that ATGL's role in regulating on-time border cell migration is independent of Pxt and PG signaling. As lipids released from LDs can serve as precursors for signaling molecules and membranes or as substrates for energy production, future work will aim to determine precisely how ATGL-dependent lipolysis is promoting on-time border cell migration. Finally, given that LD dysregulation is implicated in human diseases such as diabetes, obesity, and cancer, new insights into the diverse functions of LDs and their associated proteins are likely to lead to improved understanding of and new therapeutics for a variety of diseases.

16.

Upregulation of fatty acid synthesis genes in the liver caused by exposure to the persistent environmental toxicant, PCB52

Brynn Kyleakin Helm-Kwasny, Amanda Bullert, Hui Wang, Andrea Adamcakova-Dodd, Peter Thorne, Hans-Joachim Lehmler, James A. Ankrum, Aloysius J. Klingelhutz

University of Iowa

Polychlorinated biphenyls (PCBs) are persistent environmental toxicants that were used extensively in building materials before their intentional use was banned in the U.S. in 1979. Despite this ban, the Iowa Superfund Research Program (ISRP) has found elevated levels of volatilized lower-chlorinated PCBs in the air of older school buildings. PCB exposure has been linked to cancer, liver steatosis, cardiovascular disease, and diabetes. One PCB congener that is routinely found in school air is PCB52. The health consequences of airborne PCB52 exposure, particularly at early age, are unknown.

The liver is responsible for PCB metabolism through the expression of enzymes such as the cytochrome p450s and sulfotransferases. It is unclear how the liver metabolizes PCB52 and how PCB52 exposure via inhalation affects the liver. To begin to address these questions, a 4-week PCB52 inhalation exposure study was conducted using adolescent Sprague-Dawley rats. RNAseq analysis of liver tissue indicated that male and female rats responded differently to PCB52 exposure. Female rats showed the most extensive exposure-related changes in gene expression. Specifically, genes involved in the fatty acid biosynthesis pathway were upregulated in the livers of female PCB-exposed groups when compared to shams. Mass spectrometry analysis demonstrated that the majority of PCB52 from inhalation exposure was converted to hydroxylated forms in the liver. We hypothesize that hydroxylation of PCB52 in the liver causes dysregulation of the fatty acid biosynthesis pathway, potentially leading to nonalcoholic fatty liver disease (NAFLD). Preliminary in vitro studies using the human liver cell line HepG2 support this model and indicate that hydroxylated metabolites, but not parental PCB52, upregulate fatty acid biosynthesis genes. Further studies are underway to elucidate the mechanism(s) by which this upregulation occurs and to determine the functional consequences of these changes regarding lipid accumulation in liver cells. Our findings will be important for determining liver-specific health risks associated with early-life exposure to inhaled PCBs.

17.

Age-associated accumulation of B cells promotes macrophage inflammation and inhibits lipolysis in adipose tissue during sepsis

Anna Carey, Katie Nguyen, Pranathi Kandikonda, Victor Kruglov, Claire Bradley, Korbyn J. V. Dahlquist, Stephanie Cholensky, Whitney Swanson, Vladimir P. Badovinac, Thomas S. Griffith, Christina D. Camell

University of Minnesota

Non-canonical lipolysis induced by inflammatory cytokines or toll-like receptor ligands is required for the regulation of inflammation during endotoxemia and sepsis. Canonical lipolysis induced by catecholamines declines during aging, but the extent to which the non-canonical pathway of lipolysis is active during aging remains unknown. The immune compartment of visceral white adipose tissue (vWAT) changes during aging, including an expansion of lymphocytes, pro-inflammatory macrophage polarization, and an increase in chronic low-grade inflammation, all of which influence canonical lipolysis in old vWAT. Therefore, we aimed to define the extent to which immune cells from older hosts influence non-canonical lipolysis induced by sepsis. Here we show that models of polymicrobial sepsis and lipopolysaccharide (LPS)-induced endotoxemia induce vWAT lipolysis in young mice but fail to activate lipolytic pathways in vWAT from old mice. Characterization of vWAT B cells revealed dysfunctional activation of resident B cells during sepsis and an immunosenescent phenotype of B1 B cells that included upregulation of immuno- and metabolic suppressive pathways. Short term depletion of B2 B cells from old mice and adoptive transfer of peritoneal B cells from old mice into young recipient mice were insufficient to alter LPS-stimulated lipolysis. However, life-long deficiency of B cells increased LPS-stimulated lipolysis, suggesting the suppressive role of B cells may be indirect and requires other cell types. Pro-inflammatory macrophages and NLRP3 inflammasome activation were significantly reduced in the old B cell knockout mice. These data collectively reveal a decline in non-canonical lipolysis in vWAT during aging that is regulated by resident immune cells. Additionally, our study suggests the B cell-macrophage signaling axis may offer new approaches to resolve metabolic dysfunction in aged vWAT and attenuate septic severity in older individuals.

18.

Albumin is an important factor in the control of serum free fatty acid flux in male and female mice.

Keigo Tomoo1, Mirandia Szramowski1, Rodolfo Pinal2, Juliauna M. Meyer1, Yi Zhang1, Gregory C. Henderson1

1Department of Nutrition Science, Purdue University, West Lafayette, IN 2Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, IN

[Background] Fatty acids are primarily stored in white adipose tissue (WAT) and are continuously released into circulation by lipolysis. Excessive release of free fatty acids (FFA) and subsequent ectopic lipid deposition are central features of metabolic dysfunction. FFA are hydrophobic and bind to albumin for their dissolution into the bloodstream. Indeed, albumin-deficient (Alb-/-) mice exhibit reduced concentrations of plasma FFA, and hepatic triacylglycerol (TAG) and diacylglycerol (DAG), along with improved insulin sensitivity. However, the effect of albumin on the rate at which FFA appear in the circulation (i.e., serum FFA flux) remained unknown.

[Methods] 8 week old male and female Alb-/- mice and their wild-type (WT) littermates were intravenously injected with [U-13C]palmitate following a 16-h fast. Following the injection, the blood was collected from the tail immediately and then at 2.5, 5.0, and 7.5-min time points to obtain a single exponential equation for the time-related dilution of the tracer isotopic enrichment (IE). Labeled and unlabeled FFA were quantitated using liquid chromatography/mass spectrometry (LC/MS) for calculating FFA flux. Following the blood collection at the 7.5-min time point, epididymal WAT (eWAT) was collected for measuring adipose triglyceride lipase (ATGL), phosphorylated hormone-sensitive lipase (p-HSL), and total HSL protein expression by western blot.

[Results] There were significant main effects for genotype for FFA flux ($P = 0.001$) and FFA concentration ($P < 0.001$), with values of Alb-/- mice lower than those of WT mice. On the other hand, no significant main effect of sex nor any genotype-by-sex interaction was detected for both FFA flux and concentration. Thus, albumin deficiency similarly blunted the appearance rate of FFA in males and females. In addition, for eWAT protein levels, there was a significant genotype-by-sex interaction for the p-HSL/HSL ratio ($P < 0.05$), and a post hoc analysis showed the levels of female Alb-/- mice were significantly higher than those of female WT mice ($P < 0.05$). On the other hand, there were no significant main effects or interactions for total HSL or ATGL.

[Conclusion] The present result indicates that albumin is an important factor in determining the serum FFA flux rate in both males and females. The lower release rate of FFA into the bloodstream in Alb-/- mice was not explained by protein expression or phosphorylation of lipolytic proteins, suggesting that the lower FFA flux was likely a result of albumin deficiency per se. We conclude that the low serum FFA concentration in Alb-/- mice is a result of a lower export rate of FFA from storage sites into the circulation.

19.

The function of GDF3 on chronic inflammation during aging

In Hwa Jang, Victor Kruglov, Stephanie H. Cholensky, and Christina D. Camell

University of Minnesota

Increased visceral adiposity observed in aged individuals accompanies immune activation prior to that seen in other tissues. Notably, adipose tissue macrophages (ATM) have elevated levels of inflammation in the aged. Inflammatory ATMs support fibrosis, senescence, and metabolic dysfunction, which collectively lead to chronic inflammation during aging. Whole transcriptome analysis of ATMs identified growth differentiation factor 3 (GDF3) as a top-regulated gene by both age and NLRP3 inflammasome. Furthermore, Gdf3-deficient macrophages are less inflammatory with reduced activation of NLRP3 inflammasome.

GDF3, a member of the TGFβ family, initiates a signaling cascade that leads to phosphorylation of transcription factor SMAD2/3. GDF3-SMAD axis elicits divergent effects depending on the cellular context. ATMs are likely the primary responders to GDF3, acting in an auto/paracrine manner. However, how GDF3 affects the phenotype of aged ATM has not been investigated.

Here, we show that GDF3 plays a crucial role in the development of inflammaging by utilizing GDF3 global knockout (KO) in comparison with wildtype (WT) mouse model. Our data suggest that GDF3 induces an inflammatory phenotype in aged ATMs and impairs lipolysis with aging. Moreover, we test if inhibition of GDF3-SMAD2/3 signaling can reduce the level of inflammation in aged ATM by using a Specific Inhibitor of SMAD3 (SIS3). Our data indicate that SIS3 can decrease inflammation in aged ATMs and VAT and improve lipolytic capacity. Collectively, our study demonstrates that GDF3-SMAD2/3 axis may offer a new approach to resolving chronic inflammation with aging.

20.

The biochemical dissection of angiopoietin-like 3 function

Sydney Walker, Kelli Sylvers-Davie, Alex Dou, Shwetha Shetty, Rakshya Thapa, Bharat Bhattarai, Lucy Langmack, Kaleb Bierstedt, Francesca Reyes, Sara Park, Param Sampat, and Brandon Davies

University of Iowa

Low levels of circulating high-density lipoprotein (HDL) and high levels of circulating triglycerides are risk factors for developing cardiovascular disease. Levels of plasma lipoproteins are regulated in part by extracellular lipases, which hydrolyze lipoprotein lipids, releasing fatty acids for cellular uptake. Endothelial lipase (EL) and lipoprotein lipase (LPL) are two lipases known to regulate lipoprotein levels. EL hydrolyzes the phospholipids on high-density lipoproteins (HDL) and LPL hydrolyzes the triglycerides in triglyceride-rich lipoproteins (chylomicrons and very low-density lipoproteins (VLDL)). The activity of these lipases is highly regulated. One protein that regulates both EL and LPL is the secreted angiopoietin-like protein, ANGPTL3. ANGPTL3 inhibits EL and, when in complex with the related protein, ANGPTL8, also inhibits LPL, increasing HDL and triglyceride levels in the plasma. Recently ANGPTL3 has become a therapeutic target for reducing dyslipidemia in individuals at risk for cardiovascular disease. Although lipase inhibition by ANGPTL3 is known to be mediated by its N-terminal coiled-coil domain, the structural and functional properties that allow ANGPTL3 to specifically bind and inhibit EL or LPL remain unknown. This research aims to identify the specific residues of ANGPTL3 that mediate each of its functions and to understand its structure-function relationships.

21.

Functional characterization of plasma acylcarnitine uptake during physiological stress

Helaina Von Bank, Isabella James, Mae Hurtado-Thiele, Judith Simcox

University of Wisconsin-Madison

Acylcarnitines are intermediates of fatty acid oxidation that are increased in the plasma during acute physiological stresses as well as in chronic metabolic disease. In stresses such as cold exposure, plasma acylcarnitines serve as mobile lipid pool to support a shift from carbohydrate oxidation to fat oxidation, known as "metabolic flexibility". We found that Western diet fed mice lack the induction of acylcarnitines with cold exposure and are unable to maintain body temperature, which can be rescued with a bolus of free carnitine that stimulates acylcarnitine production. These findings indicate that acylcarnitines in the plasma are necessary for thermogenesis; despite this functional relevance, little is known about how acylcarnitines are transported into cells. Elucidating how the transport and metabolism of extracellular acylcarnitines is regulated is critical for understanding their normal physiological function as well as their potentially lipotoxic impact in disease states.

To investigate how acylcarnitine transport is regulated, we used differentiated brown adipocytes as a model for uptake, as plasma acylcarnitines are taken up by brown adipose tissue (BAT) during cold exposure. We modeled cold exposure in these cells with treatment of a B3-adrenergic receptor agonist (CL), which reflects activation of BAT by the sympathetic nervous system, and found that acylcarnitine uptake increases with CL treatment. We hypothesized that uptake is regulated though a plasma membrane transporter that is stimulated by B3-adrenergic signaling and has established affinity for carnitine. The canonical carnitine transporter OCTN2 emerged as a top candidate, as OCTN2 expression increases with CL treatment, and acylcarnitine uptake can be outcompeted with free carnitine. Using CRISPR-Cas9-mediated gene knockout, we found OCTN2 is required for CL-induced acylcarnitine uptake, suggesting that OCTN2 is necessary for uptake into brown adipocytes during cold exposure. We also found that OCTN2 was sufficient for acylcarnitine uptake and acylcarnitine-induced toxicity in Chinese hamster ovary cells. Currently, we are exploring how human variants in OCTN2 impact acylcarnitine uptake and toxicity as well as the mechanisms of acylcarnitine toxicity across different cell types. This work will expand our understanding of how circulating acylcarnitines function in healthy physiology as well as in metabolic diseases that have altered acylcarnitine profiles, including type 2 diabetes, fatty liver disease and cardiovascular disease.

22.

Electrophilic Lipid Peroxidation Products Effect Carbonyl Stress, Mitochondrial Dysfunction, and Cellular Senescence: Implications for Adipose Senescence in Aging and Obesity

T Blake Monroe1,2, Simon Vergara Santibanez1,2, Ann Hertzel1,2, Deborah Dickey1,2, Islam Berdaweel3, Ethan J. Anderson3, Paul D. Robbins1,2, Christina Camell1,2 and David Bernlohr1,2

1Dept of Biochemistry, Molecular Biology and Biophysics, University of Minnesota-Twin Cities, Minneapolis, Minnesota, 55455 2Institute for the Biology of Aging and Metabolism (IBAM), University of Minnesota-Twin Cities, Minneapolis, Minnesota, United, 55455 3Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, Fraternal Order of Eagles Diabetes Research Center, University of Iowa 52242

Accumulation of senescent cells in adipose tissue, particularly visceral depots, is increasingly becoming more appreciated as a causal factor in age- and obesity-associated metabolic dysfunction. As lipid peroxidation and pertinent drivers are shown to be associated with lifespan and age-related pathologies, we posit that resulting electrophilic lipid byproducts can incite or potentiate senescence in adipose. Produced abundantly in adipocytes by peroxidation of PUFAs, lipid aldehydes such as 4-hydroxynonenal (HNE) covalently modify nucleophilic DNA and protein moieties and herein we hypothesize that carbonyl stress exerts genotoxicity, oxidative stress, and mitochondrial dysfunction to induce the senescence program in adipose progenitor cells.

IMR90 fibroblasts and murine adipose stem cells continuously exposed to lipid aldehydes for seven days exhibit initiation of the senescence program, characterized by enhanced expression of CDKN1A (p21Cip1) among other classic senescence markers.We've termed this type of senescence Biogenic Lipid Induced Senescence, BLIS. The overall mechanism of lipid aldehyde associated senescence is multifaceted and likely to involve both DNA and mitochondrial protein modification, as we observed hallmarks of both genotoxicity and aberrant mitochondrial function concomitant with development of BLIS markers and alkylation of mitochondrial proteins. Indeed, 4-HNE exposure effects γH2AX foci and downstream p53/p21 signaling, as well as phosphorylation of AMPK and increased ADP:ATP ratios. Interestingly, we also demonstrated that formation of BAK/BAX channels in the mitochondrial outer membrane mediates the BLIS phenotype, offering an intriguing mechanistic link between carbonyl stress and BLIS. L-carnosine, a carbonyl scavenger, ameliorated the development of the senescent phenotype in cultured cells and blunted expression of p21Cip1 in visceral fat of diet-induced obese C57/Bl6J mice. Taken together, our results suggest that reactive lipid aldehydes can induce cellular senescence in human fibroblasts and adipose stem cells and that adipose senescence may be linked to lipid-mediated senescence induction in visceral fat tissue, and downstream systemic insulin resistance.

23.

Increased palmitate synthesis in metastatic breast cancer cells that target the lung

Morgan Lydia Conrad, Marjorie Anne Layosa, Dorothy Teegarden

Department of Nutrition Science, College of Health and Human Sciences, Purdue University

Breast cancer is the most common cancer among women and metastases are responsible for the majority of deaths from the disease. Dysregulated metabolism such as higher de novo fatty acid synthesis reported in metastatic cancer cells may support proliferation and survival of cancer cells. Understanding how cancer cells adapt to different conditions to regulate fatty acid synthesis during the specific steps in the metastatic cascade, such as extracellular matrix (ECM) detachment and hypoxia, may contribute to potential therapeutic strategies to prevent or reduce breast cancer progression. We utilized non-metastatic (M-Wnt) and isogenic metastatic breast cancer cells that preferentially metastasize to the lung (metM-WntLung) to examine differences in de novo fatty acid synthesis from glucose in ECM detachment and hypoxia. Under basal attached conditions, 13C6-glucose incorporation into palmitate, palmitoleate, stearate, and oleate was higher in metM-WntLung by 24%, 82%, 26%, and 89%, respectively, compared to the M-Wnt cells. Consistent with these results, mRNA abundance of ATP-citrate lyase (ACLY), which generates acetyl CoA from citrate as a substrate for de novo fatty acid synthesis, was 40% higher in metM-WntLung. In ECM detached conditions, 13C6-glucose incorporation into unsaturated fatty acids (16:1 and 18:1) was significantly reduced in metM-WntLung compared to M-Wnt cells. Interestingly, in ECM detachment, ACLY mRNA abundance was significantly reduced but pyruvate carboxylase (PC), which converts pyruvate to oxaloacetate and entry into the tricarboxylic acid cycle, was upregulated by 5-fold in metM-WntLung compared to M-Wnt cells. In contrast, in hypoxia palmitate synthesis from 13C6-glucose was 38% greater in metM-WntLung while other fatty acids were similar compared to M-Wnt cells. However, ACLY mRNA abundance was significantly lower, and PC was undetected in metM-WntLung under hypoxic conditions, suggesting upregulation of other metabolic pathways involved in higher palmitic fatty acid synthesis in metM-WntLung other than ACLY and PC in hypoxia. Thus, our results suggest that 13C6-glucose incorporation into palmitate is upregulated in basal attached conditions, ECM detachment, and hypoxia in breast cancer with preferential metastasis to the lung, potentially by regulating different metabolic enzymes depending on the stress conditions to which the cells are exposed during metastasis. Thus, inhibiting the ability of cancer cells to increase de novo fatty acid synthesis via differing targets during metastasis may contribute to the development of strategies to prevent or treat metastasis.

24.

Fatty acid availability and conversion to fatty-acyl-CoA regulates lipid droplet-mitochondrial interactions in a perilipin 5-dependent manner

Erica R. Gansemer, PhD, Mari V. Reid, & Douglas G. Mashek, PhD

University of Minnesota

Organellar interactions are critical hubs for the compartmentalization and maintenance of cellular metabolism. Based on recently characterized unique structural and functional features of cytosolic and peridroplet mitochondria, we sought to identify a mechanism regulating the formation of lipid droplet-mitochondrial interactions, and consequential changes in mitochondrial morphology and function. We discovered that fatty acid availability and subsequent conversion to fatty-acyl-CoAs promote lipid droplet-mitochondrial interactions and play a role in maintaining mitochondrial morphology, calcium load, and membrane potential. Additionally, fatty-acid availability failed to induce lipid droplet-mitochondrial interactions in the absence of the known lipid droplet tethering protein, perilipin-5, suggesting an interaction—whether direct or indirect—between perilipin-5 and fatty-acyl-CoAs. We determined that fatty-acyl-CoAs likely indirectly regulate perilipin-5 by binding to and targeting alpha/beta hydrolase domain-containing protein 5 to interact with perilipin-5 instead of ATGL. Taken together, these findings define a novel role for fatty-acyl-CoAs and alpha/beta hydrolase domain-containing protein 5 in regulating lipid droplet-mitochondrial interactions and downstream mitochondrial function.

25.

Development an LC-HRIM-MS workflow and database for untargeted plasma lipidomic analyses

Chuck Cloyd, Rachel A. Harris1, Michelle English1

Moblilion Systems

Analyzing lipid extracts is complicated by the presence of numerous isomers, which are challenging to fully characterize using traditional liquid chromatography-mass spectrometry (LC-MS) workflows. The introduction of high-resolution ion mobility (HRIM) techniques, such as structures for lossless manipulations (SLIM), enables rapid, gas-phase separation of lipids with resolving powers over 250, facilitating the identification of biologically relevant lipid isomers uncharacterized in prior analyses. The development of a full LC-HRIM-MS workflow for lipidomic analysis should enable deeper characterization of samples via these multidimensional separations. Additionally, calibration of HRIM data allows the determination of collision cross section (CCS) values for greater identification confidence. In this work, we demonstrate the application of an LC-HRIM-MS method for the creation of a lipid database that enables identification of lipids to a higher level of structural specificity than lipid headgroup and fatty acyl composition, containing 35 lipid species across multiple lipid classes expected to be present in serum, including PC, PE, TG, SM, and Cer species. Multiple forms of isomerism were included for the species in the database, such as sn regioisomers, cis/trans isomers, and double bond position isomers. This database was then applied to the untargeted analysis of a plasma extract, NIST SRM 1950. Data analysis was performed using Lipostar 2 software from Molecular Discovery, a vendor neutral, high throughput software package that enables tunable feature finding for both LC-MS and LC-IM-MS workflows. Significant attention was paid to optimization of the feature finding step for the high-resolution mobility data. Using this workflow, we were able to detect multiple lipid features corresponding to species included in the database and in some cases were able to detect specific isomers based on their measured CCS.

26.

Supraclavicular brown adipocytes originate from Tbx1+ myoprogenitors

Chenxin Gu, Zan Huang, Hai-Bin Ruan

University of Minnesota

Brown adipose tissue (BAT) dissipates energy as heat, contributing to temperature control, energy expenditure, and systemic homeostasis. In adult humans, BAT mainly exists in supraclavicular areas and its prevalence is associated with cardiometabolic health. However, the developmental origin of supraclavicular BAT remains unknown. Here, using genetic cell marking in mice, we demonstrate that supraclavicular brown adipocytes do not develop from the Pax3+/Myf5+ epaxial dermomyotome that gives rises to interscapular BAT. Instead, the Tbx1+ lineage that specifies the pharyngeal mesoderm marks the majority of supraclavicular brown adipocytes. Tbx1Cre-mediated ablation of peroxisome proliferator-activated receptor gamma (PPARγ) or PR/SET Domain 16 (PRDM16), components of the transcriptional complex for brown fat determination, leads to supraclavicular BAT paucity or dysfunction, thus rendering mice more sensitive to cold exposure. Moreover, human deep neck BAT expresses higher levels of the TBX1 gene than subcutaneous neck white adipocytes. Taken together, our observations reveal location-specific developmental origins of BAT depots and call attention to Tbx1+ lineage cells when investigating human relevant supraclavicular BAT.

27.

A potential role for adipocytes in visceral leishmaniasis

Patrick Kwadwo Nuro-Gyina, Bayan Zhanbolat, Yani Chen, Carter R Dwyer, Jacilara Alexandrino-Conceicao, Aloysius Klingelhutz and Mary E. Wilson

Departments of Internal Medicine and Microbiology & Immunology, University of Iowa and the Veterans' Affairs Medical Center, Iowa City IA

Leishmaniasis is a chronic parasitic disease in which parasites are found in host macrophages throughout the reticuloendothelial organs. Recent data indicate that many cells not thought of traditionally as immune cells contribute to the cytokine/chemokine environment locally where the parasite survives, and to the systemic immune responses. Adipocytes are versatile cells whose functions in systemic energy homeostasis and as reservoir for excess energy are well documented. Adipocytes are present in subcutaneous tissues near the parasite inoculum, but their potential involvement in immunoregulation of leishmaniasis is unknown. To determine whether and how adipocytes interact with the Leishmania species, we differentiated human preadipocytes into adipocytes and incubated them with L. infantum, L. major or L. braziliensis promastigotes. We found that the Leishmania spp. parasites were taken up by human adipocytes and transformed morphologically to the intracellular amastigote form, but this occurred differently between the species. L. infantum was taken up by 20.0% of adipocytes, whereas L. major or L. braziliensis were taken up by 11.5 or 4.8% of adipocytes, respectively. We also found parasites in both white (WAT) and brown adipose tissues (BAT) of mice upon infection with Leishmania spp., though long term (5 months) parasite load was higher in the BAT compared with the WAT of mice. RT-qPCR for candidate immunoregulatory transcripts revealed up-regulation of the IL6, IL8, IFNGR1 and IFNAR1 whereas genes transcripts involved in adipogenesis and lipogenesis such as Adiponectin, and PPAR□ were down-regulated. These data suggest a potential role for adipocytes in Leishmania spp. infection, although the roles may differ between different Leishmania species.

28.

SEX DIFFERENCES IN CANNABINOID DISTRIBUTION, METABOLISM, AND LIPID MODULATION ACROSS THE BRAIN

Clare T. Johnson, Hannah R. Bentz, Taryn Bosquez-Berger, Krishi Thacker, Shaylee Barnes, Emily Richter, Gabriel H.D. Abreu, Ken Mackie, Hui-Chen Lu, **Heather B. Bradshaw**

Indiana University

Introduction: Cannabinoids (CBs) have behavioral and physiological effects that are sex depen-dent. Our lab has previously shown that Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD) modulate CNS endogenous lipids (endolipids). Here, we investigated how CBD, THC, or the com-bination of CBD and THC would modulate endolipids across the brain of both male and female mice. Specifically, we hypothesized that CBD, THC, and their metabolites would be present in the brain at different concentrations depending on the sex of the animal and brain region inves-tigated. Additionally, we hypothesized that endolipids including N-acyl ethanolamines (NAEs) and 2-acyl-sn-glycerols would be modulated in a drug-, sex-, and region-dependent manner.

Methods: Male and female CD1 mice were injected i.p. with THC (10mg/kg, nmale=10, nfemale=10), CBD (10mg/kg, nmale=11, nfemale=9), THC+CBD (10mg/kg, nmale=11, nfemale=11), or vehicle (, nmale=8, nfemale=8). After 2hrs, animals were sacrificed, and their brains collected. Brain tissue was flash frozen on liquid nitrogen, dissected into 8 regions for lipid extraction (brainstem, hippocampus, hypothalamus, thalamus, midbrain, striatum, cortex, and cerebellum), and stored at -80˚ C until being processed. To extract lipids, samples were incubated in methanol + 5µL of 1µM internal standard (d8-AEA) for 2h in the dark on ice, then homogenized via sonication, centrifuged at 19,000G, 20˚ C for 20 min, and the lipid-containing supernatant decanted into water to make a ~25% organic solution. Lipids were partially purified from this solution using C18 solid phase extraction columns, eluting into 1-1.5mL of 65%, 75%, and 100% methanol. HPLC/MS/MS was then used to screen for ~87 lipids and calculate mol/gram concentrations for each tissue type. One-way ANOVAs with Fishers LSD post-hoc tests were used to determine significant differences in drug treatment groups within each sex, and independent t-tests were used to determine significant differences between sexes.

Results: Results showed that patterns in CB distribution were sex- and drug- dependent, though these patterns were markedly similar across the brain. Of note, in control animals, males had significantly higher levels in 2-sn-acyl glycerols in most brain regions, but NAE levels did not differ significantly from females. THC treatment primarily led to decreases in endolipids, though effects varied by brain region and sex. CBD had fewer effects on endolipids than THC or THC+CBD treatment. The effects of THC+ CBD were not wholly additive, with the combination treatment frequently causing a novel phenotype in lipid distribution.

Conclusion: These findings support the hypothesis that 10mg/kg CBD, THC, and THC+CBD affect endolipids in the brain 2hrs after i.p. administration. They also support that effects are sex- and brain region-dependent, as are concentrations of CBs and their metabolites.

29.

Physiological effects of global ATGL overexpression in mice

Mahima Devarajan, Mara Mashek, **Simone Intriago,** Peter Gutenberger, Jessica Luna Sanchez, Linshan Shang, Douglas Mashek

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota

Adipose triglyceride lipase (ATGL) is the major triglyceride lipase in most tissues. Global deletion of ATGL leads to cardiomyopathy and early death, and tissue-specific knockouts typically lead to lipid droplet accumulation and metabolic dysfunction. In contrast, models with tissue-specific ATGL overexpression commonly exhibit improvements in various physiological parameters. Previously, we have shown that drosophila overexpressing the ATGL homolog brummer exhibit increased physiological fitness and improved markers of healthspan indicated by enhanced resistance to thermal stress, increased locomotion, proteostasis, and fecundity. These studies align with a growing body of literature showing reductions in ATGL, lipolysis, and lipid metabolism with aging. Thus, to determine the effects of systemic increases in lipolysis in a mammalian model, we generated mice that globally overexpress ATGL (AKI mice). Male AKI mice had reduced body weight and adiposity despite increased food intake without changes in energy expenditure. Aged AKI mice exhibited reduced adipose tissue inflammatory macrophage inflammation and nearly undetectable hepatic lipid droplets suggesting that ATGL had robust effects on tissue specific energy metabolism and inflammatory signaling. Despite these beneficial effects, aging related neuromuscular function and lifespan do not appear to be different in the AKI mice. Overall, AKI mice exhibit some markers of improved physiological fitness, but these effects are not sufficient to extend lifespan in male mice.

30.

Prior exercise training increases oxylipins secretion from brown adipose tissue response to cold exposure

Shinsuke Nirengi1,2, Hirokazu Taniguchi3, Diego Hernández-Saavedra1, Pablo Vidal1, Lisa Baer1, Valerie Bussberg4, Michael Kiebish4, Masayuki Saito5,6, Naoki Sakane2,Kristin Stanford1.

1Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University Wexner Medical Center, Columbus, OH, USA 2Division of Preventive Medicine, Clinical Research Institute, National Hospital Organization Kyoto Medical Center, Kyoto, Japan 3Division of Applied Life Sciences, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan 4BERG, Framingham, Massachusetts 01701, USA 5Department of Nutrition, Tenshi College, Sapporo, Japan 6Department of Biomedical Sciences, School of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Exercise-training increases phosphatidylcholine (PC) containing polyunsaturated fatty acids (PUFAs) in the brown adipose tissue (BAT) in mice. The PC mainly comprises the membrane, and membrane lipids are a potential source of oxylipins. The oxylipins are bioactive lipids derived from polyunsaturated fatty acids (PUFAs) and are involved in metabolic disease. BAT releases oxylipins in response to exercise and cold exposure, and we hypothesized that one mechanism for the beneficial effects of exercise was by increasing the release of oxylipins in response to cold.

To address this, we measured oxylipins and the presence of BAT before and after 2h of cold exposure in a group of young, male athletes and sedentary, BMI matched subjects. Seven oxylipins were increased in sedentary BAT-positive subjects compared to sedentary BAT-negative subjects after cold exposure. Notably, there were 44 oxylipins that were increased in BAT-positive athletes compared to BAT-negative athletes after cold exposure.

To determine if changes in PCs contribute to the change in oxylipins, we performed a retrospective analysis on structural lipids in BAT after cold-exposure or exercise-training in mice. There were 20 PCs increased and 1 decreased with exercise, while 9 PCs were increased and 27 decreased in response to cold exposure in mice. To determine if exercise changed PC metabolism in BAT, we performed qPCR analysis in sedentary or exercise-trained mice after mild cold exposure. Genes involved in the Kennedy pathway and PUFA-cleaved from phospholipids were increased in prior trained following cold exposure mice in the BAT. These data suggest that exercise increases PC as a source of oxylipin in the membrane and cold exposure cleaves the PC to produce oxylipins.

These data indicate that exercise training prior to cold exposure increases secretion oxylipins from BAT.

31.

Impact of diet on the phenotypic and immune responses to L. infantum infection

Natalie C. Jarvis1, Grace Gutzman1, Yani Chen2, Bayan Zhanbolat2, Selma M. B. Jeronimo3,4 and Mary E. Wilson1,2,5

1Department of Microbiology and Immunology, University of Iowa, Iowa City, IA 52242, USA 2Department of Internal Medicine, University of Iowa, Iowa City, IA, USA 3Institute of Tropical Medicine, Federal University of Rio Grande do Norte, Natal, RN, Brazil 4Department of Biochemistry, Federal University of Rio Grande do Norte, Natal, Brazil 5Veterans' Affairs Medical Center, Iowa City, IA 52252, USA

Leishmaniasis refers to a group of disease caused by the Leishmania infantum, which are prevalent in many nations worldwide. Different clinical forms include cutaneous leishmaniasis (CL) which causes skin ulcers and visceral leishmaniasis (VL) which causes potentially fatal disease resembling leukemia. About 1 million people are infected annually with Leishmania spp. parasites. VL affects reticuloendothelial tissues, including the liver and spleen, with predictable kinetics of dissemination through different mouse tissues. Preliminary studies of mouse models from our lab have indicated that host metabolic state, as determined by the dietary intake of mice at the time of infection, alters the kinetics of parasite growth and dissemination in different organs. Furthermore, regional dietary changes in Brazil and resultant shifts in BMI have led our Brazilian collaborators to investigate the effects of body habitus on leishmaniasis. In a region with endemic CL due to L. braziliensis, they observed poorer outcomes after treatment of obese or overweight patients with cutaneous leishmaniasis (CL) than patients with a lower BMI. In contrast to CL, malnourishment is a predisposing factor for VL. The effects of obesity on disease are not known. Our study aimed to contrast the effects of diet-induced protein malnourishment versus obesity in a murine model of VL, and to determine the magnitude of tissues involved as a first state in defining mechanisms underlying these changes. Mice were maintained on 1 of 3 diets for 4 weeks prior to infection: a control diet, a high-fat high-cholesterol diet, and a low protein (malnourished) diet. Half of the mice from each diet group were infected intravenously via their tail vein with 1 x 106 L. infantum. DNA, RNA and protein were extracted from livers, spleens, and brown and white adipose tissue (BAT/WAT) of infected and control mice. Our plan is to document parasite loads by qPCR, tissue involvement by histology and RT-qPCR for parasite and host transcripts, and effects of infection versus diet on adipokines and cytokines expressed by adipose tissue. A link between metabolic state and the burden of Leishmania could help identify preventative or alternative clinical interventions to introduce in endemic regions such as Brazil to more effectively combat the prevalence and severity of Visceral Leishmaniasis.

32.

Loss of brown adipose-specific FXR alters energy metabolism and glucose homeostasis

Weinan Zhou, Sayeepriyadarshini Anakk

Department of Molecular and Integrative Physiology, University of Illinois Urbana-Champaign, Urbana, IL

Obesity increases the risk of a variety of metabolic diseases. Adipose tissue functions as a key regulator of whole-body energy homeostasis through the storage, mobilization, and dissipation of energy. There are two major types of adipose tissues: white and brown. White fat depot stores lipids, while brown adipose tissue burns fat and glucose to generate heat. Nuclear receptor farnesoid X receptor (FXR) transcriptionally regulates glucose and lipid metabolism in various tissues and has been associated with fat burning and brown fat function. Recently, the role for FXR in remodeling of fat-storing white adipose tissue during obesity has been demonstrated. However, the exact role of FXR in brown fat depot still remains elusive. We generated the first brown adipocyte-specific Fxr knockout (BAT-FxrKO) mouse model using UCP1-Cre. The BAT-FxrKO mice exhibited lower body weight, increased expression of genes regulating thermogenesis, and improved glucose handling compared to control mice even under normal chow fed conditions. These data suggest an unexplored inhibitory role of FXR signaling in brown fat thermogenic function and systemic energy homeostasis. But when challenged with a 60% high fat diet for 8 weeks, BAT-FxrKO mice lost the beneficial basal effects and displayed glucose intolerance, brown fat hypertrophy, and gained weight. Overall, we uncover that FXR may have distinct roles in brown adipocytes under normal and obese conditions.

33.

Lipophagy is constitutively active in pancreatic beta cells

Dalal El Ladiki, Siming Liu, Brianna Brennecke, USeong Yang, James Ankrum, Sandra Blom, Sam Stephens, Yumi Imai

University of Iowa

Lipid droplets (LDs) play a vital role in supporting beta cell function and health as they are dynamically being formed and utilized in rat and human adult beta cells. It is known that ATGL mediated lipolysis releases FA from LD to augment insulin secretion from beta cells. Lysosomal acid lipase (LAL) encoded by LIPA gene is an enzyme known to degrade neutral lipids in the lysosome. In other cells, lipophagy by lysosomal acid lipase (LAL) has been shown as an additional pathway for FA release. Therefore, we aimed to address the role LIPA in the regulation of beta cell function. LIPA activity is negatively correlated with aging in human islets($p<0.03$). Suppressing LIPA using LAL inhibitor (LAListat2), or siRNA/shRNA led to a significant increase in number and size of lipid depot in INS1 beta cell line, rat primary beta cells and human beta cells ($p<0.05$) in nutritionally sufficient condition. These three models showed that suppression of LIPA mediated lipophagy lead to an increase in TG content confirming the active role of lipophagy. Unlike in hepatocytes, the co-downregulation of ATGL and LIPA with siRNA did not prevent significant LD accumulation($p<0.05$). Even though acute LIPA suppression by lali2, did not affect GSIS, chronic LIPA suppression or downregulation lead to a significant reduction of GSIS in INS1 cells and human islets. NSG mice transplanted under their kidney capsule with shLIPA human islets showed a significantly reduced insulin response to GTT compared to mice with shScr human islets (2-Way ANOVA, $p<0.05$). To understand the observed effect of LIPA's downregulation on b cell health, we looked at the mitochondria. INS1 cells with siLIPA had a significantly decreased mitochondrial DNA compared to siScr. In addition, INS1 cells with siLIPA showed mitochondrial fragmentation compared to siScr. To conclude, pancreatic beta islet cells have a constitutively active lipophagy pathway mediated by LIPA to degrade LD that is crucial for long-term beta cell function.

34.

Deficiency of adipose FXR exacerbates hepatic lipid accumulation during obesity.

Diana Ximena Yrigoyen-Rosas, Neal Dadlani, Weinan Zhou, and Sayeepriyadarshini Anakk

University of Illinois Urbana-Champaign

Obesity affects ~20.9% of the young population and ~42.4% adults in the United States. It is characterized by excess fat accumulation and increased risk for developing chronic metabolic diseases, including non-alcoholic fatty liver disease (NAFLD). Farnesoid X receptor (FXR) is a nuclear receptor highly expressed in the liver and intestine, which regulates bile acid homeostasis and systemic glucose and lipid metabolism. Previous studies noted FXR expression in the adipose tissue and showed that this receptor can regulate differentiation of preadipocytes into adipocytes in vitro. We generated and characterized adipose-specific Fxr knockout (Ad-FxrKO) mice and found these mice exhibited adipocyte hypertrophy and systemic glucose intolerance. To investigate its role in obesity and NAFLD, we challenged Ad-FxrKO and a control-group mice with either a normal chow, 60% high fat (HFD), or high-sugar and high-fat western diet (WD) for 4 weeks. Intriguingly, we found that the loss of adipose FXR led to ectopic fat accumulation in the liver during high fat diet challenge. Hepatic lipid accumulation and the expression of lipid metabolic genes were determined by hematoxylin and eosin (H&E) and oil red O staining, as well as quantitative PCR (qPCR). We found that Ad-FxrKO exhibited increased transcript levels of beta-oxidative gene peroxisome proliferator-activated receptor (PPAR)-γ in the liver compared to the control mice upon WD but not HFD conditions. Overall, we find that loss of adipose Fxr can impinge on hepatic fat deposition. By extrapolating this data, we speculate that FXR signaling may contribute to liver adipose cross talk.

35.

Piceatannol ameliorates muscle atrophy potentially through STAT3 inhibition in an in vitro model of cancer-associated cachexia

Tongkewn Yoo, Jonathan Kershaw, and Kee-Hong Kim

Purdue University

Piceatannol, a natural resveratrol analog commonly found together in blueberries and passion fruit, has been demonstrated to have bioactivities, including antioxidant, anti-inflammatory, and anti-obesity properties. Previously, we reported an inhibitory effect of piceatannol on adipose lipolysis by suppressing lipid droplet mobilization/catabolism/hydrolysis. Cancer cachexia, a wasting syndrome, is characterized by uncontrolled body fat loss and muscle wasting which often occurs in lung, pancreatic, and colorectal cancer patients, diminishing their tolerance to chemotherapy and decreasing the survival rate. Among the proposed mechanisms contributing to cancer cachexia, STAT3 (Signal transducer and activator of transcription 3) is a major factor responsible for the increased expression of muscle-specific ubiquitin ligases, which degrade muscle proteins. We recently found a potential role of piceatannol in cancer-associated cachexia (CAC), in which piceatannol administration to animals bearing colorectal cancer was found to protect them from cancer-induced body weight loss. However, the role of piceatannol in muscle atrophy in CAC is unknown. Therefore, the objective of this study is to determine the role of piceatannol in CAC-induced muscle degradation. Here we found that piceatannol significantly suppresses myotube atrophy in C2C12 murine myotubes induced by C26 murine colon carcinoma-derived conditioned media (CM-C26). Interestingly, the inhibitory effect of piceatannol on CM-C26-induced myotube atrophy was compatible with that of STAT3 inhibitor. Taken together, in addition to the demonstrated ability of piceatannol to inhibit cancer-induced adipose lipolysis, our results implicate a novel function of piceatannol in cancer-induced muscle atrophy.

36.

Proteomic Analyses of Cytoplasmic Lipid Droplet and Whole Cell Lysates Reveal Ferroptosis-Regulation by Fatty Acid Synthesis-Derived Lipid Droplets as Major Potential Metastatic Breast Cancer Pathway

Chaylen J Andolino1, 2; Marjorie Layosa1, Kimberly K Buhman1; Dorothy Teegarden1

1Department of Nutrition Science, Purdue University, West Lafayette, IN; 2Purdue Proteomics Facility, Bindley Bioscience Center, Purdue University, West Lafayette, Indiana

Background: Breast cancer remains a serious public health concern, as it is the most diagnosed and second most fatal cancer in women in the United States. Notably, metastasis of the disease accounts for the majority of patient mortality. Metastases often have dysregulated energy metabolism, including lipid accumulation. We previously demonstrated that metastatic MCF10CA1a breast cancer cells have higher palmitate synthesis from glucose and glutamine, triacylglycerol (TAG) content, and cytoplasmic lipid droplets (CLDs) compared to non-metastatic MCF10A-ras cells. Additionally, fatty acid synthase (FASN)-derived TAG stores sustained key metastatic processes of migration and survival in detached conditions in the MCF10CA1a cells. The proteome of CLDs is diverse and often reflects their function; therefore, identifying proteins differentially associated with CLDs from vehicle-treated and FASN-inhibited MCF10CA1a breast cancer cells may uncover novel roles of CLDs in promoting metastasis.

Methods: We performed untargeted, global proteomics analysis on whole cell lysates (WCLs) and CLDs isolated by sucrose-density gradient ultracentrifugation from vehicle-treated and FASN-inhibited (TVB-3166, 20 nM, 3d) MCF10CA1a cells to determine protein pathways differentially enriched on CLDs. Each sample (20 µg protein) was delipidated, precipitated, and digested with Trypsin/Lys-C. Peptides were cleaned using C18 spin columns and analyzed by reverse-phase LC-ESI-MS/MS system coupled to the Orbitrap Fusion Lumos Mass Spectrometer. Data search, filtering, and transformation was performed using MaxQuant and Perseus. A protein was considered present when identified in ≥ two of the three biological replicates, and significantly different between groups based on LFQ (two-tailed t-test; $p<0.05$). Data containing imputed values (based on the normal distribution of other detected proteins within the sample) was used to annotate proteins via Metascape.

Results: We identified 2,416 and 3,539 proteins within CLD fractions and WCLs, respectively, from the vehicle-treated and FASN-inhibited human metastatic MCF10CA1a breast cancer cells. Of the proteins identified in CLD fractions, about 30% (717) were commonly identified within both conditions. Interestingly, 1,571 proteins were unique to the CLD fraction from vehicle-treated cells, whereas only 19 proteins were unique to the CLD fraction from FASN-inhibited cells. Proteins unique to CLDs from more migratory, vehicle-treated cells include proteins known to reduce cancer cell survival and progression, such as those involved in cell-adhesion, cell cycle regulation, and ferroptosis (cell death due to lipid peroxidation). We hypothesize that CLD association with proteins known to reduce cancer cell survival and progression prevents these proteins from functioning at their normal site of action.

To test this, we specifically focused on ferroptosis-related proteins, and hypothesized that ferroptosis is lower in the highly metastatic MCF10CA1a cells compared to FASN-inhibited, TAG-depleted cells. Although proteins that promote ferroptosis, such as STEAP3 metalloreducase, were more abundant in the CLD fraction of the more metastatic cells, their levels between the two WCLs were not different. This observation supports the hypothesis that the machinery for ferroptosis is not where it needs to be to function in metastatic cells. On the otherhand, proteins such as glutathione peroxidase 4 (GPX4), that inhibit ferroptosis, were only found in the WCL and CLDs of the vehicle-treated cells, further suggesting that ferroptosis may be protected against within the more migratory, TAG-rich cells.

Given these results, we measured differences in ferroptosis (determined by quantification of the free intracellular iron dye, FerroOrange) between the two groups. We observed that FASN-inhibited cells have nearly 30% more intracellular iron compared to the vehicle-treated cells, indicating higher levels of ferroptotic stress.Notably, although their cell viability (measured by MTT assay) is comparably reduced following additional ferroptosis-stimulation using erastin, vehicle-treated cell viability is rescued by NAC-treatment by 43%, whereas the FASN-depleted cells have an 18% increase in viability. These preliminary findings suggest that the excess FASN-derived CLDs may help protect against ferroptosis to promote metastatic breast cancer cell progression.

37.

Age-dependent changes in mouse brain and liver lipidomes

Punyatoya Panda1, Christina R. Ferreira2, Allison Schaser3, Uma K. Aryal1,2

1Department of Comparative Pathobiology, Purdue University, West Lafayette, IN 47907, USA. 2Bindley Bioscience Center, Purdue University, West Lafayette, IN 47907, USA. 3Department of Speech, Language, and Hearing Sciences, Purdue University

Aging is a major risk factor for various diseases such as cancer and neurological disorders including Alzheimer's disease. However, the mechanisms of aging are complex and remain elusive. Like genes and proteins, lipids play key structural, regulatory, and signaling roles within the cells. Therefore, characterizing lipids in various organs provides useful information for understanding their functions under different physiological or disease states. However, relatively very little is known about the composition and age-dependent changes of lipids in the brain and liver, the two most lipid-rich organs after adipose tissues. In this work, we characterized the brain and liver lipidome using two main analytical approaches: targeted shotgun profiling using Multiple Reaction Monitoring and untargeted Liquid Chromatography-tandem mass spectrometry (LC-MS/MS). The MRM profiling analysis focused on specific ion transitions corresponding to different lipid classes and fatty acid composition based on LIPID MAPS database. The untargeted LC-MS/MS analysis used product ion scans and the MONA database for lipid identification. For MRM profiling, we screened 3246 MRMs comprising of 24 different lipid classes and fatty acid composition and identified 571 MRMs in the brain and1254 MRMs in the liver. In the brain, phosphatidylcholines (PCs), phosphatidylethanolamines (PEs) and free fatty acids (FFAs) were among the most abundant classes of lipids, while in the liver, tri- and di-acylglycerols were among the most abundant ones, apart from PCs and FFAs. Statistical analysis revealed age-dependent changes in sphingomyelins, TGs and FFA in the brain, and TGs, DGs, and phospholipids classes in the liver.

Untargeted lipidomics identified 226 and 193 lipids in the brain and liver respectively, among which PCs, PEs, hexocylceramides, and DGs, were the most differentially changing lipid classes with age. In the liver, phospholipids were predominantly changing with age, including PCs, LPCs, PEs, PGs, PIs, and PSs. Lipids detected in the MRM and LC-MS/MS data showed a high degree of overlap (100% in the brain and 98% in the liver)) indicating consistency and agreement between the two analytical methods. The significantly changing lipids in the brain and liver related to aging were also consistent between the MRM profiling and the LC-MS/MS methods. In conclusion, the shotgun profiling using MRM enables simpler, faster, sensitive, and cost-effective exploratory lipid analysis workflow for characterizing the lipidome in diverse biological samples. Understanding age-dependent changes in lipid composition using this MRM method can shed light on potential biomarkers and mechanisms associated with aging.

38.

Understanding the molecular basis of postnatal heart growth defects in mice lacking β-carotene 9',10'-oxygenase (BCO2)

1,2Elisabetta Madonna, 2Youn-Kyung Kim, 2Jason Diaz, 3Chelsee Holloway, 2Loredana Quadro

1 Nutritional Science Graduate Program, 2 Department of Food Science, Rutgers Center for Lipid Research & Institute of Food Nutrition and Health, Rutgers University, New Brunswick, New Jersey, USA; 3 NIH/National Cancer Institute (NCI), Molecular Targets Program, Center for Cancer Research, NCI, Frederick, MD

Vitamin A is an essential micronutrient that supports many critical biological functions in mammals throughout the life cycle [1]. The most abundant dietary source of vitamin A is β-carotene [2]. Our lab recently demonstrated that three-month-old female mice lacking β-carotene 9',10'-oxygenase (Bco2-/-), the only enzyme of the adult heart that cleaves β-carotene into vitamin A and its derivatives, i.e., retinoids, display cardiac retinoic acid insufficiency [3]. Moreover, the heart of both male and female Bco2-/- mice is smaller (ratio heart weight/tibia length) compared to the wild-type counterpart already at 4 days of life (postnatal day 4, P4), implying that the absence of the enzyme impairs the postnatal growth of this organ. The heart grows by proliferation in the first 7 days of postnatal life, after which a shift from glucose to fatty acids as the main energy source results in cardiomyocyte cell cycle arrest and in a shift from proliferative to hypertrophic growth [4, 5]. This study aims to understand the molecular mechanism underlying the heart phenotype of the Bco2-/- mice. As the fat content and composition of the maternal milk have been indicated as among the main drivers of postnatal cardiomyocyte cell cycle arrest [5], ongoing experiments seek to assess fat content in the mammary glands of the mutant mothers. Additional studies in progress aim at determining whether cardiomyocyte number and dimensions are different between WT and Bco2-/- mice as well as at assessing potential perturbations in key proliferative signaling pathways in the heart of the mutant vs. WT mice at P4. Based on the current findings, we propose that the small heart phenotype of the Bco2-/- mice is due to defects in both proliferation and hypertrophy of the cardiomyocytes.

39.

Inhibition of Toxoplasma gondii by linoleic acid is host cell type-dependent

Nicole D. Hryckowian, Caitlin Zinda, Martin T. Kelty, Sung Chul Park, Nancy P. Keller, and Laura J. Knoll

University of Wisconsin-Madison

The obligate intracellular parasite Toxoplasma gondii causes toxoplasmosis, a dangerous disease for pregnant or immunocompromised people. T. gondii can infect and replicate in any warm-blooded cell tested to date, but most of our knowledge about T. gondii cell biology comes from just one host cell type: human foreskin fibroblasts (HFFs). To expand our knowledge of host-parasite interactions, we study T. gondii in intestinal epithelial cells, the first site of host-parasite contact following oral infection. Recently, we found that lipid metabolism differs dramatically in intestinal epithelial cells (Caco-2s) relative to HFFs. Highly metabolic Caco-2 cells are permissive to T. gondii growth even when treated with linoleic acid (LA), a polyunsaturated fatty acid (PUFA) that normally kills parasites in HFFs. Caco-2 cells appear to sequester LA away from the parasite, possibly by incorporating LA into host membranes or lipid droplets. Our current efforts are to: 1) identify Caco-2-specific metabolic pathways that prevent parasite uptake of LA and 2) extend our studies to non-cancerous intestinal cell lines. Our findings will improve our understanding of host-parasite interactions in the gut, an understudied but important part of the T. gondii life cycle.