3rd Annual Big Ten Academic Alliance Lipid Conference

September 27, 2019
Memorial Hall, McNamara Alumni Center,
University of Minnesota

Program

8:00-8:20 Check-in and poster setup

8:20-8:30 Introduction and welcome

8:30-9:00 Brandon Davies, PhD
Assistant Professor, University of Iowa
ANGPTL control of lipases and lipoproteins

9:00-9:30 Sayeepriyadarshini Anakk, PhD
Assistant Professor, University of Illinois
Bile acid Signaling in fat: a new sequel to the old soap

9:30-10:00 Loredana Quadro, PhD
Associate Professor, Rutgers University
The role of beta-carotene metabolism in maternal cardiac remodeling

10:00-10:30 Coffee break/poster browsing

10:30-11:00 Heather Bradshaw, PhD
Associate Professor, Indiana University
Investigating the lipoamine lipidome: effects of enzymes, receptors, and cannabinoid pharmacology

11:00-11:30 Wayne Riekhof, PhD
Assistant Professor, University of Nebraska
Membrane remodeling contributes to virulence in pathogenic fungi

11:30-12:00 Rob Stahelin, PhD
Professor, Purdue University
Lipid dependent assembly and budding of the Ebola virus

12:00-2:00 Lunch/posters

2:00-2:30 Brian Parks, PhD
Assistant Professor, University of Wisconsin
An integrative genetic approach to discover genes that regulate cholesterol metabolism

2:30-3:00 Charles Burant, PhD
Professor, University of Michigan
Mechanisms underlying mitochondrial fatty acid oxidative capacity, metabolic health and longevity

3:00-3:30 Peter Crawford, MD
Professor, University of Minnesota
Multi-dimensional roles of ketone bodies in fuel metabolism, signaling, and therapeutics

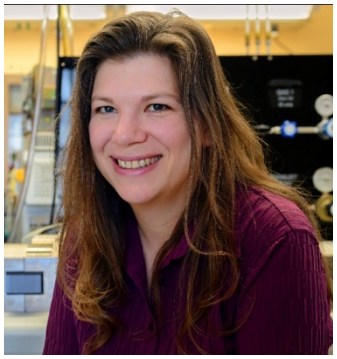
Sponsors

University of Minnesota
Biochemistry, Molecular Biology and Biophysics Department

Speaker Biographies

Sayeepriyadarshini Anakk, Ph.D. University of Illinois
Our laboratory is focused on understanding the role for bile acid signaling in regulating metabolism both during normal as well as diseased states. Bile acids are amphipathic molecules that are synthesized from cholesterol and are typically known for their role in fat digestion. Over the last decade, bile acids are recognized for their signaling through nuclear receptor, Farnesoid X receptor (FXR) and G-protein coupled receptor, TGR5. We are currently investigating how bile acids and nuclear receptor signaling coordinate energy homeostasis using cell-based systems and genetically engineered mouse models.

Charles F. Burant, M.D., Ph.D. University of Michigan.
Dr. Burant is Professor of Internal Medicine and the Robert C. and Veronica Atkins Professor of Metabolism, Section of Metabolism Endocrinology and Diabetes. He also has appointments in Integrative Physiology and Computational Medicine and Biology in the U-M Medical School and Human Nutrition, School of Public Health. He is the Director of the Taubman Research Institute which support biomedical studies a UM. Dr. Burant's research program centers on the interaction between genetics and environmental factors in the development of obesity, insulin resistance and diabetes and how intrinsic cardiorespiratory fitness can modulate risk of metabolic diseases. His studies integrate metabolomic and lipidomic profiling with other 'omics technologies to understand the emergent clinical phenotypes and use them to predict individual responses to diet, exercise and weight loss with a goal to individualize interventions to improve a person's metabolic health.



Heather B. Bradshaw, Ph.D. Indiana University
My research program combines the fields of neurophysiology, behavior, and lipid biochemistry to understand how lipid signaling drives changes in cellular communication. A central focus of my research relies on lipidomics discovery and characterization of endogenous lipids using mass spectrometric and signaling assays with a special emphasis on understanding their biosynthesis and metabolism. Ongoing investigations of the endogenous cannabinoid signaling molecule, *N*-arachidonoyl ethanolamine (Anandamide), drove the discovery of more than 80 endogenous structural analogs called "Lipoamines", which we have shown to be produced in the brain and periphery in both vertebrates and invertebrates. Recently, we have demonstrated that lipoamines and related lipids, prostaglandins, are differentially regulated by each of the enzymes and receptors associated with the endogenous cannabinoid system and that the active compounds in Cannabis, THC and CBD, likewise regulate these classes of lipids. We have shown that many of CBD's effects on the brain lipid profile is dependent on a specific enzyme, NAPE-PLD.

Peter A. Crawford, M.D. University of Minnesota
We leverage recent advances in stable isotope tracer based NMR and mass spectrometry-based untargeted metabolomics technologies to study metabolism on a systems level, with computational analyses of high resolution mass spectrometry datasets steadily increasing in importance. Most of these analyses proceed through provocations of fatty acid oxidation and ketone metabolism in genetically manipulated mouse models. Complex in vivo phenotyping methodologies are strategically aligned with these sophisticated chemical profiling platforms to generate high resolution phenotypic pictures, focusing on obesity, diabetes, NAFLD/NASH, and heart failure/CHF.

Brandon S. Davies, Ph.D. University of Iowa
Brandon is an Associate Professor in the Department of Biochemistry at the University of Iowa, and a member of the Fraternal Order of Eagles Diabetes Research Center, the Obesity Research and Education Initiative, and the Abboud Cardiovascular Research Center. He performed his graduate studies in the lab of Jasper Rine at the University of California, Berkeley studying the transcriptional regulation of ergosterol biosynthesis. Dr. Davies then did his postdoctoral training under the mentorship of Stephen Young at the University of California, Los Angeles, where he worked to understand the critical role of GPIHBP1 (a GPI-anchored protein of capillary endothelial cells) in the metabolism of triglyceride-rich lipoproteins. Dr. Davies started his independent lab at the University of Iowa in 2012. Currently, his lab studies triglyceride metabolism and lipid partitioning.

Brian W. Parks, Ph.D. University of Wisconsin-Madison
Brian is an Assistant Professor in the Department of Nutritional Sciences at the University of Wisconsin-Madison. Brian grew up in Virginia and completed his undergraduate degree in Biology at the University of Virginia's College at Wise. After working for a year in the biotechnology industry, Brian pursued doctoral studies in the lab of Janusz Kabarowski at the University of Alabama at Birmingham where he studied atherosclerosis and lipoprotein metabolism. Next, Brian performed postdoctoral studies in the lab of Jake Lusis at UCLA developing a systems genetics approach in the mouse to understand how diet and genetics interact to contribute to obesity and diabetes. In his own lab, Brian uses systems genetics based approaches to uncover the biological pathways and genes that mediate dietary interactions and ultimately lead to the development of common metabolic diseases.

Loredana Quadro, Ph.D. Rutgers University

Loredana is a Professor of Food Science and member of the Rutgers Center for Lipid Research (RCLR) and of the Institute of Food Nutrition and Health (IFNH) at Rutgers University. She received her B.S. degree from the School of Biology at the University of Naples (Italy) and her Ph.D. degree in Biotechnology from the School of Medicine at the University of University of Naples (Italy). Her postdoctoral training was in Nutritional Biochemistry at Columbia University in New York. Dr. Quadro's research aims at understanding the mechanisms of vitamin A and carotenoids absorption, transport and metabolism in mammalian tissues by using genetically modified mouse models. A major focus of her research is on the maternal-fetal metabolism of vitamin A and its carotenoid precursor beta-carotene with the ultimate goal to understand how to prevent or improve congenital defects as well as maternal pathological conditions associated with both the deficiency and excess of the vitamin.

Wayne R. Riekhof, Ph.D. University of Nebraska-Lincoln

Wayne received a BS in Biochemistry from the University of Missouri in 1999, and did his PhD work on lipid metabolism in plants, algae, and photosynthetic bacteria with Christoph Benning in the DOE Plant Research Lab and Department of Biochemistry and Molecular Biology at Michigan State University, graduating in 2004. From there he moved to a post-doctoral position with Dennis Voelker at National Jewish Health, Denver, CO, and initiated work on membrane lipid trafficking and lysophospholipid transport and metabolism in Saccharomyces cerevisiae. Since 2012 he has been an Assistant and now Associate Professor in the School of Biological Sciences, University of Nebraska - Lincoln. Current work in the Riekhof lab centers around membrane lipid biosynthesis and remodeling in fungal pathogens; nutritional control of lipid and central carbon metabolism in green algae; the identification and characterization of novel antifungal compounds from plants; and mutualistic metabolic and signaling interactions between green algae, melanized fungi, and bacteria in rock- and soil-surface biocrusts.

Rob V. Stahelin, Ph.D. Purdue University

Rob received a PhD in chemistry from the University of Illinois-Chicago, studying the structural basis of lipid-protein interactions. During postdoctoral work at the University of Illinois-Chicago, he investigated the mechanisms with which bioactive lipid signals recruit peripheral proteins in cell signaling and membrane trafficking. Rob moved to South Bend, IN in 2006 to work as an Assistant Professor in Biochemistry and Molecular Biology at IU School of Medicine-South Bend and as an Adjunct Assistant Professor in Chemistry and Biochemistry at the University of Notre Dame. In 2017, Rob moved his lab to Purdue University where he is the Retter Professor of Pharmacy studying how lipid-enveloped viruses replicate and assemble from the plasma membrane of cells.

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Lipid storage in starved *Tetrahymena thermophila*

Byunghyun Ahn and Laura Listenberger
St. Olaf College, Northfield, MN

Lipid droplets are increasingly recognized as important and dynamic organelles in all cells. The pathways contributing to the formation and degradation of lipid droplets have been well-studied in a variety of organisms, including mouse, human, roundworm, fruit flies, and yeast. Our experiments aim to determine the cellular pathways that contribute to lipid droplet homeostasis in *Tetrahymena thermophila,* a single-celled ciliate. In this study, we demonstrate an increase neutral lipid storage in *Tetrahymena* following starvation. Lipid droplet number and size increase within 3 hours of starvation, remain elevated for an additional 72 hours, and then begin to decline. Pulse chase experiments using a fluorescent fatty acid analogue (Red C12) show that lipid trafficking to lipid droplets in starved *Tetrahymena* occurs along a similar time frame. We hypothesize that *Tetrahymena* mobilize lipids in lipid droplets to serve as substrates for beta oxidation. However, our results suggest that lipophagy does not play a role in this process. Monodansylcadaverine, a fluorescent label for autophagosomes, fails to colocalize with lipid droplets in starved *Tetrahymena.* Future studies will use inhibitors of cytosolic lipases and/or lipophagy to investigate the pathways of lipid droplet metabolism in *Tetrahymena.* We have also identified several novel lipid droplet-associated proteins from starved *Tetrahymena* following lipid droplet isolation, SDS-PAGE and mass spectrometry. Ongoing analysis of these proteins will provide further insight into mechanisms of lipid storage in starved *Tetrahymena thermophila.*

Lipid Atlas of the Human Calf Using Advanced Fast Magnetic Resonance Spectroscopic Imaging

Ahmad Alhulail[1,2], Pingyu Xia[1], Debra A. Patterson[1,3], Xiaopeng Zhou[1], M. Albert Thomas[4], Uzay E Emir[1,5], Ulrike Dydak[1,3]
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Musculoskeletal triglyceride (lipid) exists either inside (IMCL) or outside the muscle cells (EMCL) with different saturation. While they serve as an energy source, the content elevation of some of their components has been related to several common diseases, such as type 2 diabetes and cardiovascular disease[1]. The in-vitro quantification methods can measure these lipids, but their information represents only a small muscle volume. Although conventional imaging techniques can map and quantify the total fat content, they cannot differentiate between their components. The differences in chemical saturation, chain length, and location of lipid components relative to the cells result in a differentiated frequency of their protons that allows proton magnetic resonance spectroscopy (1H-MRS) to detect their distinct signals noninvasively. However, a single voxel MRS cannot map the lipid's distribution. Alternatively, MRS-imaging (MRSI) can cover a larger area with multiple voxels, but conventional MRSI requires a long acquisition time (15-60 minutes).
We implemented an advanced MRSI technique[2] to acquire high-quality data of lipids from many voxels in shorter scan time. In-vivo lower-leg scans were performed to validate this technique. The results show that our technique can measure the content of eight lipid components in only ~3 minutes and generate separate maps for each of them (Figure 1&2). The quantification results are represented as fat-fraction (FF= lipid-specific signal/total signal).
This technique can be used with other applications such as quantifying lipids in the bone marrow to evaluate the bone density, or to assess the liver’s intrahepatic lipid to assess hepatic steatosis.

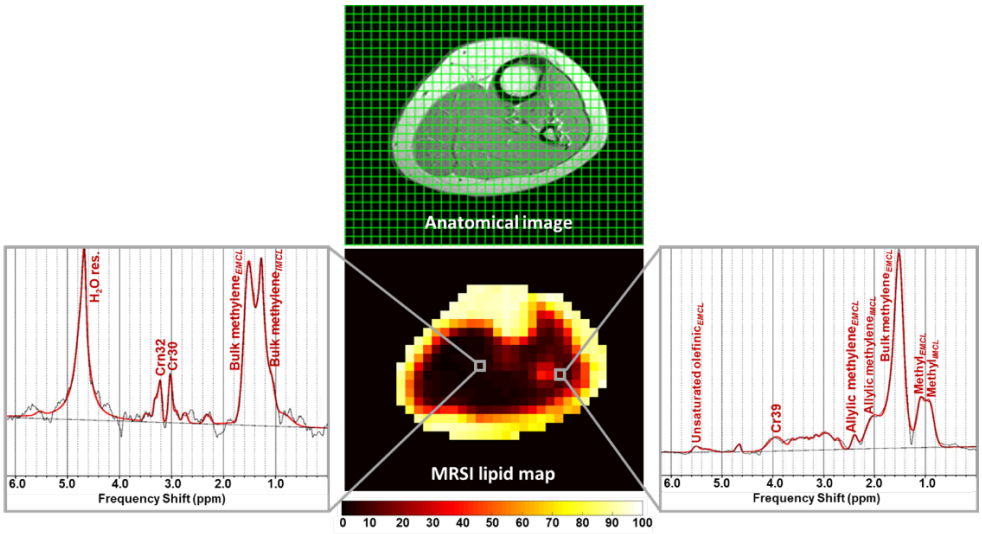

FIGURE 1 Lipid spectra samples from different location within the calf muscles (cross-section view). H_2O res. stands for the residual water signal. Other metabolites than lipids can also be detected such as CH_3 and CH_2 groups of creatine that resonate at 3.0 ppm (Cr30) and 3.9 ppm (Cr39), respectively, in addition to the CH_3 group of carnitine (Crn32)

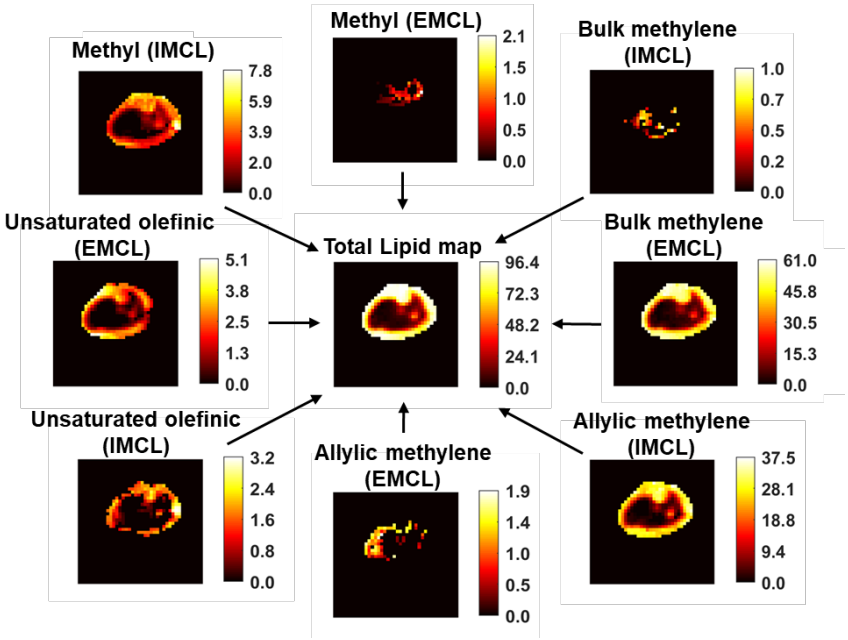

Figure 2 Lipid atlas of human calf muscles (cross-section view). In addition to the total fat map, the intramyocellular (IMCL) and extramyocellular lipid (EMCL) components were separately mapped. The scale next to each map indicates the fat fraction (FF =lipid-specific component/total tissue) values from 0 to maximum in percent.

References:

1. Abbasi, Fahim, et al. "Relationship between obesity, insulin resistance, and coronary heart disease risk." *Journal of the American College of Cardiology* 40.5 (2002): 937-943.

2. Steel, Adam, et al. "Metabolite-cycled density-weighted concentric rings k-space trajectory (DW-CRT) enables high-resolution 1 H magnetic resonance spectroscopic imaging at 3-Tesla." *Scientific reports* 8.1 (2018): 7792.

Beta-carotene delays atherosclerosis progression by reducing hepatic VLDL secretion

Jaume Amengual[1], Xiaoyun Wu[1], Ivan Pinos[1], Felix Zhou[2], Benjamin Abraham[1], Tessa Barrett[2], Johannes von Lintig[3], Edward A Fisher[2]
[1]Department of Food Sciences and Human Nutrition, University of Illinois Urbana-Champaign, and [2]Department of Medicine, New York University, and [3]Department of Pharmacology, Case Western Reserve University.

A positive association between beta-carotene (BC)-rich food intake and a reduction in metabolic disorder incidence, including heart disease, is prevalent in humans. Similarly, preclinical research finds dietary BC supplementation to reduce atherosclerosis progression. These beneficial outcomes are attributed to the antioxidant effects of BC and/or its role as retinoic acid precursor, which is produced by the activity of the enzyme beta-carotene oxygenase 1 (BCO1). To ascertain how BC reduces atherosclerosis progression, and the mechanisms involved we utilized $Ldlr^{-/-}$ mice lacking the enzyme BCO1 ($Bco1^{-/-}$).
BC-fed $Ldlr^{-/-}$ mice showed a significant reduction in plaque size when compared to control diet, which positively correlated with a decrease in plasma cholesterol and triglyceride levels (approximately 35% for both). These mice showed delayed atherosclerosis progression, determined by the total macrophage (CD68+ cells) and lipid content (ORO staining) in the plaque, which could not be explained by the effect of vitamin A in the intestinal cholesterol absorption, measured using the fecal dual-isotope ratio method. We also observed for the first time that in the absence of BCO1 ($Ldlr^{-/-}$ /$Bco1^{-/-}$ mice), the beneficial effects of BC on atherosclerosis were abrogated, implicating vitamin A production in atherosclerosis, and discarding any role of the parent BC in this process.
To elucidate the mechanism of action behind the effects of BC on atherosclerosis progression, we used radioisotope labeling techniques to perform lipoprotein production assays in hepatocytes exposed to retinoic acid, the active metabolite derived from BC. We show that retinoic acid decreases VLDL lipidation and particle number (approximately 25% for both). Similarly, a single dose of retinoic acid was sufficient to reproduce the effects observed in cell culture, unveiling retinoic acid and its precursor BC as important modulators of VLDL secretion.
Overall here we demonstrate that BC, a retinoic acid precursor in mammals, reduces atherosclerosis progression by reducing hepatic lipoprotein secretion, providing a mechanistic explanation to the positive effects of BC intake in humans.

Ebola matrix protein VP40 membrane binding specificity goes beyond the lipid headgroup

Souad Amiar*, Kristen A. Johnson‡, Robert V. Stahelin*
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Ebola virus (EBOV) causes an acute and serious illness, which is often fatal. The EBOV was in the spotlight from 2014-2016 with the biggest outbreak in Western Africa giving rise to more than 11,000 deaths. Last year, Democratic Republic of the Congo (DRC) declared two Ebola infection outbreaks with 591 confirmed cases. Moreover, the current Ebola outbreak is reaching over than 2,790 cases (65% death rate) in DRC and this date, there are no Food and Drug Administration (FDA)-approved drugs and/or vaccines. Fortunately, two glycoprotein-targeting antibodies (mAb114 and REGN-EB3) are currently in clinical trials and are being used for their effective EBOV spread reduction. Last July, EBOV outbreak in DRC was recognized as a public health emergency of international concern by the World Health Organization (WHO) and the disease is listed as an urgent need for accelerated research and development. EBOV particles consist of seven structural proteins involved in different virus replication steps. VP40 is the major viral matrix protein and the most abundant protein in EBOV particles. It is involved in various viral life cycle processes such as, transaction and replication regulation, but also in the virus particle formation and sorting from the host cell . VP40 is a peripheral protein localized at the inner leaflet of the host cell plasma membrane (PM). It forms a high affinity dimer, then oligomerizes at the PM to form a hexamer and larger filamentous structure that constitute the viral matrix. PM recruitment and oligomerization involves specific interactions of VP40 with phosphatidylserine (PS) and the phosphatidylinositol (4,5)-bisphosphate PI(4,5)P_2. It is suggested that eVP40 trafficking to the PM is mediated by electrostatic interactions that occur between the anionic lipids such as PS and the basic patch of the eVP40 C-terminal domain. PS is the most abundant anionic constituent of the PM inner leaflet. In addition to the heterogeneity of phospholipid species, cholesterol levels, and phosphatidylinositol phosphate species, the plasma membrane has varying microdomains with different profiles of membrane fluidity defined by the lipid packing level, the saturation of fatty acids and cholesterol distribution. Membrane fluidity is an important parameter for the correct membrane functionality and it is continuously remodeled by budding and fission processes.In this study,we investigated whetherVP40 has a dependence on membrane fluidity for assembly of the viral matrix layer. We have shown that VP40 has additional requirements for lipid binding beyond the PS and PI(4,5)P_2headgroup. Using with different FDA approved drugs that increase the plasma membrane fluidity, we demonstrated that VP40 oligomerization is sensitive to changes in membrane fluidity in live cells which abolish the emergence of virus like particles on the surface of VP40 expressing cells. In addition, in vitro assay highlighted that VP40 lipid binding is enhanced in ordered membranes and nearly abolished in disordered membranes. Together this work describes a novel membrane sensing mechanism of VP40.

Using Targeted Lipidomics to Identify Chemotherapy and Diet Induced Changes in Heart Oxylipins

Austin Angelotti[1]; Deena Snoke[1]; Kate Ormiston, RD[1]; Rachel Cole, MS RD[1]; Maryam Lustberg, MD MPH[2]; John Newman, PhD[3,4]; Kamil Borkowski, PhD[5]; Tonya Orchard, PhD RD[6]; Martha Belury, PhD RD[6]
[1]OSU Nutrition PhD Program, Graduate School, The Ohio State University, Columbus, OH; [2]Department of Internal Medicine, The Ohio State University, Columbus, OH; [3]USDA-ARS-Western Human Nutrition Research Center, Davis, CA; [4]Department of Nutrition University of California Davis, Davis, CA; [5]West Coast Metabolomics Center, Genome Center, University of California Davis, Davis, CA; [6]Department of Human Sciences, The Ohio State University, Columbus, OH

As cancer treatments become more efficacious, cancer patients are living longer. Anthracyclines, a class of chemotherapeutic agents, are commonly used because of their potent antineoplastic activity. However, toxic side-effects of anthracyclines can cause cardiovascular dysfunction in >20% of treated patients. Currently, there are no routine treatments against anthracycline cardiotoxicity. Oxidized fatty acid metabolites (oxylipins), such as epoxides, are recognized for their anti-inflammatory and cardioprotective properties. With the possibility that oxylipins may be a therapeutic target for reducing cardiotoxicity, mice were assigned to consume one of two diets (a low or high omega-3 diet). Mice were further randomized to receive anthracycline chemotherapy or a saline control. Seventeen days after the last chemotherapy injection, hearts were flash frozen, oxylipins were saponified and analyzed by LC-MS/MS. Of the oxylipins quantified, none of them showed significant differences based on chemotherapy administration, 17 showed significant differences based on dietary changes, and 14 showed a significant interaction between chemotherapy and diet. Based on these results, diet has a significant role in changing oxylipin concentrations in the heart. Further studies are needed to determine whether diet induced changes in oxylipin concentrations can prevent cardiotoxicity.

Azelaic acid enhances longevity at low temperature via promoting fatty acid desaturation in *Caenorhabditis elelgans*

Juan Bai [1,2], Miran Jang [1], Yuan Zhang [1,3], Hanqiu Xu [1], Kee-hong Kim [1]
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Azelaic acid (AzA), a naturally occurring α,ω-dicarboxylic acid in wheat, rye, barley and sorghum, has been reported to exert anti-inflammatory and anti-oxidation property. AzA has long been used as an antiacne drug by inhibiting bacterial DNA synthesis. Recently, its role in reducing high fat diet-induced adiposity through activation of olfactory receptor 544 has been reported. However, its physiological role in environmental stress (e.g., fasting and cold) and aging is unknown. Using *C. elegans* as a whole-system invertebrate animal model, we demonstrated that AzA showed no effects on the pumping rates, locomotive activities and growth rate. Supplement with AzA didn't alter the lifespan of *C. elegans* under normal condition, while it resulted in a significant extension of worms' survival under cold and oxidative stressed condition. Despite no effect of AzA elicited on the overall fat accumulation under cold stress, AzA treatment resulted in marked increase in expression of fatty acid desaturases genes *fat-1, fat-5* and *fat-7*, with a decrease in lipolysis related genes such as *aak-2* and *atgl-1*. Moreover, the effects of AzA on prolonging the survival under cold condition were abolished in the *fat-1, fat-5, fat-7* and *aak-2* mutants. Taken together, our results suggested that azelaic acid, a functional supplement could ameliorate cold stress in *C. elegans*.

Roles of ACATs in lipolysis and survival in fasted *Caenorhabditis elelgans*

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ACAT catalyzes the formation of CE from FC and FA. Dysregulated ACAT1 and 2 activities are shown to treat cardiovascular diseases, indicating blockage of ACAT as a therapeutic solution for this disease. We recently suggested a potential role of avasimibe (Ava), an inhibitor of ACATs, in lipid metabolism and obesity. However, its role in systemic lipid metabolism and aging are still unknown. In the present study, we aimed to investigate the influences of avasimibe on lipid metabolism and its potential causality with the survival under stressful conditions in *C. elegans* model. Our results indicated that Ava showed no effects on food intake and locomotive activities in *C. elegans*. However, Ava treatment resulted in decreased lipogenesis and elevated lipolysis. Consistent with these findings, we confirmed that Ava upregulated mRNA levels of genes involved in lipolysis such as *aak-2* and *atgl-1* without changing the *daf-16* expression in fasted worms. In addition, the effects of Ava on prolonging the survival under fasting were abolished in the *aak-2, atgl-1* and *daf-16* mutants. Our study highlights an essential role of ACAT in fat mobilization and aging of *C. elegans* in the absence of food.

A New Targeting Strategy to Prevent Egress of the Ebola Virus

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The Ebola virus (EBOV) is a filamentous lipid-enveloped virus that causes up to a 90% mortality rate. In 2014, there was an outbreak in Western Africa that killed over 11,000 people. This devastation was in part due to the lack of effective treatments for those who were exposed to the virus. The treatment options currently in use include siRNA, monoclonal antibodies, and plasma transfusions; however, none of these approaches have been approved by the FDA. There is a promising new drug strategy, however, that involves stapled peptides to block key protein interactions. Stapled peptides have successfully been developed to inhibit p53 binding to a negative regulator and thus preventing cancer. Our lab has shown that the EBOV viral protein VP40 is the sole requirement for viral egress and budding. This process occurs when VP40 is able to oligomerize and bind to the plasma membrane. Point mutations at the VP40 binding interface prevent oligomerization and viral egress. It is our hypothesis that a stapled peptide that is able to bind to this oligomerization interface will prevent oligomerization and viral egress. In conjunction with this work, structure guided drug design will be employed by first determining the structure of VP40, in particular when it is bound to a lipid membrane. It is anticipated that this study will lead to the development of a new and effective therapeutic against EBOV.

Consumer acceptance and changes in fatty acid profiles after consumption of healthy cookies enriched with linoleic acid for 2 weeks: A randomized double-blinded placebo-controlled study

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Higher blood levels of the essential fatty acid, linoleic acid (LA), are associated with a reduced risk of cardiovascular disease (CVD) and type 2 diabetes (T2DM). Unfortunately, many US dietary oils once high in LA are now high in oleic acid (OA) and low in LA, making it challenging for adults to increase LA intake. This study examines the extent in which healthy cookies enriched with LA can alter plasma, erythrocytes and peripheral blood mononuclear cells (PBMCs) LA levels after 2 weeks of daily consumption. 84 healthy adults were randomly assigned to consume one healthy cookie per day either rich in LA (7.5g) or in OA (8.1g) for two weeks. 42 participants were randomly assigned to each group and all but one completed the study. 66% of the participants were female. The mean age was 32.6 years and the mean BMI was 25.8. There was no difference in the overall acceptability of the LA and OA healthy cookies. Both were rated as “liked moderately”. There was a significant difference in the change of LA from week 0 to week 2 in the plasma, erythrocytes and PBMCs between the LA and the OA healthy cookie groups. In the LA healthy cookie group, the increase in LA was largest in plasma, followed by erythrocytes and then PBMCs. Healthy cookies are a food-based approach to increasing LA in the diet and in the blood. Future long-term studies are needed to assess the ability of healthy cookie consumption to alter CVD and T2DM risk.

Fatty Acids Generated via Lipophagy Are Effluxed Prior to Their Subsequent Metabolism

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Hepatic lipid droplets (LDs) are the defining characteristic of NAFLD and a hallmark of insulin resistance and Type 2 Diabetes. LD catabolism is driven by cytosolic lipases such as adipose triglyceride lipase (ATGL)-mediated lipolysis or through autophagy of LDs, a process known as lipophagy. However, the interplay between these pathways has only recently been explored. Our laboratory has shown that hepatic ATGL promotes autophagy/lipophagy via sirtuin 1 (SIRT1) signaling. Moreover, autophagy/lipophagy is required for ATGL-mediated LD degradation and fatty acid (FA) oxidation in the liver. These findings suggest a regulatory circuitry of ATGL-SIRT1-lipophagy that controls LD degradation. Additional work has explored the mechanisms to the extent of FAs are trafficked following lipophagy. Our data show lysosomes efflux fatty acids extracellularly following their release from triglycerides via lysosomal lipase. The efflux of FAs requires lysosomal fusion to the plasma membrane suggesting that FA are not released directly into the cytosol. Inhibition of autophagosome-lysosome fusion or lysosomal acid lipase prevents FA efflux and trafficking to neighbor cells. In addition, pharmacological inhibition of fatty acid reuptake significantly increases FA in media and decreases the number of intracellular LDs. These findings suggest a lipophagy-driven FA efflux-reuptake loop prior to the substantial bioenergetics process in the nutrient deprivation state. Moreover, studies in primary hepatocytes and in situ perfused livers reveal that FA efflux occurs prior to the subsequent oxidation or metabolism of hydrolyzed FAs in response to nutrient deprivation or ATGL overexpression. Taken together, our data propose a new framework for LD catabolism and FA trafficking. Given the importance of LDs and fatty acid trafficking and signaling, ongoing work is to further explore the regulation of fatty acid efflux and the downstream signaling/metabolic consequences of this biological pathway that would contribute to disease etiology.

Hepatoma cell response to saturated fatty acids

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Hepatocellular Carcinoma (HCC), a major type of primary liver cancer, is the third leading cause of cancer-related mortality, globally. The main causes of HCC are Hepatitis C and B viral infections, followed by excessive alcohol consumption, but the fastest increasing cause of this cancer is due to metabolic disorders, such as non-alcoholic fatty liver disease (NAFLD). Metabolic-disease-associated HCC has been linked to biological processes, such as chronic inflammation due to pro-inflammatory cytokine secretion and lipotoxicity. Our project investigates the cytokines secreted by hepatoma cells in response to excess saturated fatty acids. An antibody array indicated that the cytokines CINC-1 and VEGF are secreted in response to palmitate treatment. In our experiments, we have observed a 3-fold increase in the secretion of CINC-1 in hepatoma cells treated with palmitate compared to a vehicle control. This increase was not observed in AML-12 hepatocytes, suggesting that it is specific to hepatoma cells. Elevated CINC-1 levels began within two hours of palmitate treatment and were also dose-dependent. When co-treated with the unsaturated fatty acid oleate, or in the absence of glucose, CINC-1 secretion was reduced. We are currently investigating the mechanisms involved in the production of CINC-1 in response to palmitate treatment. A better understanding of the progression of HCC is important to improve treatment options as well as metastatic prevention.

Whole-Body Liver Fatty Acid-Binding Protein (LFABP) Ablation in C57BL/6 Mice Differentially Affects the Volume and Histological Features of Adipose Depots

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Liver fatty acid-binding protein (LFABP) is expressed in proximal small intestine and liver, contributing to cellular fatty acid (FA) transport and metabolism and affecting whole-body energy homeostasis. On a high fat diet (HFD), LFABP$^{-/-}$ mice become more obese than their wild-type (WT) counterparts, but they display a metabolically healthy obese (MHO) phenotype characterized by normoglycemia, normoinsulinemia and increased endurance exercise capacity (1). In the skeletal muscle, increased availability of triacylglycerols (TAGs) along with high mitochondrial efficiency of energy production have been found, which presumably fuel the exercising muscles (2). Additionally, increased intramuscular peroxisome proliferator-activated receptor gamma (PPARγ) coactivator 1 alpha (PGC1α) and PPARα mRNA levels, and increased circulating FA levels, likely coming from adipose tissue, imply an increase in FA delivery and β-oxidation in LFABP$^{-/-}$ skeletal muscle (2). To explore the potential role of adipose tissue in contributing to the improved exercise capacity of LFABP$^{-/-}$ skeletal muscle and the MHO phenotype, here we focused on the volume of various adipose depots and their histological features in LFABP$^{-/-}$ compared to WT mice. Preliminary results show that LFABP$^{-/-}$ mice have higher levels of interscapular brown adipose tissue (iBAT) compared to the WT controls. Also, H&E staining revealed that LFABP$^{-/-}$ mice have relatively more intensely stained iBAT, compared to WT mice, presumably due to greater sympathetic innervation and blood delivery. Interestingly, the results also suggest that the inguinal white adipose tissue (iWAT) of LFABP$^{-/-}$ mice, although bigger, consists of smaller adipocytes; these are more insulin-sensitive compared to larger white adipocytes (3), possibly contributing to the MHO phenotype of these mice.

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β-carotene Regulation of Microsomal Triglyceride Transfer Protein in the Intestine: a possible role for ISX

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Low dietary intake of the main vitamin A precursor, β-carotene (BC), is associated with major health problems. Absorption and utilization of dietary BC are highly regulated by the action of the intestine-specific homeobox transcription factor (Isx) which contains a RAR binding motif in its promoter region so that its expression is induced by the BC metabolite retinoic acid (RA). Upon induction, Isx suppresses gene expression of the intestinal scavenger receptor class B type 1 (Scarb1) and the β -carotene-15,15 ′ -dioxygenase (Bco1), which encode proteins that respectively mediate the uptake of carotenoids and their conversion into retinoids. This negative feedback regulation controls the utilization of dietary BC for retinoid production depending on demand and availability. Previous data from our laboratory, indicated that the transfer of β-carotene from the placenta to the fetal circulation is mediated by lipoproteins and regulated by the availability of the provitamin A precursor with a feed-forward mechanism. We obtained evidence that this feed-forward mechanism also takes place in the intestine. In Isx-/- mice uncontrolled Scarb1 and Bco1 gene expression leads to increased BC uptake and retinoid production in the intestine. Moreover, whereas in the jejenum of the WT mice, upon BC oral administration, Isx was upregulated and mRNA levels of the microsomal triglyceride transfer protein (Mtp) were not perturbed, in mice lacking Isx the transcription of Mtp was significantly enhanced. Overall these data support our hypothesis that Isx might play a critical role in the regulation of intestinal lipoprotein biogenesis upon BC intake.

Influences of genetic selection on hepatic and serum lipidomes of prepartum and postpartum Holstein cows

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Contemporary Holstein (CH) dairy cattle achieved high milk yield from genetic selection in the past decades, but they are more vulnerable to metabolic dysfunctions and morbidities than their counterpart - unselected Holstein (UH), especially during transition and early lactation periods. Lipids are essential metabolites for sustaining and regulating energy homeostasis and milk production in dairy cattle. However, influences of genetic selection on the lipidome of Holstein cow were not well defined. In this study, serum and liver samples were harvested from UH and CH cows between 5 weeks prepartum and 10 weeks postpartum, and analyzed by liquid chromatography-mass spectrometry-based lipidomic analysis. The result showed a time-dependent shift of serum lipid profile in both UH and CH cows, represented by the dramatic decrease of triglycerides (TGs) in prepartum samples and the gradual increases of phospholipids, including phosphatidylcholines (PC), phosphatidylethanolamines (PE), LysoPCs, and sphingomyelins, in postpartum samples. The scale of these changes in CH cows were greater than that in UH cows. Analysis of liver samples also showed that UH cows had a more stable hepatic lipidomes compared to CH cows during parturition and lactation. UH cows had higher abundances of phospholipids containing polyunsaturated fatty acids (PUFA), while CH cows had higher abundances of TGs containing saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA). Overall, these observations warrants further analysis on the influences of genetic selection on the expression of genes and enzymes involved in lipid metabolism, including fatty acid synthesis and elongation, lipoprotein, and transport of fatty acids, TGs, phospholipids.

ACOT1 deficiency attenuates high-fat diet induced fat mass gain and non-alcoholic fatty liver disease

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Acyl-CoA thioesterase 1 (ACOT1) is a cytosolic and nuclear thioesterase that catalyzes the hydrolysis of long-chain acyl-CoAs to free fatty acids and coenzyme A. Prior work from our laboratory has shown that hepatic ACOT1 generates fatty acid ligands that activate peroxisome proliferator receptor alpha to couple fatty acid supply with oxidative metabolism during fasting. However, the impact of global, chronic ACOT1 knockout on energy metabolism during low (LFD) or high-fat diet (HFD) feeding in mice has not been characterized. Here we report that ACOT1 knockout mice are resistant to HFD induced fat mass accretion and non-alcoholic fatty liver disease (NAFLD), effects that were associated with an increase in O_2 consumption and energy expenditure without alterations in substrate metabolism, physical activity levels, or food intake. In white adipose tissue (WAT) of HFD fed mice ACOT1 deficiency did not modify mitochondrial content, but increased uncoupling protein 2 (UCP2) protein abundance, while reducing 4-Hydroxynonenal (4-HNE), a marker of oxidative stress. In the liver, ACOT1 deficiency reduced markers of mitochondrial content in HFD mice but did not modify UCP2 or 4-HNE protein abundance. In brown adipose tissue, ACOT1 deficiency modestly reduced 4-HNE protein abundance in HFD mice, while UCP2 protein abundance was upregulated in LFD mice, but not HFD mice. These data suggest ACOT1 deficiency in WAT induces UCP2 expression to reduce oxidative stress during HFD feeding, an effect that increases energy expenditure to prevent weight gain and NAFLD. Targeting ACOT1 represents a potential novel therapy to prevent weight gain and NAFLD during HFD.

Inhibition of de novo lipogenesis targets androgen receptor signaling in castration-resistant prostate cancer

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A hallmark of prostate cancer progression is dysregulation of lipid metabolism via overexpression of fatty acid synthase (FASN), a key enzyme in de novo fatty acid synthesis. Metastatic castration resistant prostate cancer (mCRPC) develops resistance to inhibitors of androgen receptor (AR) signaling through a variety of mechanisms, including the emergence of the constitutively active AR variant V7 (AR-V7). Here, we studied an irreversible FASN inhibitor (IPI-9119) and demonstrated that selective FASN inhibition results in reduced protein expression and transcriptional activity of both full-length AR (AR-FL) and AR-V7. Activation of the endoplasmic reticulum stress response resulting in reduced protein synthesis was involved in IPI-9119–mediated inhibition of the AR pathway. These findings provide a compelling rationale for the use of FASN inhibitors in mCRPCs, including those overexpressing AR-V7.

Marginal vitamin A deficiency perturbs intestinal functions and fecal microbiome: insights from a mouse model

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The essential nutrient vitamin A (VA) modulates immunity, inflammation and cell proliferation. Thus, the VA status has a profound impact on intestinal health. Indeed, VA deficiency (VAD) is often associated with pathological conditions of the gut. Additionally, a healthy intestine is critical to support a “healthy” microbiome, which in turn helps maintaining proper intestinal functions in a feedback loop. Emerging data suggest that marginal VAD maybe be more frequent that previously anticipated among population with poor diet. We investigated the impact of marginal VAD on intestinal barrier morphology, functions and microbiome perturbations, by using a tunable mouse model of VAD. *Lrat*$^{-/-}$*Rbp*$^{-/-}$ mice are unable to store and mobilize hepatic vitamin A store. Thus, they develop marginal VAD, when maintained on a VA-sufficient diet, and severe VAD when deprived of dietary VA. At 6 weeks of age, *Lrat*$^{-/-}$*Rbp*$^{-/-}$ and WT mice were placed on either VA sufficient or deficient diet for 28 days. Our results confirm that fecal dysbiosis and perturbation in intestinal morphology and functions are concomitant with VAD and indicate that the VAD status (sufficient *vs.* marginal/severe), rather than the amount of VA in the diet *per se*, might be a major discriminant of the intestinal microbiota. Moreover, the taxonomic footprint of the fecal bacteria community appeared to be already compromised under marginal VAD but it was not further affected by the severity of the deficiency. In contrast, a clear separation based on the degree of VAD emerged when microbial functionalities were evaluated. Thus, marginal VAD compromises gut health.

Investigation of Assembly and Budding of Filovirus

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Ebola virus disease, first recognized in 1976 in the Democratic Republic of the Congo, is a serious and often fatal illness in humans and nonhuman primates caused by infection with one of five Ebola virus species (four of which can infect humans). The virus is spread through direct contact with the bodily fluids of a sick person and can cause fever, headache, muscle pain, weakness, fatigue, diarrhea, vomiting, stomach pain and hemorrhage.
Ebola virus is part of the Filoviridae family, which also includes Marburg virus. Marburg virus disease was first recognized in 1967 and is characterized by the same symptoms and transmission routes as Ebola virus disease. There are two known species of Marburg virus that can cause disease in humans and nonhuman primates.
We are investigating how the Ebola and Marburg viruses assemble at the plasma membrane of human cells. In particular, we study their matrix proteins which form the viral particle, independent of other viral proteins. We use a variety of biochemical and biophysical techniques to study these viral proteins.

Piceatannol, a natural stilbene, extends the lifespan of *Caenorhabditis elegans* under fasting through inhibition of lipolysis

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Lipolysis is the catabolic process that hydrolyzes triglyceride (TG) to free fatty acids (FFAs) and glycerol under negative energy balance such as fasting. In adipocytes, adipose TG lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase play key roles in a series of TG hydrolysis reactions in mammals. However, overly activated adipose lipolysis is believed to contribute to link between obesity and systemic inflammation and oxidative stress. In our previous research, we demonstrated that piceatannol (PIC), a natural resveratrol analogue, inhibited adipogenesis in 3T3-L1 adipocytes and lipogenesis in *Caenorhabditis elegans*. Furthermore, we showed that PIC extends the lifespan of *C.elegans* via the insulin/IGF-1 signaling. However, the effects of PIC on lipid metabolism during fasting state is unknown. In this study, we demonstrated that PIC-treated *C.elegans* exhibited suppressed lipolysis under fasting by showing increased lipid accumulation and TG levels with decreased free glycerol release. Consistent with these findings, PIC treatment resulted in decreased mRNA levels of genes involved lipolysis such as *atgl-1, hosl-1*, and *aak-2* in fasted *C.elegans*. Also, PIC treatment augmented fasting-induced lifespan of *C.elegans* by an increase *daf-16* gene expression. However, the positive modulating of PIC on lifespan extension in fasting was abolished when *atgl-1*, *aak-2*, and *daf-16* mutants were treated PIC. Collectively, our results indicate that PIC contributes to extending the lifespan through regulating lipolysis-mediated fat and energy metabolism of *C.elegans* during fasting state.

GPR55 Deletion Causes Decreases In CNS Prostaglandins And Regio-Specific Changes In Lipoamines

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G-protein-coupled receptor 55 (GPR55) has been identified as part of the endocannabinoid signaling system because of its binding affinity for both endogenous and synthetic cannabinoids. It is found in tissues ranging from the central nervous system to the periphery and has demonstrated therapeutic potential in that GPR55 antagonists decrease prostaglandin levels *in vitro*. However, the impact of GPR55 on lipid signaling more generally has yet to be explored. To determine how GPR55 may influence the regulation of lipid signaling molecules associated with the endogenous cannabinoid system in the central nervous system, both globally and in a regio-specific manner, 8 different brain regions were analyzed for levels of Anandamide and its structural analogs (fatty acids conjugated to amines referred to here as lipoamines), 2-arachidonyl glycerol and its structural analogs, free fatty acids including arachidonic acid, and prostaglandins (PGs) in WT and GPR55 knock out mice. Lipids were partially purified from methanolic extracts using C-18 solid extraction columns, and eluants screened through a large lipid library (~85 individual species) using HPLC/MS/MS. Analysis revealed changes in both endocannabinoids, lipoamines, and PGs. Importantly, PG levels decreased in 7 of the 8 brain areas examined, which supports previous data that GPR55 antagonists decrease levels of PG *in vitro*. These data extend our understanding of the interconnected endocannabinoid lipidome and provide further evidence for the relationship between cannabinoid signaling and PG signaling.

Functional Analysis of Common Human ANGPTL3 Mutants

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Dyslipidemia contributes to the risk of developing cardiovascular disease, a leading cause of death in the United States. Two key proteins that regulate lipid metabolism are lipoprotein lipase (LPL) and endothelial lipase (EL). The key role of lipoprotein lipase is the cleavage of triglycerides into free fatty acids for uptake into tissues while EL hydrolyzes the phospholipids of HDL, a lipoprotein that facilitates reverse cholesterol transport. Angiopoietin-like 3 (ANGPTL3) is an endogenous inhibitor of both EL and LPL. Complete deficiency in ANGPTL3 is associated with decreased plasma triglycerides and may present a protective effect against cardiovascular disease. Several common human mutations of ANGPTL3 (G56V, K63T, M259T, F295L, R332Q) also alter plasma triglycerides levels. Constructs of the G56V, K63T, M259T, F295L, and R332Q mutants were created using inFusion cloning. Strep-tagged mutants were transfected into 293T cells and cell media and lysate was collected. We tested the expression and secretion of these mutants through western blotting and their ability to inhibit EL through EL inhibition assays. Our results have shown that F295L and R332Q mutants are not expressed. In comparison, the G56V, K63T, and M259T mutants express but show no difference in EL inhibition compared to wild type human ANGPTL3. In the future, we plan to test LPL inhibition by these mutants and the ability of the mutant proteins to interact with ANGPTL8, an important cofactor.

β-cell OGT is required for acute and adaptive lipid potentiation of insulin secretion

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A small number of *in vitro* studies have linked the nutrient-sensitive protein modifier, O-GlcNAc transferase (OGT), to increased β-oxidation and shown its activity (protein O-GlcNAcylation) to be upregulated by saturated fatty acids, suggesting an unrecognized role in mediating cellular responses to hyperlipidemia. We previously showed that OGT loss in insulin-producing β-cells leads to a mouse model of diabetes, a disease for which obesity is a major susceptibility factor. Presently, we report that protein O-GlcNAcylation in β-cell-housing pancreatic islets was increased in mice after 6 weeks of high-fat-diet (HFD), concomitant with a transient period of potentiated insulin secretion characteristic of early obesity adaptation to maintain euglycemia. Secretory potentiation was absent in islets from 18 weeks HFD mice or obese human males, which also showed decreased O-GlcNAcylation and OGT levels compared to lean controls. Furthermore, a partial loss of β-cell OGT in mice prevented obesity-precipitated insulin hypersecretion, *in vivo* and *in vitro*, while its complete loss resulted in the failure of palmitate to acutely potentiate islet stimulus-secretion coupling. A transcriptomic analysis of βOGT KO islets revealed differentially expressed genes and upstream regulators with major roles in β-cell lipid metabolism and secretory function, including the ER Ca^{2+}-ATPase SERCA2, a putative OGT target whose loss we confirmed by western blot. We show that pharmacological activation of SERCA2 by CDN1163 completely rescues palmitate secretory potentiation in βOGT KO mouse islets. These data support our hypothesis that lipid-driven OGT activity mediates β-cell hypersecretion during the compensatory pre-diabetic phase of early obesity adaptation.

Lipid droplet-derived monounsaturated fatty acids traffic via PLIN5 to allosterically activate SIRT1

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Lipid droplets (LDs) provide a reservoir for triacylglycerol storage and are a central hub for fatty acid trafficking and signaling in cells. Lipolytic of triacylglycerol promotes mitochondrial biogenesis and oxidative metabolism via a SIRT1/PGC-1α/PPARα-dependent pathway through an unknown mechanism. Herein, we identify that monounsaturated fatty acids (MUFAs) allosterically activate SIRT1 towards select peptide-substrates such as PGC-1α. MUFAs enhance PGC-1α/PPARα signaling and promote oxidative metabolism in cells and animal models in a SIRT1 dependent manner. Moreover, we characterize the LD protein perilipin 5 (PLIN5), which is known to enhance mitochondrial biogenesis and function, to be a fatty acid binding protein that preferentially binds LD-derived monounsaturated fatty acids (MUFAs) and traffics them to the nucleus following cAMP/PKA-mediated lipolytic stimulation. Ablation of PLIN5 blocks the effects of MUFAs. Thus, these studies identify the first-known endogenous allosteric modulators of SIRT1 and characterize a LD-nuclear signaling axis that underlies the known metabolic benefits of MUFAs.

Protein binding to lipid droplets with altered ratios of surface phospholipids

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Alcoholic Fatty Liver Disease (AFLD) is characterized by an increase in lipid droplets in the liver after acute ingestion of alcohol. Previous experiments with a rat model of AFLD show hepatic lipid droplets contain altered levels of the predominant classes of phospholipids. Specifically, hepatic lipid droplets from AFLD rats were found with lower amounts of phosphatidylcholine (PC) relative to phosphatidylethanolamine (PE). Our experiments aim to determine if the altered phospholipid composition of AFLD lipid droplets causes differences in the numbers or types of lipid droplet-associated proteins. We developed an *in vitro* binding assay to explore protein binding to model lipid droplets with defined PC:PE ratios. In preliminary experiments, we found increased levels of perilipin 2 and perilipin 3 bound to the lipid droplets that model the AFLD conditions (with lower ratios of PC:PE). We have also examined protein binding to lipid droplets in cultured cells. AML12 cells cultured in choline-deficient media simulate the altered phospholipid composition of AFLD lipid droplets. We isolated lipid droplets from AML12 cells cultured with and without choline and examined proteins of interest by SDS-PAGE and western blotting. We found equivalent levels of rab18 and lanosterol synthase in lipid droplet fractions from cells grown with and without choline. Alternatively, perilipin 1, perilipin 2, and CIDE-C are predominantly found in lipid droplet fractions from cells grown without choline (with lower ratios of PC:PE). The proteins that are most influenced by lipid droplet surface composition are all predicted to associate with lipid droplets through amphipathic alpha helices.

High-throughput Targeted Lipidomics Analysis of Dihydroceramide Desaturase-1 (DES1) Knockout Mice

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Amide column chemistry was chosen for lipid class separation and minimize isomeric interference. The target list of lipids is comprehensive, covering most major lipid classes and categories, and MRMs were selected to cover lipids containing fatty acids with 14 to 22 carbons and 0 to 6 double bonds. The method is customizable, so new lipid categories, classes and molecular species can be added to the MRMs list. This method provides quantitative measurement of over 1150 different lipid molecular species in a rapid, highly reproducible manner. The sMRM Pro Builder template which was developed to streamline the method optimization process, enables assigning the retention time, optimize dwell weight and set window size per MRM to enhanced coverage and sensitivity of the method. This optimization improved results quality especially on low abundant lipids.

Liver and eWAT tissues were harvested from dihydroceramide desaturase-1 (DES1) knockout mice. DES1 is the enzyme responsible for inserting the 4,5-trans-double bond into the sphingolipid backbone causing the dihydroceramide conversion to ceramide. While both of these classes of lipids are lower in abundance in the chosen tissues, this method shows significant changes in these lipid classes in a quantitative manner. However, lipids from another 17 classes were not changing.

Lipid standards from 19 different classes, which are either heavy isotopic labeled lipids or odd chain lipids, served as internal standard. This method provided extensive lipid class coverages including, CE, CER, DCER, HCER, LCER, TAG, DAG, MAG, LPC, PC, LPE, PE, LPG, PG, LPI, PI, LPS and PS.

Comparing Binding Affinities of Various Monounsaturated Fatty Acids for Fatty Acid Binding Proteins 4 and 5

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Fatty-acid-binding-proteins (FABPs) have been found to play key roles in metabolic homeostasis by facilitating the transportation of critical lipids. One particular example, FABP4, has proven to be a molecular link between obesity and metabolic disease, as it is found primarily in adipose cells and has correlation with long-term inflammation and regulating insulin action. Among the wide variety of lipids that FABPs bind to, the lipokine C16:1n7-palmitoleate is a signaling molecule derived from adipose tissue that assists in regulating metabolic homeostasis and muscle insulin action. Considering the overlap in the processes both macromolecules are involved in, FABP4 shows a higher binding affinity for C16:1n7-palmitoleate when compared to other fatty acids. Furthermore, this preference for C16:1n7-palmitoleate prevails when placed next to other isomeric forms of this monounsaturated fatty acid. This data can be later applied when considering the evaluation of pathways that involve C16:1n7-palmitoleate and FABP4, and how their relative interactions may affect one another and the pathway as a whole. Overall, this knowledge and its applications expands on our understanding on the connections between obesity and metabolic diseases.

Brummer-catalyzed lipolysis promotes lifespan and healthspan in *Drosophila melanogaster*

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Our research focuses on adipose triglyceride lipase (ATGL), the rate-limiting enzyme of lipolysis in most tissues. Using *Drosophila* as a well-established model of aging, we studied the effect of overexpression of *bmm* (*brummer*, the *Drosophila* ortholog of *ATGL*) on lifespan and healthspan. We employed non-inducible and inducible GAL4 drivers crossed with a UAS-*bmm* line to study the effects of global overexpression of *bmm*. We've found that *bmm* transgenic flies (*Daughtless-Gal4*/UAS-*bmm*) had increased median lifespan (9.1±1.7% in females, and 10.9±1.6% in males, $p<0.05$) compared with controls (*Daughtless-Gal4/+*). *Bmm* overexpression significantly increased the lifetime fecundity (8.4±0.3%, $p<0.001$). *Bmm* overexpression robustly improved locomotion, a hallmark of improved aging, in both males and females. In addition, *Bmm* overexpression significantly decreased senescence compared with control flies, as indicated by less ß-galactosidase staining in ovaries and the intestines, and decreased level of insoluble ubiquitinated protein aggregation in muscle tissues. Taken together, these data for the first time show that Brummer can promote lifespan and healthspan in *Drosophila*, further highlighting the important role of lipolysis in the regulation of the aging process. Since healthspan-promoting interventions such as caloric restriction, exercise and fasting are all known to promote lipolysis, these data suggest that the enhanced lipolysis may be an important mechanism contributing to their beneficial effects.

Sexually dimorphic effects of 7,8-DHF on body weight and intestinal microbiome

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Globally, increasing prevalence of adult and pediatric obesity is a significant concern in development of metabolic diseases such as cardiovascular, type 2 diabetes, and hepatic steatosis. In the context of this rapidly growing public health challenge, the market for nutritional products and natural supplements is increasing. However, studies of safety, efficacy, and mechanisms of action are limited. One such natural supplement that shows promise as a therapy for obesity is the flavonoid, 7,8-dihydroxyflavone (DHF). DHF is a naturally occurring flavonoid, enriched in *Godmania*, *Tridax*, and *Primula* species. Studies in our lab and others have demonstrated that orally-administered DHF attenuates weight gain in a sex-dependent manner, such that female mice are largely protected from high-fat diet induced weight gain, but male mice do not display a similar protection. The mechanisms for this sex specific role were previously unknown. However, we report that DHF stably remodels the female, but not male, intestinal microbiome, prior to divergence in body weight. Importantly, longitudinal analyses indicated changes in the intestinal microbiome precede alterations in body weight in female mice, pointing towards a causative role for these microbial alterations in modulating weight gain. These results are the first demonstration of a sexually-dimorphic effect of this clinically relevant natural compound. Importantly, they point to a role for sex-dependent remodeling of the intestinal microbiome as a potential mechanism of action and have implications not only to the use of DHF in weight management but also to investigations of its use in prevention of neurocognitive decline and other pathologies.

Linoleate-rich cardiolipin is altered in skeletal muscle of mice fed diets differing in dietary fat quality

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Cardiolipin (CL) is a phospholipid integral to mitochondrial inner membrane structure and function in cardiac and skeletal muscle. Little is known about how diet influences CL in skeletal muscle, which may thereby affect muscle metabolism. We investigated how different dietary oils impacted CL species and muscle architecture in healthy C57 BL6/J mice fed AIN-93M diets containing 6%wt of palm (PO), linoleate-rich safflower (LO), or oleate-rich safflower (OO) oils for 5 weeks. Mice fed the LO diet exhibited a shift towards smaller muscle fiber cross-sectional area. Despite no changes in total CL, there was an increase in the percent of m/z 1448 (tetralineoyl-CL) in mice fed the LO diet VS the OO diet but not the PO diet. A loss of linoleate-containing CL species and an increase in oleate-containing species has an unfavorable impact on mitochondrial energetics. We found that mice consuming OO or PO diets displayed shifts towards oleate-containing CL species when compared to the LO diet group. Further, mice fed OO diet had greater relative amounts of oxidized CL species. Although citrate synthase activity between groups was similar, there was a significant positive relationship between citrate synthase activity and both m/z 1448 and total CL in only mice fed the LO diet. These findings indicate that in healthy mice, diet influences shifts in LA-rich CL that may impact skeletal muscle metabolism. Because skeletal muscle drives glucose disposal, it is important to further investigate these findings in the context of metabolic disease.

Proteomic identification of novel membrane lipid binding proteins in human cells by mass spectrometry

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Cellular membranes are composed of thousands of different lipids which include three major lipid classes: glycerolipids, sterols and sphingolipids. Not only do lipids build up the physical structures of biological membranes such as organelle membranes and plasma membrane creating a separated space and platform for bioactive reactions, but many of these lipids are signaling messengers involved in various physiological events, such as ceramide, ceramide-1-phosphate (C1P), sphingosine-1-phosphate (S1P) and phosphotidic acid (PA). These lipids usually interact with protein targets as secondary messengers in signaling cascades during various physiological processes. However, many of these binding proteins have yet to be discovered meaning their function and potential druggability are unknown. Here, the overarching aim is to identify new membrane lipid interacting proteins in human cells, so that selective protein candidates can be assessed as lipid biosensors or drug candidates.

Inhibition of Endothelial Lipase by ANGPTL3

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High plasma triglyceride levels and low high-density lipoprotein (HDL) cholesterol levels are risk factors for atherosclerosis and cardiovascular disease. Both plasma triglyceride and HDL levels are regulated in part by the circulating inhibitor angiopoietin-like 3 (ANGPTL3). ANGPTL3 acts on HDL by inhibiting the phospholipase endothelial lipase (EL), which hydrolyzes the phospholipids of HDL, thus decreasing plasma HDL levels. ANGPTL3 regulates plasma triglycerides by inhibiting lipoprotein lipase (LPL), the lipase primarily responsible for the clearance of triglycerides from the circulation. In this study, we characterized ANGPTL3 inhibition of EL and investigated the role of angiopoietin-like 8 (ANGPTL8) in EL inhibition. We found that ANGPTL3 inhibits EL in dose- and temperature-dependent manner. Heparin prevents this inhibition and the binding of EL to heparan-sulfate proteoglycans on the surface of endothelial cells also protects against ANGPTL3-mediated inhibition. Our lab has previously found that ANGPTL3 must form a complex with ANGPTL8 to efficiently inhibit LPL. However, here we found that ANGPTL8 did not significantly alter the binding or the inhibition of EL by ANGPTL3, indicating that ANGPTL8 is not necessary for EL inhibition.

Nervonic Acid: a Potential Therapy to Delay Onset of Cerebral Adrenoleukodystrophy

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Adrenoleukodystrophy (ALD), a rare X-linked disorder is caused by a defective peroxisomal transporter protein, encoded by *ABCD1* gene. This results in accumulation of very long-chain fatty acids (VLCFA, 26:0), an important factor in the nerve injury associated with ALD. This disease is associated with diverse neurological phenotypes with no phenotype-genotype correlations. The most devastating outcome is for patients with cerebral ALD, with approximately 50% boys succumbing to their disease within 5 years after onset of symptoms. Currently there is no approved treatment available to pre-symptomatic patients that can normalize VLCFA levels, so as to delay or arrest disease progression. It has been shown previously that dietary monounsaturated fatty acids such as erucic acid can decrease VLCFA accumulation. We hypothesized that another monounsaturated fatty acid, nervonic acid will have therapeutic efficacy similar to erucic acid. Our objective is to determine whether nervonic acid can significantly decrease C26:0 in cultured fibroblasts derived from patients. ALD fibroblasts were treated with increasing concentrations of erucic and nervonic acid and the total lipid fatty acid profile measured using validated gas chromatography-mass spectrometric methods. We observed that nervonic acid decreased C26:0 and the total lipid saturated VLCFA similar to erucic acid. This effect was concentration dependent and consistent in both ALD cell lines tested. We also examined the effects on complex lipids such as lyso-phosphatidylcholines and sphingomyelins, which will be discussed. Results from this study will provide information on the optimum concentrations of nervonic acid needed to show a significant response in pre-clinical studies.

Global Lipidomic Analysis of Lipid Droplets Derived from Young and Geriatric Mouse Livers Reveals Distinct Signatures of Aging

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Lipid droplets (LDs) are highly dynamic lipid-rich cellular organelles that play a critical role in intracellular lipid storage and metabolism. There is growing evidence that dysfunction in lipid droplet regulation can contribute to the development of diseases, in particular LDs have been shown to be key contributors to the metabolic syndrome of aging. Age-related alterations in the lipid composition of LDs could be contributing to the altered lipid metabolism that is a hallmark feature of aging. Characterization of the changes in the LD lipidome during aging will provide a phenotypic signature to characterize the impact of altered LD composition on metabolism. In the present study, we have applied an untargeted lipidomics workflow incorporating liquid chromatography coupled with high-resolution tandem mass spectrometry (LC-HRMS/MS), in both positive and negative polarity, to characterize lipidomic profiles in LDs isolated from gender-matched young and geriatric female C57BL/6J mouse livers (young, n = 4, ~35 weeks and geriatric, n = 4, ~115 weeks). The application of univariate and multivariate statistical analysis indicated that triglycerides, phosphatidylcholines, and phosphatidylethanolamines provided the best phenotypic signature to distinguish LDs from each group. The unique features that distinguish each group will be selected as future targets for multiple reaction monitoring (MRM) MS experiments for validation through absolute metabolite quantitation. We demonstrate that LDs derived from mouse liver are sensitive responders to the stresses of aging, developing a distinct lipid signature, indicating a potentially unexplored role in age-related metabolic disorders.

The role of Aldolase C in cholesterol metabolism

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Excess refined dietary carbohydrates have a negative impact on human health by contributing to elevated levels of plasma lipids and the development of cardiovascular disease (CVD). Plasma lipid levels and CVD risk are known to be highly heritable. Through a unique multi-omics approach designed to identify candidate genes that contribute to cholesterol levels in humans we identified the Aldolase C (*Aldoc*) gene. *Aldoc* belongs to a family of glycolytic aldolase enzymes that perform a critical step in the catabolism of both glucose and fructose. Through the catabolism of fructose and glucose, *Aldoc* contributes to the production of pyruvate and eventually acetyl-CoA, the basic building block of cholesterol. Mouse cholesterol feeding studies established that hepatic *Aldoc* is transcriptionally regulated by dietary cholesterol. In vitro validation studies established that *Aldoc* is necessary to maintain cholesterol biosynthesis under cholesterol depleted conditions. Additionally, we found that *Aldoc* has a specific role in the conversion of glucose to cholesterol in hepatocytes. Future in vitro studies will establish the molecular mechanisms by which cholesterol regulates *Aldoc* expression. *Aldoc* is a glycolytic enzyme with the capability of regulating cholesterol metabolism and may be a novel therapeutic target for reducing the conversion of refined carbohydrates to cholesterol and therefore reduce CVD risk.

Aorta intima resident macrophages control atherosclerotic lesion initiation and require monocyte recruitment for plaque progression

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A better understanding of immune cells in the arterial intima during homeostasis may improve our understanding of how diseases of the arterial intima, like atherosclerosis, arise. The healthy intima is thought to be populated by vascular dendritic cells (DC) that, during hypercholesterolemia, initiate atherosclerosis by being the first to accumulate cholesterol. Whether these cells remain key players in later stages of disease is unknown. Using single-cell gene profiling and fluorescence-based lineage tracing approaches, we reveal these cells are not DCs but instead specialized aorta intima resident macrophages (Mac^{AIR}) that depend upon Csf-1 and are sustained by local proliferation. Mac^{AIR} were found to comprise the earliest lipid-loaded foamy cells in plaque and promote lesion progression through an inflammasome-mediated pathway. However, local Mac^{AIR} proliferation during plaque progression is limited. After three months of hypercholesterolemia, Mac^{AIR} were lost from plaques and overtaken by recruited monocytes, which subsequently induce Mac^{AIR}-defining genes. These data redefine the lineage of intimal phagocytes, the origins of foamy macrophages in early atherosclerotic lesions, and suggest that local proliferation is not robust enough to sustain generations of macrophages during plaque progression as in homeostasis.

High-fat Diets and Exercise Modify Colonic Epithelial Stress and Inflammation in Male and Female Mice

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Endoplasmic reticulum (ER) stress is implicated in the pathogenesis of inflammatory bowel disease. Experimentally high fat diets (HFDs) induce colonic epithelial stress and inflammation which may be attenuated by exercise. PURPOSE: The purpose of this study was to elucidate the effects of exercise in ameliorating HFD-induced ER stress in male and female animals. METHODS: Fifty-six (n=7/group) 6-week old C57BL/6NTac male and female mice were randomly assigned to the following groups: (1) control-diet sedentary (CDS, 10% fat diet, Research Diets); (2) very high-fat diet sedentary (VHFS, 60% fat, Research Diets); (3) control-diet exercise (CDX); and (4) very high-fat diet exercise (VHFX) for 12 weeks. Mice had *ad libitum* access to food and water. Exercised mice had free access to a running wheel. Colon sections were fixed for immunohistochemistry for cyclooxygenase-2 (COX-2), an inflammatory marker, or snap frozen for qRT-PCR to assess the unfolded protein response and western blot for eIF2α and phosphorylated (p)-eIF2α following sacrifice. One-way ANOVA with Tukey's post-test was used to analyze group means for each sex. A p value of ≤ 0.05 was considered statistically significant. RESULTS: COX-2 expression was reduced in both exercised groups (CDX and VHFX) in males and females. In females, exercise attenuated HFD-induced suppression of Atf6 and Ire1β expression, but this was not seen in males. p-eIF2α/eIF2α ratio was reduced in both male, but not female, exercised groups compared to VHFS. CONCLUSION: This data shows that HFDs suppress gene expression of ER membrane protein sensors in females and exercise attenuates epithelial stress in males.

Sulfotransferase SULT2B1b inhibition potently stimulates the expression of immunomodulatory genes in prostate cancer cells

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SULT2B1b (sulfotransferase family cytosolic 2B member 1b) catalyzes the addition of a sulfate group to cholesterol and its hydroxylated derivatives, oxysterols. We have previously demonstrated that SULT2B1b is essential for the survival of prostate cancer cells. The purpose of the current study is to elucidate these underlying mechanisms and to identify novel functions for this enzyme. We have found that, in both LNCaP and enzalutamide-resistant C4-2R prostate cancer cells, interferon response pathways and TNF signaling pathway were highly enriched with SULT2B1b knockdown (KD). Consistently, genes which belong to these pathways were among the most upregulated. SULT2B1b-regulated immunomodulatory gene expression may be mediated through TBK1/IRF3, as inhibition of these proteins partially blocked the immunomodulatory gene induction. In addition to the cell death and the unique gene expression pattern, DNA damage was detected after SULT2B1b KD. We then investigated which products mediate the function of this enzyme. It was found that both cholesterol sulfate (CS) and 25-hydroxycholesterol sulfate (25HCS), a sulfated oxysterol, were able to reverse the DNA damage and cell death induced by SULT2B1b inhibition. However, only 25HCS blocked the induction of immunomodulatory gene expression, indicating that SULT2B1b function may be carried out through both CS and sulfated oxysterols with the latter being more critical in the regulation of immunomodulatory genes. In summary, these findings suggest that SULT2B1b inhibition may not only directly target prostate cancer cells and induce cell death, it may also alter the behavior of immune cells in the tumor microenvironment through the induction of immunomodulatory genes.

Regional Differences in Cytoplasmic Lipid Droplet Characteristics and Proteomes in Mouse Small Intestine in Response to Dietary Fat

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The absorptive cells of the small intestine, enterocytes, contribute to postprandial blood lipid levels by packaging and secreting lipids on chylomicrons. Enterocytes along the entire length of the intestine are capable of secreting lipids on chylomicrons. One of the steps in this process is the temporary storage of resynthesized triglycerides in cytoplasmic lipid droplets (CLDs). Although the fate of triglycerides in CLDs is uncertain, most is thought to be hydrolyzed, re-esterified to triglyceride and packaged on chylomicrons for secretion at later times. The characteristics of CLDs may influence CLD metabolism or the specific roles of each intestinal region during dietary fat absorption. However, it is unknown whether the characteristics or proteomes of CLDs differ in enterocytes of each intestinal region in the physiological response to dietary fat. To address this, we utilized transmission electron microscopy and proteomic analysis to assess the characteristics and proteomes of CLDs present in the proximal, middle, and distal regions of the small intestine of mice two hours after an oil bolus. We identified a greater percentage of smaller CLDs, less triglyceride storage, and more secreted lipid between cells in the distal region compared to the proximal and middle regions. Proteins involved in lipid metabolism were differentially present in the CLD fraction of each region. Interestingly, we identified proteins involved in bile acid metabolism exclusively in the CLD fraction of the distal region. Regional differences in CLD characteristics and their proteins may reflect the specific activities of each region and how each responds to dietary fat.

The HDL mimetic peptide 4F mitigates cerebral amyloid angiopathy in transgenic APPswDI mice

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Cerebral amyloid angiopathy (CAA) features amyloid-β (Aβ) deposition in small arteries and capillaries of the cerebral cortex and the leptomeninges, and constitutes one of the pathological hallmarks of Alzheimer's disease (AD). CAA is associated with cerebrovascular dysfunction and cognitive impairment. Substantial evidence has shown that high levels of high-density lipoprotein (HDL), and its main protein component, apoA-I, are associated with superior cognitive function in the elderly; in AD mice, our previous studies have shown that overexpression of human apoA-I rescues cognitive function by attenuating CAA and neuroinflammation. 4F is an 18 amino-acid HDL mimetic peptide advanced into cardiovascular clinical trials. Our preliminary data have shown that 4F inhibits Aβ aggregation. The present study was undertaken to investigate whether acute/chronic treatment with 4F mitigates CAA and associated cognitive deficits and neuropathologies in the transgenic APPswDI mouse model of CAA/AD. APPswDI mice were treated with i.p. injections of D-4F, the D-isomer that exhibits higher bioavailability and longer half-life. Two cohorts of age- and sex-matched APPswDI mice received either 1-week (acute) or 12-week (chronic) daily treatments of D-4F or vehicle (PBS). After acute treatment, soluble Aβ was significantly reduced in the D-4F treated mouse brains; consistently, D-4F treatment trends toward decreased amyloid deposition and microglia recruitment in cortical/hippocampal regions. In the chronic study, D-4F was shown to rescue CAA-associated memory deficits in a sex-dependent manner in APPswDI model. Additional analyses are underway to unravel the molecular mechanisms underlying effects of D-4F. These findings suggest that HDL memetic peptides could be potentially therapeutic to mitigate CAA/AD.

Key words: Alzheimer's disease; Cerebral amyloid angiopathy; HDL; Mimetic Peptide; Transgenic mice; Neuroinflamation

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FXR regulates metabolic function of fat depots during obesity

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Obesity is a major epidemic and is associated with excess calorie intake leading to adipose tissue expansion and metabolic dysfunction. Farnesoid X receptor (FXR) is a nuclear receptor that transcriptionally regulates lipid and glucose metabolism. Despite several studies revealing FXR expression in the adipose tissue, its exact role in this tissue remains elusive. We determined that both primary pre- and post-differentiated adipocytes from inguinal white (WAT) and brown (BAT) adipose tissue express *Fxr* transcripts. Notably, adipose-specific *Fxr* knockout (Ad-FxrKO) mice recently generated in our laboratory exhibit glucose intolerance, increased fat accumulation and concomitant reduction in the expression of lipolytic genes in both WAT and BAT when challenged with a high-fat diet. These data suggest that adipose-specific FXR may play a crucial role in regulating fat accumulation and subsequent glucose homeostasis during diet-induced obesity. Since BAT is specialized for non-shivering thermogenesis by generating heat through uncoupled mitochondrial respiration, we investigated the role for FXR in brown adipocyte mitochondrial function. We found that when *Fxr* is deleted, genes involved in BAT mitochondrial β-oxidation, biosynthesis and respiratory chain complex are down-regulated. Conversely, FXR activation by its endogenous bile acid ligand, chenodeoxycholic acid increases mitochondrial membrane potential of primary differentiated brown adipocytes *in vitro*, suggesting a key role for FXR in controlling brown adipocyte mitochondrial function and a potential role in thermogenesis. Overall, we demonstrate that FXR can modulate fat break down and regulate mitochondrial functions in the adipose tissue.

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