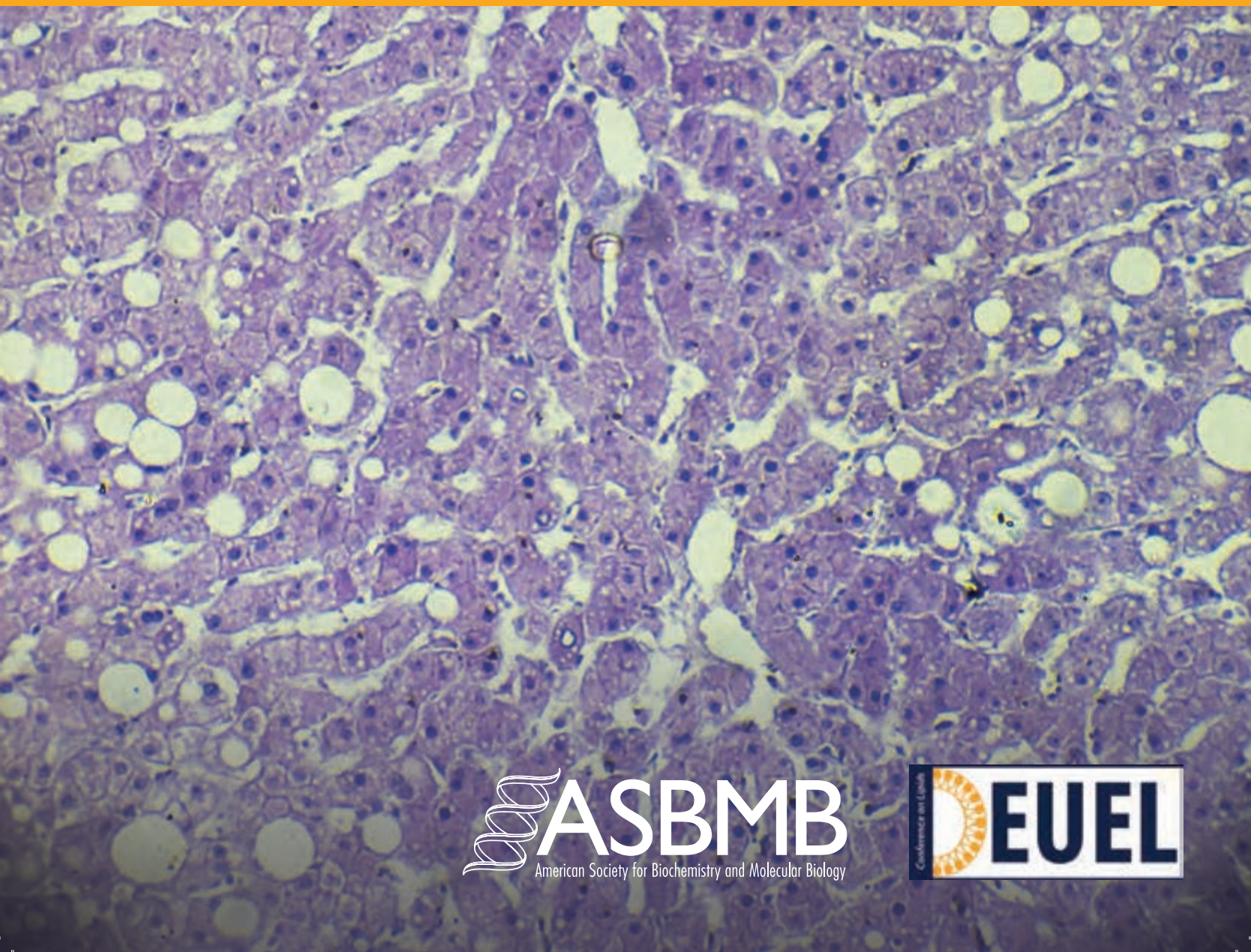
ASBMB DEUEL CONFERENCE ON LIPIDS

Fat in Liver and Beyond

March 3 – 6, 2020
Coronado, Calif.



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DEUEL

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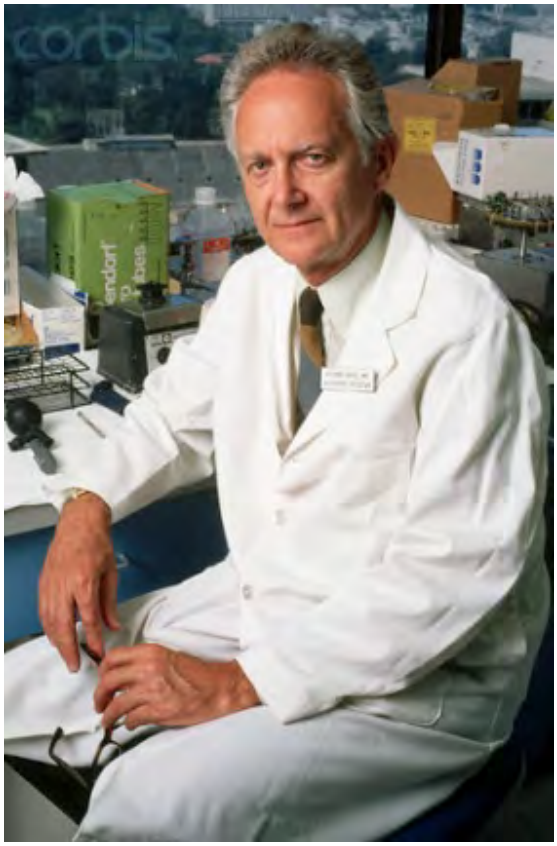
The ASBMB-Deuel Conference on Lipids, March 3-6, 2020

Hotel del Coronado, Coronado, Calif.

The Deuel Conference on Lipids was organized in 1955 by a small group of eminent West Coast investigators who were interested in lipid metabolism. Their goal was to establish a high-quality conference on lipids within the western part of the country, akin to forums provided by the Gordon Conferences on the east coast. Shortly after the Conference was organized, one of the founders, Dr. Harry Deuel, died—and the conference was named in his memory. The two-and-one-half day conference includes up to five scientific sessions, with an eminent lipid scientist chairing each session. Each session includes three to four original scientific presentations followed by in-depth discussions of the topic.

The relatively small size of the audience, a round-table format, and the absence of videotaping or recording, encourage informality and the free interchange of new hypotheses and scientific data. Lively discussions by conference participants are the highlight of the meeting.



The Havel Lecture was named after Dr. Richard J. Havel because he has done more than anyone else to keep the Conference vibrant. For many years, he organized the meeting, and each year he has energized the scientific discussions.

Dr. Havel is known to many as **"Mr. Lipoprotein, USA."** And has unraveled the complex metabolism of plasma lipoproteins. As a Clinical Associate in the laboratory of Christian Anfinsen at the National Institute of Health (1953-1956) he published a manuscript on the complex metabolism of the plasma lipoproteins beginning with his pioneering work in the Anfinsen lab at the National Heart Institute in Bethesda, Maryland, where he was one of the first Clinical Associates from 1953–1956. This manuscript is one of the most frequently cited papers in the scientific literature, rivaling Lowry's paper on protein measurement.

Dr. Havel has published over 300 manuscripts. The quality of his publications is reflected in his election to the National Academy of Sciences in 1983; the Institute of Medicine in 1989; and the American Academy of Arts and Sciences in 1992. He has received many other honors including the Bristol-Myers Squibb Award for Distinguished Achievement in Nutrition Research and the Distinguished Achievement Award from the American Heart Association Council on Arteriosclerosis.

The first Havel Lecture was held on March 6, 2002, at the 2002 Deuel Conference on Lipids in Borrego Springs, California.

2020 HAVEL AWARD LECTURE

Jennifer Lippincott-Schwartz, HHMI Janelia Research Campus

"*Dynamics of membrane trafficking, sorting and compartmentalization within eukaryotic cells"*

PAST HAVEL AWARDEES

2019		**Jake Lusis, University of California, Los Angeles** "The power of natural variation: Sex differences and mitochondrial functions"	2018		**Michael Czech, University of Massachusetts Medical School** *"Crosstalk between fat metabolism and neuronal signaling"*
2017		**Peter Tontonoz, University of California, Los Angeles** *"Transcriptional control of lipid metabolism in physiology and disease"*	2016		**Sir Stephen O'Rahilly, University of Cambridge** *"Obesity and insulin resistance; lessons from human genetics"*
2015		**Thomas Sudhof, Stanford University** *"Brown & Goldstein-inspired science off field: lipid membrane fusion at the synapse"*	2014		**Rudolf Zechner, University of Graz** *"Lipolysis - more than just the breakdown of fat"*
2013		**Rick Lifton, Yale University** *"From human genetics to validated therapeutic targets"*	2012		**Gokhan Hotamisligil, Harvard University** *"Inflammation, endoplasmic reticulum stress and lipids: emerging networks regulating metabolism"*
2011		**Christopher K. Glass, University of California, San Diego** *"Oxysterol regulation of macrophage gene expression"*	2010		**David J. Mangelsdorf, University of Texas Southwestern Medical Center** *"Nuclear receptor control of lipid metabolism"*

2009		**Stephen G. Young, University of California, Los Angeles** *"Adventures in lipid metabolism"*	2008		**Helen H. Hobbs, University of Texas Southwestern Medical Center** *"Going to extremes to identify genetic variations contributing to cardiovascular risk*
2007		**" Ronald Evans, The Salk Institute** *"PPARdelta and the marathon mouse: running around physiology*	2006		**David Russell, University of Texas Southwestern Medical Center**
2005		**Johann Deisenhofer, University of Texas Southwestern Medical Center, HHMI** *"Structure of the LDL receptor"*	2004		**Jeffrey M. Friedman, Rockefeller University** *"Oxysterol regulation of macrophage gene expression"*
2003		**Bruce Spiegelman, Harvard Medical School** *"Transcriptional control of energy and glucose metabolism"*	2002	 	**Michael S. Brown and Joseph L. Goldstein, University of Texas Southwestern Medical Center** *"SREBPs: Master regulators of lipid metabolism"*

Schedule At-a-Glance

	Tuesday March 3	Wednesday, March 4	Thursday March 6		Friday March 7
7AM		**Breakfast** 7-8:15 a.m.	**Breakfast** 7-8:15 a.m.	**Board Meeting** 7:00 - 8:15	**Breakfast** 7-8:15 a.m.
8AM					
		Session 1 8:45 -10:15 a.m.	**Session 3** 8:45 – 10 a.m.		**Session 5** 9-10:30 a.m.
9AM					
C10AM		**Coffee Break** 10:15 – 10:45 a.m.	**Coffee Break** 10:15-10:45 a.m.		
		Session 1 cont. 10:45 – 11:45	**Session 3 cont.** 10:45 – 11:45		
11AM		**Free Time** 11:45 a.m.–5 p.m.	**Free Time** 11:45 a.m. – 5 p.m.		
12PM					
1PM					
2PM					
3PM	**Registration** 3:00 - 5:00 p.m.				
4PM					
5PM	**Opening Reception, Dinner** 5-7 p.m.	**Poster Session 1** 5:00 - 6:00	**Poster Session 2** 5:00 - 6:00		
6PM		**Dinner** 6-7:15 p.m.	**Dinner** 6-7:15		
7PM	**The Havel Lecture** 7:30 p.m. – 8:30	**Session 2** 7:30 – 9:30 p.m.	**Session 4** 7:30 - 9:30 p.m.		
8PM					
9PM					

Deuel Conference on Lipids
March 3-6, 2020
Hotel del Coronado, Coronado, CA

Program Co-Chairs: Jay Horton, University of Texas, Southwestern
Alan Tall, Columbia University

Tuesday, March 3

3:00-5:00 p.m. Meeting Registration, Garden Patio

5:00-6:00 p.m. Opening Reception, Garden Patio

6:00-7:00 p.m. Dinner, Ballroom

7:30-7:35 p.m. **Welcome: Jay Horton, University of Texas, Southwestern**

7:35-7:40 p.m. **The Havel Lecture Introduction – Ballroom**

7:40-8:30 p.m. **The Havel Lecture**

Introduction: Stephen Young, University of California, Los Angeles
Dynamics of membrane trafficking, sorting and compartmentalization within eukaryotic cells
Jennifer Lippincott-Schwartz, HHMI Janelia Research Campus

Wednesday, March 4

7-8:15 a.m. Breakfast, Windsor Lawn

Session 1: **Genetics of NAFLD**
8:45-11:45 a.m.
Upper Grand Ballroom

Session Chair: Jay Horton, University of Texas, Southwestern

8:45-9:15 a.m. **Fatty Liver disease: a tale of two genes**
Helen Hobbs, University of Texas Southwestern Medical Center

9:15–9:45 a.m. **Use of GWAS to identify genes causing NASH**
Elizabeth Speliotes, University of Michigan

9:45-10:15 a.m. **New pathways for cellular and systemic lipid transport**
Peter Tontonoz, University of California, Los Angeles

10:15-10:45 a.m. **Break**

Wednesday, March 4, continued

10:45-11:15 a.m.	**Regulation of the degradation of SREBP2** Arun Radhakrishnan, University of Texas, Southwestern
11:15-11:30 a.m.	**Hepatic Deletion of Mboat7 (Lpiat1) Causes Activation of SREBP-1c and Fatty Liver** Matthew Mitsche, University of Texas, Southwestern
11:30-11:45 a.m.	**DYRK1B Triggers de-novo Lipogenesis by Activating mTORC2 Complex and is an Attractive Target for the Treatment of Non-Alcoholic Fatty Liver Disease** Arya Mani, Yale University
11:45 a.m.-5 p.m.	Free Time
5-6 p.m.	Poster Session I, Upper Grand Ballroom
6-7:15 p.m.	Dinner, Empress Room
Session 2: **7:30-9:30 p.m.** **Upper Grand Ballroom**	**Role of Lipids in NASH**
Session Chair:	Alan Tall, Columbia University
7:30-8 p.m.	*Journal of Lipid Research Award Lecture* **Hepatocyte cholesterol signaling in TAZ-induced NASH fibrosis** Ira Tabas, Columbia University
8:00-8:30 p.m.	**Lipid droplets and non-alcoholic fatty liver disease** Robert Farese, Harvard School of Public Health
8:30-9 p.m.	*Journal of Clinical Investigation Award Lecture* **Leveraging large-scale genomics and EHR-linked biobanks to understand NAFLD/NASH risk and identify novel therapeutic targets** Noura Abul-Husn, Mount Sinai School of Medicine
9-9:15 p.m.	**Loss of function of an atypical AAA+ ATPase, torsinA, results in severe NAFLD on a chow diet with marked reductions in VLDL secretion and accumulation of lipid droplets within the nucleus and ER** Henry Ginsberg, Columbia University

Wednesday, March 4, continued

9:15-9:30 p.m. **Neutralization of Oxidized Phospholipids Ameliorates Non-alcoholic Steatohepatitis**
Xiaoli Sun, University of California, San Diego

Thursday, March 5

7-8:15 a.m. Breakfast, Windsor Lawn

Session 3: **Fatty liver, TRLs and atherosclerosis**
8:45-11:45 a.m.
Upper Grand Ballroom

Session Chair: Rebecca Haeusler, Columbia University

8:45-9:15 a.m. **DGAT2 inhibition for the treatment of NASH**
Morris Birnbaum, Pfizer, Inc.

9:15-9:45 a.m. **A New Era for Treatment of Hypertriglyceridemia; or Not?**
Henry Ginsberg, Columbia University

9:45-10:15 a.m. **Role of de novo lipogenesis in NAFLD**
Sam Klein, Washington University in St. Louis

10:15-10:45 a.m. Break

10:45-11:15 am **The Multifaceted Roles of Adipose Tissue for Diabetes and Fatty Liver**
Phil Scherer, UT Southwestern

11:15-11:30 a.m. **Role of Liver-derived Angiopoietin-like protein 4 in metabolic diseases**
Abhishek Singh, Yale University

11:30-11:45 a.m. **Identifying tissue-specific actions of Angiopoietin-like 4 during high fat diet feeding in mice**
Kathryn Spitler, University of Iowa

11:45 a.m.-5 pm Free Time

Thursday, March 5, continued

5-6 p.m.	Poster Session II, Upper Grand Ballroom
6-7:15 p.m.	Dinner, Empress Room

Session 4: **Fat to NASH and beyond**
7:30-9:30 p.m.
Upper Grand Ballroom

Session Chair:	Ira Tabas, Columbia University
7:30-8 p.m.	**NASH, Fibrosis and HCC – Diagnostic and Therapeutic Frontiers** Scott Friedman, Mount Sinai School of Medicine
8-8:30	**Notch activation induces NASH-associated fibrosis** Utpal Pajvani, Columbia University
8:30-9 p.m.	**Innate immune signaling in NASH/ASH** Wajahat Mehal, Yale University
9-9:15 p.m.	**Deletion of hepatic Ppp1r3b causes dysregulated glucose and lipid metabolism that precedes steatosis in mice** Kate Townsend Creasy, University of Pennsylvania
9:15-9:30 p.m.	**TMEM55B regulates hepatic fatty acid oxidation** Yuanyuan Qin, University of California, San Francisco

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Friday, March 6

7-8:15 a.m.	Breakfast, Windsor Lawn

Session 5: **Emerging Therapies for NASH**
8:45-10:15 a.m.
Upper Grand Ballroom

Session Chair:	Joseph Witztum, University of California, San Diego
9-9:30 a.m.	**Combination therapies for NASH** Chinweike Ukomadu, Novartis Institutes for Biomedical Research

Friday, March 7, continued

9:30-10 a.m. **Characterization of FGF19 analogues for NASH**
Lei Ling, NGM Biopharmaceuticals

10-10:30 a.m. **ACC inhibition for the treatment of NASH**
Chuhan Chung, Gilead Sciences, Inc.

1	DYRK1B Triggers de-novo Lipogenesis by Activating mTORC2 Complex and is an Attractive Target for the Treatment of Non-Alcoholic Fatty Liver Disease
2	Loss of function of an atypical AAA+ ATPase, torsinA, results in severe NAFLD on a chow diet with marked reductions in VLDL secretion and accumulation of lipid droplets within the nucleus and ER.
3	The Microtubule, Perilipin-2, Lipid Droplet Axis - a Potential Therapeutic Target for Fatty Liver Disease
4	Hepatic Deletion of Mboat7 (Lpiat1) Causes Activation of SREBP-1c and Fatty Liver
5	Proprotein Convertase 7 (PCSK7) Reduces apoA-V Levels
6	Membrane type 1 matrix metalloproteinase promotes ectodomain shedding of low-density lipoprotein receptor and accelerates the development of atherosclerosis.
7	New insights into the sigma-2 receptor, an enigmatic membrane protein that regulates cellular cholesterol homeostasis
8	Identification of lipidomic signatures and a novel phenotype in response to long chain n-3 polyunsaturated fatty acid supplementation in humans
9	Role of Liver-derived Angiopoietin-like protein 4 in metabolic diseases
10	GLP-1R agonist inhibits hepatic lipogenesis via β-catenin signaling
11	Eicosapentaenoic Acid (EPA) Chain Length and Degree of Unsaturation Leads to Inhibition of Oxidation of Small Dense LDL and Membrane Cholesterol Domains as Compared to Related Fatty Acids
12	Eicosapentaenoic Acid (EPA) has a Stable Membrane Location Associated with Slower Dissociation Kinetics as Compared to Fenofibric Acid and Nicotinic Acid
13	Eicosapentaenoic Acid Improved Nitric Oxide Bioavailability in Human Endothelial Cells in Contrast to Docosahexaenoic Acid In Vitro
14	Contrasting Effects of Eicosapentaenoic Acid (EPA) and Arachidonic Acid (AA) Containing Phospholipids on Membrane Structure: X-ray Diffraction Analysis
15	Decoding disease-associated changes in gene expression of diverse hepatic macrophages
16	Neutralization of Oxidized Phospholipids Ameliorates Non-alcoholic Steatohepatitis
17	The Role of Arylacetamide Deacetylase (AADAC) in Hepatic Lipid Homeostasis
18	Functional characterization of the MBOAT7 O-acyltransferase activity
19	Identifying tissue-specific actions of Angiopoietin-like 4 during high fat diet feeding in mice
20	Genome-wide CRISPR-Cas9 knockout screen to dissect ceramide cytotoxicity
21	Dynamic regulation of de novo lipogenesis by internal RNA modifications
22	Haploid genetic screens identify SPRING/C12ORF49 as a novel determinant of SREBP signaling and cholesterol metabolism
23	A human liver chimeric mouse model for non-alcoholic fatty liver disease
24	Development of inhibitors for human Adipose Triglyceride Lipase (ATGL)
25	Novel mechanisms controlling adipose tissue heterogeneity
26	Insulin signaling in the liver maintains cholesterol homeostasis by suppressing cholesterol absorption in the gut
27	TMEM55B regulates hepatic fatty acid oxidation
28	Altered humoral immune response in the gut during non-alcoholic fatty liver disease in hypercholesterolemic mice.
29	Chop/Ddit3 depletion in pancreatic β-cells alleviates ER stress and corrects hepatic steatosis
30	Identifying novel genes involved in hepatic steatosis through bioinformatics
31	Conditional deletion of Pdia1 reveals its multifaceted role in fatty liver disease and its potential as a novel anti-fibrotic therapeutic target
32	Identification of distinct residues in the carboxy-terminal domain of SREBP2 that control interaction with Scap and degradation by proteasomes
33	Large-Scale Multi-Omics Integration Identified Liver Pyruvate Kinase as a Novel Sex-Specific Candidate in NAFLD/NASH
34	Functional and biophysical investigation of the Scap-SREBP pathway

35	Acute effect of long-chain and medium-chain saturated fatty acids on hepatic energy metabolism.
36	Reduced Fatty Acid Desaturase 1 Function Alters Glucose Metabolism and Leads to Hepatic Stellate Cell Activation
37	HDL induces ADAM metallopeptidase domain 17 (ADAM17) to modulate lipid- and inflammatory macrophage phenotypes
38	Inhibition of Endothelial Lipase by ANGPTL3
39	Intestinal Apolipoprotein A1 deletion reduces total and HDL lipoprotein mass and alters bile acid composition in mice
40	Lipin 1 Regulates Adipocyte Lipogenic Capacity and Systemic Insulin Sensitivity on High Fat Diet.
41	Monoacylglycerol acyltransferase 1 knockout mice are not protected from obesity-related metabolic disease.
42	Progress Towards Standardizing Metagenomics: Application of Metagenomic Reference Materials to Develop a Reproducible Microbial Lysis Methodology with Minimum Bias
43	Deletion of hepatic Ppp1r3b causes dysregulated glucose and lipid metabolism that precedes steatosis in mice.
44	Fish oil-derived furan fatty acids: new players in the regulation of metabolic syndrome
45	Identification of Mitochondrial Pyruvate Carrier Inhibitors to Treat Nonalcoholic Fatty Liver Disease
46	Elucidating the physiological function of FIT2 in murine ER homeostasis and lipid storage
47	Adipose tissue lipolysis provides signals that modulate hepatic gene expression program
48	Insulin signaling in the liver promotes lipogenesis but suppresses inflammation
49	An AMPK-Caspase-6 Axis Controls Liver Damage in Nonalcoholic Steatohepatitis
50	The nuclear receptor FXR regulates hepatic lipids via a reduction in lipid absorption
51	Interrogating Liver Macrophage LXR Signaling in Health and Non-Alcoholic Fatty Liver Disease
52	Human plasma lipidome and its link to non-alcoholic fatty liver disease

1

DYRK1B Triggers de-novo Lipogenesis by Activating mTORC2 Complex and is an Attractive Target for the Treatment of Non-Alcoholic Fatty Liver Disease

Neha Bhat[1], Mohsen Fathzadeh[1], Gerald I. Shulman[1], Arya Mani[1]

[1]Yale University

The mechanisms underlying increased insulin-induced hepatic de-novo lipogenesis (DNL) in the presence of insulin resistance in individuals with Metabolic syndrome (MetS) and Non-alcoholic fatty liver disease (NAFLD) are not understood. Rare independent mutations in the DYRK1B gene have been associated with MetS. In this study, we show that the encoded kinase is a nutrient-sensing protein that is upregulated in the liver of mice with diet-induced NAFLD and individuals with advanced NAFLD. The elevated expression of Dyrk1b in the mouse liver resulted in enhanced de-novo lipogenesis (DNL) hepatic fatty acid uptake, triglycerides secretion and hepatic Glucose Production (HGP), while it reduced canonical Insulin signaling. Strikingly, disruption of Dyrk1b conferred protection against hepatic steatosis, hyperlipidemia and insulin resistance. This exciting finding prompted a comprehensive systems-biology approach, which revealed that Dyrk1b facilitates DNL by binding, and activating mTORC2 complex via displacing its inhibitor Fkbp12 from the complex and inducing its auto-phosphorylation. These findings provide key insights into the mechanisms that trigger DNL in the setting of hepatic insulin resistance and identify Dyrk1b as an attractive therapeutic target for NAFLD and MetS.

2

Loss of function of an atypical AAA+ ATPase, torsinA, results in severe NAFLD on a chow diet with marked reductions in VLDL secretion and accumulation of lipid droplets within the nucleus and ER.

Henry N. Ginsberg[1], Ji-Yeon Shin[1], Antonio Hernandez-Ono[1], Tatyana Fedetova[1], Cecilia Ostlund[1], Michael Lee[1], Sarah Gibeley[1], Chun-Chi Liang[2], William T. Dauer[3], Howard J. Worman[1]

[1]Vagelos College of Physicians and Surgeons, Columbia University, New York, New York, USA, [2]University of Michigan Medical School, Ann Arbor, Michigan,, USA, [3]University of Texas Southwestern Medical Center, Dallas, Texas, USA

Deciphering novel pathways regulating liver lipid content has profound implications for understanding the pathophysiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Recent evidence suggests that the nuclear envelope is a site of regulation of lipid metabolism but there is limited appreciation of the responsible mechanisms and molecular components within this organelle. We show that conditional hepatocyte deletion (KO) of the inner nuclear membrane protein, lamina-associated polypeptide 1 (LAP1), caused a moderate reduction in the secretion of VLDL apoB100 and TG, and steatosis both in vivo and in primary hepatocytes from LAP1 KO mice. The steatosis comprised both cytosolic and intra-nuclear lipid droplet accumulation. LAP1 binds to and activates torsinA, an AAA+ ATPase that resides in the perinuclear space and continuous main ER. TorsinA has no intrinsic ATPase activity but is activated by both LAP1 and an ER-localized protein, LULL1. Deletion of torsinA from mouse hepatocytes caused much greater reductions in VLDL apoB100 and TG secretion, and profound steatosis compared to the LAP1 KO mouse. Unexepectedly, there was marked sequestration of small lipid droplets within the ER. Both of these mutant mouse lines developed hepatic

steatosis and subsequent steatohepatitis on a regular chow diet in the absence of whole-body insulin resistance or obesity. Our results establish an essential role for the AAA+ATPase activity of the nuclear envelope-localized LAP1:torsinA complex in hepatic VLDL secretion, and both lipid droplet formation and distribution. These models will also enable study of the conversion of stimple steatosis to steatohepatitis in the absence of the metabolic abnormalities associated with insulin resistance and obesity.

3

The Microtubule, Perilipin-2, Lipid Droplet Axis - a Potential Therapeutic Target for Fatty Liver Disease

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[1]Mayo Clinic, Rochester, MN 55905

Hepatic steatosis is the most common precursor of liver diseases which necessitate transplantation and also the most common reason allografts are rejected from the donor pool. Annually, >1,300 steatotic donor allografts cannot be utilized due to risk of primary nonfunction. 35% of willing, living donors are not acceptable due to having their own non-cirrhotic, hepatic steatosis. As such, strategies to reverse fatty liver are important for patients at risk for end stage liver disease as well as to increase the pool of usable livers for transplantation. With the ultimate goal of rapidly delipidating steatotic donor livers in the time between allograft harvest and recipient transplantation, we have discovered interactions between microtubules and lipid droplets (LD) may be therapeutic targets to rehabilitate fatty livers. Using immunoprecipitation, we show that perilipin-2 (PLIN2), a LD-associated protein which regulates fat storage and utilization within hepatocytes, interacts with microtubules. Further, this interaction is disrupted by nocodazole, a microtubule targeting agent (MTA). We show treatment of primary, steatotic hepatocytes with MTAs leads to uncoupling of PLIN2 from the LD surface. Under basal conditions, PLIN2 occupies up to 97% of the LD surface however treatment with the MTA epothilone A decreases PLIN2's surface occupancy to 75% ($p=0.03$) perhaps exposing vulnerabilities for the action of cytosolic lipases. Using static and live-cell imaging, we demonstrate that nocodazole at non-toxic concentrations promotes liposecretion, the loss of LDs from steatotic primary hepatocytes. Using the precision cut liver slice ex-vivo model, steatotic liver tissue treated with nocodazole for 6 hours shows a >50% reduction in hepatic lipid compared to vehicle treated tissue (0.17 vs 0.41 LD/nuclei ratio, $p=0.05$) with no loss in tissue viability (71 vs 73 pmol ATP/ug protein, $p=0.4$). These data pave the way for further studies aimed at salvaging steatotic liver allografts for transplantation.

4

Hepatic Deletion of Mboat7 (Lpiat1) Causes Activation of SREBP-1c and Fatty Liver

Matthew A. Mitsche[1], Mingfeng Xia[1], Preethi Chandrasekaran[1], Xiaorong Fu[1]

[1]University of Texas Southwestern Medical Center

Genetic variants that increase the risk of fatty liver disease (FLD) and cirrhosis have recently been identified in the intergenic region between membrane bound O-acyltransferase domain-containing 7 (MBOAT7) and transmembrane channel-like 4 (TMC4). To elucidate the link between these genetic variants and FLD we characterized Mboat7 liver-specific knock-out mice (Mboat7 LSKO). These mice developed fatty livers on a chow diet and had increased plasma markers of hepatic inflammation, consistent with the human phenotype. Hepatic deletion of Mboat7 caused a depletion of 20-carbon polyunsaturated fatty acids in phosphatidylinositols (PIs). The change in PI composition was associated with a marked increase in de novo lipogenesis caused by increased SREBP-1c, which transcriptionally activated the protein machinery responsible for fatty acid biosynthesis. This study shows that PI composition has an important role in regulation hepatic fat synthesis and that inactivation of hepatic Mboat7 can lead to FLD.

5

Proprotein Convertase 7 (PCSK7) Reduces apoA-V Levels

Nabil G. Seidah[1], Yahya Ashraf[1], Stephanie Duval[1], Vatsal Sachan[1], Annik Prat[1], Robert S. Kiss[2]

[1]Montreal Clinical Research Institute, affiliated to the University of Montreal, [2]McGill University

The locus of the human proprotein convertase subtilisin-kexin type-7 (PC7) gene (*PCSK7*) is on chromosome 11q23.3 close to the gene-cluster *POA5/APOA4/APOC3/APOA1*, a region implicated in the regulation of lipoprotein metabolism. A GWAS reported the association of *PCSK7* SNPs with plasma triglycerides (TG), and exome sequencing of African-Americans revealed the association of a low-frequency coding variant of PC7 (R504H; SNP rs142953140) with a ~30% TG-reduction. Another *PCSK7* SNP rs508487 is in linkage disequilibrium with a promoter-variant of the liver-derived apolipoprotein A-V (apoA-V), an indirect activator of the lipoprotein lipase (LpL), and is associated with elevated TG-levels. We thus hypothesized that PC7 regulates the levels/activity of apoA-V. Studies in the human hepatic cell line HuH7 revealed that wild-type (WT) PC7 and its endoplasmic reticulum (ER)-retained forms bind to and enhance the degradation of human apoA-V in acidic lysosomes in a non-enzymatic fashion. PC7-induced degradation of apoA-V is inhibited by bafilomycin-A1, and the alkalinizing agents: chloroquine and NH_4Cl. Thus, the PC7-induced apoA-V degradation implicates an ER-lysosomal communication inhibited by bafilomycin-A1. *In vitro*, the natural R504H mutant enhances PC7 Ser_{505}-phosphorylation at the structurally-exposed Ser-X-Glu_{507} motif recognized by the secretory kinase Fam20C. Co-expression of the phosphomimetic PC7-S505E with apoA-V resulted in lower degradation compared to WT, suggesting that Ser_{505} phosphorylation of PC7 lowers TG levels via reduced apoA-V degradation. Notably, the natural G185C mutant of apoA-V, which has been associated with high levels of TGs, is ~10-fold better degraded by PC7 compared to WT ApoA-V. In agreement, in *Pcsk7*$^{-/-}$ mice fed high-fat diet, plasma apoA-V levels and adipocyte LpL activity are increased, providing an *in vivo* mechanistic link for a role of liver PC7 in enhanced TG storage in adipocytes. In conclusion, we present biochemical and genetic evidence for the implication of mouse PC7 in TG metabolism *via* its diet-dependent regulation of apoA-V and adipose tissue hypertrophy, revealing that the absence of PC7 results in higher circulating apoA-V levels associated with enhanced LpL activity in adipocytes. Future studies in human should address the possible protective role of PC7 LOF mutations in enhancing apoA-V levels and hence reducing circulating TG levels, which could be pharmacologically beneficial.

6

Membrane type 1 matrix metalloproteinase promotes ectodomain shedding of low-density lipoprotein receptor and accelerates the development of atherosclerosis.

Dawei Zhang[1]

[1]University of Alberta, Edmonton, AB, Canada T6R 2G3

Low-density lipoprotein receptor (LDLR)-mediated cellular LDL uptake is the main pathway for plasma LDL cholesterol (LDL-C) clearance. It has been documented that LDLR can be proteolytically cleaved to release its soluble ectodomain (sLDLR) into extracellular milieu. Plasma sLDLR levels are positively correlated with plasma LDL-C levels. Membrane type 1-matix metalloproteinase (MT1-MMP) is a Zn2+-dependent endopeptidase that can cleave extracellular matrix and non-matrix substrates. However, the proteinase responsible for LDLR cleavage and the role of MT1-MMP in LDLR shedding are unknown. We found that knockdown of MT1-MMP increased cellular LDLR abundance and reduced the levels of sLDLR in cultured hepatocytes. LDLR and MT1-MMP were co-immunoprecipitated and co-localized. Consistently, mice lacking hepatic MT1-MMP displayed an increase in liver LDLR levels and a reduction in plasma levels of sLDLR, HDL-cholesterol, and non-HDL cholesterol. Opposite effects were observed when MT1-MMP was overexpressed. Moreover, we demonstrated that overexpression of MT1-MMP significantly increased atherosclerotic lesion area in apolipoprotein (apo) E knockout mice. In addition, we found that the majority of circulating sLDLR were associated with apoB and apoE-containing lipoproteins in both mouse and human plasma. Plasma levels of sLDLR were significantly increased in subjects with high plasma LDL-C levels. Thus, we demonstrate that MT1-MMP promotes ectodomain shedding of hepatic LDLR, thereby regulating plasma cholesterol levels and the development of atherosclerosis.

7

New insights into the sigma-2 receptor, an enigmatic membrane protein that regulates cellular cholesterol homeostasis

Assaf Alon[1], Andrew C. Kruse[1]

[1]Harvard Medical School

S2R is a poorly understood transmembrane protein implicated in Alzheimer's disease and schizophrenia. Unlike other pharmacologically defined receptors, S2R has eluded molecular cloning since its discovery and the gene that codes for the receptor remained unknown, precluding the use of all modern molecular biology tools for its study. We cloned S2R by biochemical purification from calf-liver tissue, revealing its identity as TMEM97, an endoplasmic reticulum-resident transmembrane protein. We show that TMEM97 possesses the full suite of molecular properties that define S2R. TMEM97 is a SREBP target gene that regulates cellular cholesterol homeostasis and controls the levels of the sterol transporter NPC1. The sigma-2 receptor is genetically related to the mammalian Δ8-9 sterol isomerases EBP. Although the sigma-2 receptor cannot catalyze the isomerization reaction, it has the conserved amino acids required for catalysis. These amino acids are likely needed to specifically identify its physiological ligand, most likely a sterol signaling molecule. Cloning the sigma-2 receptor resolves a longstanding mystery and will enable therapeutic targeting of this potential drug target.

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Identification of lipidomic signatures and a novel phenotype in response to long chain n-3 polyunsaturated fatty acid supplementation in humans

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Supplementation with long chain n-3 polyunsaturated fatty acids (LCn-3) is used clinically to reduce total circulating triacylglycerol (TAG) concentrations. However, interindividual variability exists; some individuals do not show a decrease in TAG. Moreover, supplementation has variable effects upon plasma cholesterol concentrations. To determine which lipid species contribute to this variability, we performed a targeted, mass spectrometry (MS), infusion-based lipidomic analysis on plasma samples obtained from a clinical study in which participants were supplemented with 3 g/day of LCn-3 in the form of fish oil capsules over a 6-week period. TAG species (brutto structure) and cholesteryl esters (CE) were quantified for 130 participants pre- and post-supplementation. Based upon the change of total TAG concentrations following supplementation determined by MS, participants were segregated into three potential responder phenotypes: (1) positive responder (R^+; TAG decrease < 10%), (2) non-responder (NR; TAG changes +/- 10%), and (3) negative responder (R^-; TAG increase > 10%) representing 87/130 (67%), 23/130 (18%), and 20/130 (15%) of the study samples, respectively. Sparse partial least squares discriminant analysis (SPLSDA) allowed for separation of the three phenotypes with component 1 (31% of variance) attributed to change in TAG 50-53:X with 0-3 desaturations with R^+ having reductions in these TAG. Separation along component 2 (8.3% of variance) identified lower mass TAG 46-48:X with 1-3 desaturations likely containing 14:0. This latter effect impacted mostly NR and R^- phenotypes. Using the TAG responder phenotype for grouping, we performed SPLDA analysis for CE responses. Surprisingly, we observed that distinction of the TAG responder phenotypes qualitatively applies to CE in which separation along component 1 (65% of variance) was due to differences in CE 18:0, 18:1, 14:0, and others. Plotting percent change for individual CE and for total CE demonstrated that LCn-3 intake either elevated or left unchanged CE for the NR, R^-, and R^+ phenotypes. However, the R^+ phenotype also contained a large number of participants that had reductions in CE. In summary, these data identify lipidomic signatures (TAG and CE) associated with LCn-3 response phenotypes and identify a novel phenotype based upon CE changes.

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Role of Liver-derived Angiopoietin-like protein 4 in metabolic diseases

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An exaggerated response of postprandial circulating triglyceride-rich lipoproteins (TRL) remnants and the accumulation of cholesterol remnant particles represent a risk factor for type 2 diabetes and coronary artery disease (CAD). Under physiological conditions, liver regulates systemic lipid and glucose metabolism to maintain whole-body energy homeostasis. Dysregulation of lipid metabolism in the liver can often lead to T2D and CAD. In particular, high levels of triacylglycerol (TAG) are associated with increased risk of T2D and atherosclerosis. Angiopoietin-like protein 4 (ANGPTL4) is a secretory protein predominantly expressed in adipose and liver and is known to modulate plasma TAG levels by inhibiting lipoprotein lipase (LPL) activity. Several human genetic studies have shown that loss of function of ANGPTL4 improves systemic lipid and glucose metabolism and associated with lower risk of T2D and CAD. Since, global ANGPTL4 knockout (KO) mice showed severe systemic metabolic complications upon high-fat diet feeding, largely due to gut inflammation, limiting our understanding about the contribution of ANGPTL4 in metabolic dysfunction, therefore to circumvent the deleterious effect of ANGPTL4, we developed liver-specific ANGPTL4 KO mouse model. Our present studies demonstrate that ANGPTL4 deficiency in hepatocytes facilitates the catabolism of TRL remnants in the liver via increased hepatic lipase (HL) activity, thus lowering circulating lipid levels. In addition, depletion of hepatocyte ANGPTL4 improves hepatic lipid metabolic function through reducing lipid biosynthesis and increasing fatty acid oxidation in the liver. These effects are likely dependent on ROS mediated AMPK signaling. As a consequence of deletion of hepatocyte ANGPTL4 protected obesity-induced glucose intolerance with a reduction in body weight, liver steatosis, and atherosclerosis in mice.

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GLP-1R agonist inhibits hepatic lipogenesis via β-catenin signaling

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Non-alcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease worldwide in part due to obesity epidemic and insulin resistance. The mechanisms underlying the non-alcoholic steatosis formation are poorly understood and little information is available on the pathways that are responsible for the progressive hepatocellular damage that follows lipids accumulation. Recently, much research has focused on the identification of the Wnt/β-catenin signaling pathway that is play a significant role in the physiology and pathology of liver and up to half of hepatocellular carcinoma (HCC) patients have activation of the Wnt/β-catenin signaling. However, to the best of our knowledge, a role for Wnt/β-catenin signaling in exendin-4-mediated protection against hepatic lipogenesis has not been described. The aim of our study is to investigate whether the beneficial effect of GLP-1R agonist on hepatic steatosis is mediated by Wnt/β-catenin signaling and to test the hypothesis that exendin-4-mediated activation of β-catenin signaling plays a crucial role in the inhibition of hepatic lipogenesis. HepG2 human hepatoma cells were treated with Oleic Acid (OA) to induce steatosis, the total triglycerides levels were increased in a dose-dependent and the expression levels of perilipin family members were upregulated in cells treated with PA. For our in vitro model of hepatic steatosis, HepG2 cells were treated with OA in the presence or absence of exendin-4. Our results showed that OA increased the expression of lipogenic genes, such as SREBP-1c, SCD1, CPT1A FAS, FABP1 and four, FOXA1 and ACC and triglyceride synthesis-involved genes, such as DGAT1 and DGAT2. Moreover, exendin-4 treatment increased the expression of phosphorylated glycogen synthase kinase-3 beta (GSK-3β) in the cytosolic fraction and the expression of β-catenin and transcription factor 4 (TCF4) in the nuclear fraction. In addition, siRNA-mediated inhibition of β-catenin upregulated the expression of lipogenic transcription factors. The protective effects of exendin-4 on intracellular triglyceride content and total triglyceride levels were not observed in cells treated with the β-catenin inhibitor IWR-1. These data suggest that exendin-4 treatment improves hepatic steatosis by inhibiting lipogenesis via activation of Wnt/β-catenin signaling

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Eicosapentaenoic Acid (EPA) Chain Length and Degree of Unsaturation Leads to Inhibition of Oxidation of Small Dense LDL and Membrane Cholesterol Domains as Compared to Related Fatty Acids

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LDL and cell membrane oxidation are causatively related to inflammation, cholesterol crystal formation and endothelial dysfunction in atherosclerosis. Small dense LDL (sdLDL) is especially atherogenic due to high susceptibility to oxidation and vessel penetration. In clinical trials the omega-3 fatty acid (FA) eicosapentaenoic acid (EPA, 20:5, ω-3) significantly reduced oxidized LDL in patients with hypertriglyceridemia by unknown mechanisms. We compared EPA effects to related FAs of varying chain length and unsaturation on oxidation of sdLDL and model membranes, and on cholesterol crystal domains. We compared EPA to the following FAs: stearic (SA, 18:0), oleic (OA, 18:1, ω-9), linoleic (LA, 18:2, ω-6), alpha-linolenic (ALA, 18:3, ω-3), eicosanoic (EA, 20:0), eicosatrienoic (ETE, 20:3, ω-3), arachidonic (AA, 20:4, ω-6), docosapentaenoic (DPA, 22:5, ω-3), and docosahexaenoic (DHA, 22:6, ω-3). Human sdLDL or model membranes of cholesterol and 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC) were preincubated with FAs followed by copper-induced oxidation. Malondialdehyde (MDA) or lipid hydroperoxides (LOOH) levels measured oxidation; small-angle X-ray diffraction assessed cholesterol domain formation under hyperglycemic conditions. After 40 minutes, EPA reduced MDA levels 70% compared to vehicle ($p<0.001$). Lesser inhibition was observed with DHA, DPA, ETE, and ALA (33%, 34%, 32%, and 16%, respectively; all $p<0.001$ versus vehicle). At 60 minutes, EPA remained the most active FA, inhibiting oxidation by 44% ($p<0.001$) compared to vehicle. Only DPA and DHA also showed significant activity at 60 minutes, reducing MDA formation by 9% and 7%, respectively ($p<0.05$) while the remaining FAs had no antioxidant activity. Similar relative FA effects were observed in model membranes; EPA inhibited LOOH formation by 77% versus vehicle. DHA, DPA, ETE, and ALA inhibited oxidation by 11, 48, 36, and 27%, respectively ($p<0.05$ for DHA; $p<0.001$ for other treatments). Finally, EPA and DPA more substantially inhibited cholesterol crystal domain formation than the other FAs examined. These data suggest a preferred molecular conformation for EPA due to its combination of hydrocarbon length and number of double bonds, possibly allowing it to insert efficiently and effectively into lipoprotein particles. Reduced sdLDL and membrane oxidation may provide a rationale for greater LDL clearance and reduced inflammation with EPA treatment.

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Eicosapentaenoic Acid (EPA) has a Stable Membrane Location Associated with Slower Dissociation Kinetics as Compared to Fenofibric Acid and Nicotinic Acid

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Eicosapentaenoic acid (EPA), an omega-3 fatty acid (O3FA), has been associated with reduced cardiovascular risk that may extend beyond triglyceride (TG)-lowering, including reductions in membrane and lipoprotein oxidation. The objective of this research project was to compare the membrane location of EPA to widely used TG-lowering agents fenofibric acid (FF) and nicotinic acid (NA). We correlated the membrane location of these agents with their dissociation or "wash-out" kinetics. Small angle x-ray diffraction approaches were used to compare the membrane interactions of EPA, NA, and FF in vesicles reconstituted from 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and cholesterol (C) at a 0.3 C:POPC mole ratio. Electron density profiles (electrons/Å3 vs Å) were calculated from the diffraction data to determine the precise drug distribution and membrane width or d-space, at 1 Å resolution. The wash-out kinetics of these agents were measured using rapid centrifugation methods combined UV/Vis spectrophotometry. The results showed that EPA had a well-defined and relatively extended conformation in the membrane hydrocarbon core as evidenced by a broad increase in electron density ±15Å from the membrane center. By contrast, FF and NA were limited to surface headgroup interactions. EPA-treated samples had a similar d-space value compared to vehicle (56 ± 0.3 vs 57 ± 0.3 Å) while the d-space values for FF and NA samples were greater at 60 ± 2 Å and 58 ± 0.3 Å, respectively. Finally, EPA wash-out from the membrane was much slower than that of FA or NA. After 15 minutes, only 27 ± 6% of EPA was removed from the membrane as compared to 98 ± 1% and 86 ± 2% for FF and NA, respectively. This indicates that EPA, unlike other TG-lowering agents, is able to intercalate into membranes where it may exert pleiotropic effects beyond TG-lowering such as membrane stability and inhibition of lipid oxidation. These distinct membrane interactions of EPA may contribute to atheroprotective benefits beyond TG-lowering.

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Eicosapentaenoic Acid Improved Nitric Oxide Bioavailability in Human Endothelial Cells in Contrast to Docosahexaenoic Acid In Vitro

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Treatment with prescription eicosapentaenoic acid (EPA) has been associated with reduced cardiovascular risk due to atherosclerotic disease compared to combination omega-3 fatty acid (O3FA) treatments that include docosahexaenoic acid (DHA). A possible mechanism of benefit of EPA may be improved endothelial cell (EC) function, as evidenced by increased nitric oxide (NO) release and decreased nitro-oxidative ($ONOO^-$) stress. The effects of EPA may be distinct from other O3FAs such as DHA due to differences in structure, metabolism and antioxidant potency. In this study, human umbilical vein endothelial cells were pretreated with EPA or DHA at equimolar levels (10 μM). Following treatment, the cells were stimulated with calcium ionophore and assayed for the ratio of NO and $ONOO^-$ release, an indicator of endothelial nitric oxide synthase (eNOS) coupling, using tandem porphyrinic nanosensors. The results showed that ECs treated with EPA had significantly greater NO release following stimulation as compared to vehicle by 16% ($p<0.01$). By contrast, DHA did not significantly improve NO production. ECs treated with EPA also showed a reduction in $ONOO^-$ release by 11%. EPA caused a pronounced improvement in the NO/$ONOO^-$ release ratio by 30% (3.80 ± 0.28 vs 2.92 ± 0.15; $p<0.05$) that was not observed with DHA (14% increase; 3.34 ± 0.19 vs 2.92 ± 0.15). Thus, EPA may improve vascular endothelial function, distinct from DHA, due to potential differences in their effects on signal transduction.

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Contrasting Effects of Eicosapentaenoic Acid (EPA) and Arachidonic Acid (AA) Containing Phospholipids on Membrane Structure: X-ray Diffraction Analysis

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The omega-3 fatty acid, eicosapentaenoic acid (EPA), inhibits membrane lipid oxidation and cholesterol crystalline domain formation in a manner opposite to the omega-6 fatty acid, arachidonic acid (AA). The molecular basis for these distinct effects of EPA versus AA is not understood but may be due to their influence on membrane structure as a constituent of phospholipids (PL). Small angle x-ray diffraction approaches were used to compare the effects of 1-palmitoyl-2-eicosapentaenoyl-sn-glycero-3-phosphocholine (PEPC) versus 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (PAPC) on model membrane structure in the absence and presence of cholesterol (C) at a 0.3:1 C:PL ratio. As a control, we also evaluated membranes consisting of oleic acid (OA) or 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). Electron density profiles (electrons/Å3 vs Å) generated from the diffraction data were used to determine membrane structure, including its width or d-space, at 1 Å resolution. The results showed that membranes composed of PEPC and PAPC had similar membrane widths of 46 ± 2 Å and 46 ± 2 Å, respectively, in the absence of cholesterol. In the presence of cholesterol, however, the membrane width of PEPC (49 ± 1 Å) was 5 Å and 8 Å less, respectively, than PAPC (54 ± 1 Å) and POPC (57 ± 1 Å). PEPC also had a broader and more pronounced change in electron density throughout the hydrocarbon core (±10 Å from center of the membrane) compared to both PAPC and POPC membranes. These data indicate that PL composed of EPA form membranes in the presence of cholesterol with a width and electron density that is distinct from those formed by AA- and OA-containing PL, conceivably due to the number and location of EPA's double bonds. The distinct effects of EPA-containing PL on membrane structure may provide a potential mechanism for its beneficial effects of membrane organization and signal transduction under conditions of disease.

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Decoding disease-associated changes in gene expression of diverse hepatic macrophages

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Tissue macrophages contribute beneficially to host defense but also play pathological roles in a diverse range of human diseases. Varied macrophage phenotypes are often found in a diseased tissue, but our understanding of the mechanisms that control diversification remains underdeveloped. Here we used a combination of genetic, genomic, and imaging approaches to investigate the origins and epigenetic trajectories of hepatic macrophages during diet-induced non-alcoholic steatohepatitis (NASH). Development of NASH was linked to emergence of three recruited macrophage (RM) subsets associated with different niches. Comparing chromatin accessibility of the enhancer-like regions of these macrophage subsets supported predictions of increased NFkB and RUNX transcriptional activity in RMs and increased activity of Liver X receptors (LXR) in Kupffer cells. Focused studies on Kupffer cell enhancer changes during NASH revealed that while detection of global alterations in chromatin accessibility were modest, significant changes in activity occurred at more than 7,700 enhancers. Consequently, NASH led to partial loss of Kupffer cell transcriptional identity, increased expression of *Trem2* and *Cd9*, and cell death. Kupffer cell loss was compensated by gain of adjacent monocyte derived macrophages that exhibited convergent epigenomes, transcriptomes, and function. Mechanistically, NASH-induced epigenetic changes in Kupffer cells were driven by hierarchical activity of ATF3 which reprogrammed Kupffer cell specific functions of LXRs. These enhancer sites had gained recruitment of the histone acetyltransferase P300 and associated histone acetylation. Together our findings reveal mechanisms by which disease-associated environmental signals instruct resident and recruited macrophages to acquire distinct programs of gene expression and corresponding phenotypes. Further, this approach could serve as an experimental framework for extending mechanistic understanding of cellular function in many tissues.

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Neutralization of Oxidized Phospholipids Ameliorates Non-alcoholic Steatohepatitis

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Oxidized phospholipids (OxPLs), which arise due to oxidative stress, are proinflammatory and proatherogenic,but their roles in non-alcoholic steatohepatitis(NASH) are unknown. Here, we show that OxPLs accumulate in human and mouse NASH. Using a transgenic mouse that expresses a functional single-chain variable fragment of E06, a natural antibody that neutralizes OxPLs, we demonstrate the causal role of OxPLs in NASH. Targeting OxPLs in hyperlipidemic Ldlr-/- mice improved multiple aspects of NASH, including steatosis, inflammation, fibrosis, hepatocyte death, and progression to hepatocellular carcinoma. Mechanistically, we found that OxPLs promote ROS accumulation to induce mitochondrial dysfunction in hepatocytes. Neutralizing OxPLs in AMLN-diet-fed Ldlr-/- mice reduced oxidative stress, improved hepatic and adipose-tissue mitochondrial function, and fatty-acid oxidation. These results suggest targeting OxPLs may be an effective therapeutic strategy for NASH.

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The Role of Arylacetamide Deacetylase (AADAC) in Hepatic Lipid Homeostasis

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The liver plays a central role in maintaining the whole-body lipid and energy homeostasis. Hepatic triacylglycerol (TG) and cholesteryl ester (CE) synthesis, lipolysis and transport are regulated by nutritional status. Fasting increases hepatic TG/CE storage and this storage is rapidly depleted during re-feeding. The lipolytic processes responsible for TG/CE turnover during re-feeding have not yet been characterized. We have identified a novel liver lipase arylacetamide deacetylase (AADAC) that is localized in the endoplasmic reticulum and exhibits hydrolytic activity toward diacylglycerol, TG and CE. We have found that AADAC is involved in the hepatic TG/CE turnover during the fasting/re-feeding transition. TG/CE depletion was abolished during the re-feeding period in AADAC deficient mice. AADAC knockout mice accumulated significantly more hepatic neutral lipids than wild type mice when challenged with western-type diet, and exhibited accelerated progression of liver steatosis in the long-term western-type diet feeding study. These findings indicate that AADAC plays an important role in hepatic lipid metabolism and that dysregulation of AADAC activity leads to fatty liver.

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Functional characterization of the MBOAT7 O-acyltransferase activity

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Background: Human membrane bound O-acyltransferase domain-containing 7 (MBOAT7), also known as lysophosphatidylinositol acyltransferase 1 (LPIAT1), is an enzyme involved in the acyl-chain remodeling of phospholipids via the Lands' cycle. The *MBOAT7 rs641738* variant has been associated with the entire spectrum of non-alcoholic fatty liver diseases (NAFLD) and brain disorders, but very little is known about the MBOAT7 activity. The determination of the MBOAT7 substrate specificity and enzymatic activity are crucial to understand the role of the protein in the development of NAFLD.

Methods: To investigate the MBOAT7 O-acyltransferase activity, human wild type MBOAT7 and three MBOAT7 mutants missing in the catalytic residues (N321A, H356A, N321A+H356A) were produced into *Pichia pastoris*, a heterologous system for protein production. MBOAT7 proteins were purified using Ni-affinity chromatography. The enzymatic activity of MBOAT7 wild type and mutants was assessed measuring the incorporation of radiolabeled fatty acids into lipid acceptors. Moreover, the *MBOAT7* gene expression was measured in human hepatocytes incubated with fatty acids with different degrees of saturation.

Results: We found that MBOAT7 preferentially transferred polyunsaturated fatty acids (PUFAs) to lysophosphatidylinositol (LPI). On the contrary, MBOAT7 showed lower enzymatic activity for transferring unsaturated fatty acids, regardless the lipid substrate. MBOAT7 mutants showed decreased O-acyltransferase activity. Furthermore, the incubation of hepatocytes with PUFAs increased the *MBOAT7* gene expression level.

Conclusions: Thus, MBOAT7 catalyzes the transfer of PUFAs to lipid acceptors. MBOAT7 shows the highest affinity for LPI, and missense mutations at the MBOAT7 putative catalytic dyad inhibit the O-acyltransferase activity of the protein. Our findings support the hypothesis that the association between the *MBOAT7 rs641738* variant and the increased risk of NAFLD is mediated by changes in the hepatic phosphatidylinositol acyl-chain remodeling. The understanding of the enzymatic activity of MBOAT7 may help developing new strategies for the treatment of NAFLD.

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Identifying tissue-specific actions of Angiopoietin-like 4 during high fat diet feeding in mice

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Elevated plasma triglyceride levels are associated with metabolic diseases including diabetes and obesity. Angiopoietin-like 4 (ANGPTL4) is a regulator of plasma triglyceride levels through its inhibition on lipoprotein lipase (LPL). ANGPTL4-deficient mice have decreased plasma triglyceride levels and we previously observed that these mice have increased triglyceride uptake into adipose tissues, identifying a role of ANGPTL4 in adipose tissue. ANGPTL4 is also expressed in other metabolically active tissues, especially the liver. The role of ANGPTL4 in these other tissues remains unclear. Therefore, we generated adipose and liver-specific Angptl4 ($Angptl4^{AdipoKO}$, $Angptl4^{LivKO}$) knockout mice. We hypothesized that adipose derived ANGPTL4 is responsible for the TG phenotypes observed in whole body knockout mice. Indeed, $Angptl4^{AdipoKO}$ mice recapitulated the triglyceride phenotypes of whole-body deficiency, whereas liver-specific loss of ANGPTL4 had no effect on plasma triglyceride levels or triglyceride uptake. We also examine how deficiency in liver or adipose ANGPTL4 would affect the metabolic disturbances induced by high-fat feeding. These mice were put on a high fat diet (60% kCal/fat) for either 12 weeks or 6 months. Metabolic phenotyping, gene expression, LPL activity and triglyceride uptake were measured in these mice. $Angptl4^{AdipoKO}$ mice gained more weight after 6 months of HFD-feeding and enhanced adipose LPL activity compared to littermates. Interestingly, we found that high-fat feeding dampened the increased triglyceride uptake into adipose seen in $Angptl4^{AdipoKO}$ mice fed a chow diet. We also found that for mice fed a HFD, $Angptl4^{AdipoKO}$ mice initially had improved glucose tolerance and insulin sensitivity, but over time this improvement disappeared. Conversely, $Angptl4^{LivKO}$ mice initially showed no difference in glucose tolerance and insulin sensitivity after 12 weeks of HFD feeding, but at the end of 6 months of HFD feeding these mice had improved glucose tolerance despite no apparent difference in triglyceride phenotypes. showed signs of improvement in both these areas. We concluded that adipose derived Angptl4 is responsible for triglyceride partitioning and regulation of plasma triglycerides, while both adipose and liver derived Angptl4 may play a role in glucose homeostasis.

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Genome-wide CRISPR-Cas9 knockout screen to dissect ceramide cytotoxicity

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Ceramides are a class of sphingolipids tightly linked to a variety of pathological conditions. In metabolic tissues, elevated ceramide levels inhibit PI3K and Akt activities, leading to insulin resistance. Aberrant ceramide levels can also induce cell death. It remains poorly characterized how ceramide accumulation impairs cellular functions. In this work, we performed a genome wide CRISPR-Cas9 genetic screen to identify regulators of ceramide-induced cell death in mouse preadipocytes. The screen isolated known mediators of ceramide metabolism such as ceramide synthase (CERS5) but most of the identified genes were not previously linked to ceramide cytotoxicity. We anticipate that these genes regulate ceramide-linked proapoptotic and antiapoptotic pathways. This work may also shed light on other ceramide-linked activities such as insulin resistance.

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Dynamic regulation of de novo lipogenesis by internal RNA modifications

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N^6-Methyladenosine (m6A) is the most common chemical modification present on eukaryotic RNA. Multiple lines of evidence have shown that m6A plays a critical role in cell fate determination including stem cell renewal, however, the contributions of internal RNA modifications on metabolic control is less well explored. Here, we mapped the m6A landscape in mouse liver showing that RNA modifications are dynamically regulated in response to diet, segregate lipid rich versus control livers and strongly enrich lipogenic genes. Liver-specific deletion of m6A installing machinery resulted in an increase in fatty acid biosynthetic proteins and hepatic triglyceride levels. Our studies reveal insights into the mode of regulation of m6A and physiologic contributions in lipid homeostasis. In summary, we find that the epitranscriptome is dynamically regulated in response to diet and is essential for proper maintenance of metabolic homeostasis.

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Haploid genetic screens identify SPRING/C12ORF49 as a novel determinant of SREBP signaling and cholesterol metabolism

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The sterol-regulatory element binding proteins (SREBP) are central transcriptional regulators of lipid metabolism. Using haploid genetic screens we identify the SREBP Regulating Gene (SPRING/C12ORF49) as a novel determinant of the SREBP pathway. SPRING is a glycosylated Golgi-resident membrane protein and its ablation in Hap1 cells, Hepa1-6 hepatoma cells, and primary murine hepatocytes reduces SREBP signaling. In mice, Spring deletion is embryonic lethal. Yet like in cells, silencing of hepatic Spring expression attenuates the SREBP response. Mechanistically, attenuated SREBP signaling in SPRING-KO cells results from reduced SREBP cleavage-activating protein (SCAP) and its mislocalization to the Golgi irrespective of the cellular sterol status. Consistent with limited functional SCAP in SPRING-KO cells, reintroducing SCAP restores SREBP-dependent signaling and function. Moreover, in line with the role of SREBP in tumor growth, a wide range of tumor cell lines display dependency on SPRING expression. In conclusion, we identify SPRING as a previously unrecognized modulator of SREBP signaling.

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A human liver chimeric mouse model for non-alcoholic fatty liver disease

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Non-alcoholic fatty liver disease (NAFLD) is an emerging public health burden and is characterized by excess neutral lipid accumulation within the liver. Many animal models have been developed to study the features and molecular changes underlying human NAFLD pathogenesis. Despite their relevance for studies on liver physiology, mouse models only poorly recapitulate human NAFLD and are therefore not ideal for validation of therapeutics. To address this disparity between both species, we developed a human liver chimeric mouse model of diet-induced NAFLD. Livers of TIRF (Transgene-free *Il2rg*-/-*Rag2*-/-*Fah*-/-; mixed background) mice were successfully repopulated with human hepatocytes (>60% human chimerism). When fed a Western-type diet (WD) (43 and 40% calories from carbohydrates and fat, respectively) for 12 weeks, xenograft mice developed hepatic steatosis in the absence of steatohepatitis. Whereas murine hepatocytes displayed a normal histopathology, human hepatocytes exhibited lipid droplet accumulation, suggesting increased susceptibility to lipid-related metabolic changes in the setting of overnutrition. Unbiased metabolomics, lipidomics, and transcriptomics revealed molecular signatures mimicking NAFLD patients. Upon WD-feeding, hepatic amounts of cholesteryl-esters were increased (13-fold increase) despite a marked suppression of pathways of cholesterol metabolism at transcriptomic level. There was a 54% increase in hepatic triacylglycerols and an overall increase in the degree of unsaturation of fatty acids esterified to neutral lipids and phospholipids. The NAFLD xenograft mice also revealed transcriptional discrepancies between human and mouse hepatocytes exposed to identical nutritional challenges. Notably, human differentially expressed genes (DEGs) showed little overlap with mouse DEGs, indicating that human and mouse transcriptional signatures differ dramatically. Taken together, these data suggest that, under conditions of overnutrition, human liver chimeric mice recapitulate human NAFLD pathology. We anticipate that this first NAFLD xenograft model will elucidate critical gaps in our understanding of the disease and accelerate the development and validation of therapies for NAFLD.

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Development of inhibitors for human Adipose Triglyceride Lipase (ATGL)

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In obesity, elevated circulating fatty acids provoke adverse metabolic effects including non-alcoholic fatty liver disease (NAFLD) and insulin resistance, hallmarks of metabolic and cardiovascular complications of adipose tissues expansion. Adipose Triglyceride Lipase (ATGL) is the major triglyceride lipase in adipose tissue and critically determines the concentration of fatty acids in the circulation. We previously demonstrated that pharmacological inhibition of ATGL via the small-molecule inhibitor Atglistatin reduces obesity and insulin resistance and protects from ectopic lipid accumulation and NAFLD in diet- and genetically-induced obese mice. Hence, our findings demonstrate an important contribution of ATGL mediated lipolysis to the development of obesity and metabolic disorders and identify ATGL as interesting target for drug development. Since Atglistatin is a selective inhibitor for murine but not human ATGL, we synthesized and characterized inhibitors for human ATGL. In the present study we investigated efficacy and safety of human ATGL inhibitors, paving the way for a novel strategy to treat obesity-associated comorbidities like NAFLD and insulin resistance.

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Novel mechanisms controlling adipose tissue heterogeneity

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Adipocyte development and differentiation have an important role in chemical energy storage, and host metabolism homeostasis during nutrient overload. The adipocyte life cycle involves a complex and highly orchestrated program of gene expression. However, the in vivo detailed mechanisms underlying the molecular regulation are incompletely understood. Recent work from our group found that Bhlhb9 is a nutrient-responsive gene in a genetic and diet-induced obesity model, and its expression is rescued by PPARg-agonist treatment. Additionally, in vitro experiments demonstrated that Bhlhb9 is involved in the early commitment of preadipocytes and driving the gene expression of fully differentiated adipocytes. Whether these changes in adipogenesis also have an impact in adipose heterogeneity remains unclear. Using a newly developed conditional knockout mouse of Bhlhb9 in preadipocytes we are beginning to understand the metabolic programs regulated by Bhlhb9. Despite no weight gain or expansion of white adipose tissue, Bhlhb9F/YPPARg+/tTA;TRE-Cre mice presented increase in the stromal cell population in comparison to Bhlhb9F/YPPARg+/tTA mice. Such increase in the number of progenitors was also observed by single-cell RNA sequencing technology. These mesenchymal progenitor cells highly express dipeptidyl peptidase–4 (DPP4), and Actin Alpha 2, Smooth Muscle (Acta2). However, the increase in this population does not lead to hyperplasia, as histological analysis showed hypertrophic adipocytes and fibrosis in both inguinal and epididymal adipose tissue. Together this work highlights a role of Bhlhb9 influencing adipogenesis and adipose tissue heterogeneity. Furthermore, the impact of such cellular and molecular reprograming into host physiology may contribute to the development of obesity related disease.

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Insulin signaling in the liver maintains cholesterol homeostasis by suppressing cholesterol absorption in the gut

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Poor glycemic control in type 1 diabetes is associated with increased levels of pro-atherogenic apolipoprotein B (ApoB)-associated cholesterol and high risk of cardiovascular disease, although the mechanisms remain unclear. Here, we show that serum markers of cholesterol absorption are increased in subjects with type 1 diabetes and positively associated with the marker for glycemic control, hemoglobin A1c. Similarly, the ablation of hepatic insulin signaling in mice increases cholesterol absorption and hepatic cholesterol, leading to a compensatory decrease in cholesterol synthesis. On a Western diet, this results in marked increases of ApoB-associated cholesterol. Importantly, these changes in cholesterol metabolism are all FoxO1-dependent. In parallel, ezetimibe treatment of subjects with type 1 diabetes lowered cholesterol absorption markers, increased cholesterol synthesis markers, and reduced LDL-cholesterol. These findings suggest that the primary abnormality in cholesterol metabolism in type 1 diabetes is an increase in cholesterol absorption, and challenge the current paradigm of using inhibitors of cholesterol synthesis, rather than inhibitors of cholesterol absorption, as the primary treatment for hyperlipidemia in type 1 diabetes.

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TMEM55B regulates hepatic fatty acid oxidation

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Mitochondrial fatty acid oxidation is the primary mechanism by which cells metabolize lipids to produce energy. Mitochondrial dysfunction or impaired fatty acid transport into mitochondria leads to hepatic steatosis in both humans and murine models. Previously we identified transmembrane protein 55B (TMEM55B), a PI(4,5)P2phosphatase, as a sterol-regulated gene that modulates cellular and plasma cholesterol levels in hepatoma cells lines and in C57BL/6J mice, respectively. Recently TMEM55B has been reported to regulate lysosome position and function. Considering the emerging role of the lysosome in lipid metabolism, especially fatty acid trafficking, we investigated the effect of TMEM55B on fatty acid oxidation in human hepatoma cell lines and murine primary hepatocytes. Treatment ofHepG2 cellswith siRNA targeting TMEM55B(siTMEM55B) resulted in a 50% reduction in the endogenous mitochondrial oxygen consumption rate (OCR) compared with scrambled siRNA control. Incubation of cells with 10 nM palmitic acid significantly increased OCR by 32% in control ($p<0.01$), but not in siTMEM55Btreated cells. Similarly, primary hepatocytes from Tmem55bknockout (Tmem55b-/-) mice had a 37% decrease in OCR compared to those from Tmem55b+/+mice ($p<0.001$). TMEM55Bknockdown in HepG2 cells also resulted in increased intracellular triglyceride levels as assessed by Nile red staining with flow cytometry (~29%) and confocal microscopy (~63%). Although the total number of lipid droplets per cell were similar after TMEM55Bknockdown compared to control, there were significant differences in lipid droplet size with an increase in the number of larger lipid droplets and a decrease in the number of smaller lipid droplets. Finally, there was no effect of TMEM55Bknockdown on cellular fatty acid uptake using Bodipy labelled C16 fatty acid in vitro, or on hepatic mitochondrial oxidative phosphorylation protein levels by immunoblot in vivo. These findings suggest that defects in fatty acid oxidation observed upon TMEM55Bknockdown are not attributed to differences in transport of fatty acids into the cells or mitochondrial function, but rather impaired intracellular fatty acid trafficking to the mitochondria. Together these findings identify TMEM55B as a novel regulator of fatty acid oxidation and hepatic triglyceride accumulation.

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Altered humoral immune response in the gut during non-alcoholic fatty liver disease in hypercholesterolemic mice.

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Background: Although changes in the composition and number of intestinal bacteria, known as dysbiosis, is known to be implicated in non-alcoholic fatty liver disease (NAFLD), the underlying mechanisms by which dysbiosis influences disease progression are not fully understood. A key strategy to maintain intestinal homeostasis and generate immune protection against invading pathogens is via the production and secretion of immunoglobulin A (IgA) in the gut. Here, our aim was to address the intestinal humoral immune response during NAFLD.

Methods: Hypercholesterolemic low-density lipoprotein deficient (*Ldlr-/-*) mice on high-fat, high-cholesterol (HFC) diet for 5 weeks are used as established model for early stage fatty liver disease with human-like lipid profile. Results: Hepatic steatosis and inflammation in *Ldlr-/-* mice on HFC diet was associated with a reduction in plasma IgA titers, while systemic IgM levels were increased compared to chow-fed mice. Importantly, we observed that HFC feeding of germfree *Ldlr-/-* mice, which have lower baseline IgA titers compared to conventionally housed *Ldlr-/-* mice, does not alter plasma IgA levels. In association with lowered systemic IgA titers after HFC, we found a significant reduction in the frequency of IgA+ plasma cells (CD138+) in Peyer's Patches and lamina propria (LP) cells of the small intestine, while no difference was found in the large intestine. Accordingly, the percentage of IgM+ and IgA-IgM- plasma cells (CD138+) were increased, which might suggest diminished class switching towards IgA. In line, mRNA expression of A Proliferation Inducing Ligand (*April*) was reduced in small intestinal LP cells of *Ldlr-/-* mice on HFC compared to controls. Interestingly, intestinal levels of secretory IgA, IgM and IgG1 were elevated in HFC-fed *Ldlr-/-* mice, which was associated with a pro-inflammatory state in the small intestine as indicated by higher mRNA levels of Tumor Necrosis Factor alpha (*TNF-α*) and Interleukin-1 beta (*Il-1β*), and decreased Interleukin-10 (*Il-10*). Additionally, HFC leads to weakened antimicrobial defense as shown by reduced *Claudin-1* and Regenerating Islet-derived Protein 3 gamma (*Reg3g*) mRNA levels in the small intestine.

Conclusion: Our results highlight an imbalance in intestinal humoral immunity during hypercholesterolemia, which might enhance disease progression due to intestinal inflammation and impaired antimicrobial defense.

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Chop/Ddit3 depletion in pancreatic β-cells alleviates ER stress and corrects hepatic steatosis

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Type 2 diabetes (T2D) is a metabolic disorder characterized by hyperglycemia, hyperinsulinemia and insulin resistance (IR). During the early phase of T2D, insulin synthesis and secretion by pancreatic β cells is enhanced, which can lead to proinsulin misfolding that aggravates endoplasmic reticulum (ER) homeostasis in β cells. Moreover, increased insulin in the circulation may contribute to fatty liver disease. Medical interventions aimed at alleviating ER stress in β cells while maintaining optimal insulin secretion are therefore an attractive therapeutic strategy for T2D. Previously, we demonstrated that germline Chop/Ddit3 gene deletion preserved β cells in high fat diet (HFD) fed mice and in leptin receptor-deficient db/db mice. In the current study, we further investigated whether targeting Chop specifically in murine β cells confers therapeutic benefits. First, we show that Chop deletion alleviates ER stress in β cells and delays glucose-stimulated insulin secretion (GSIS) in aged HFD fed mice. Second, importantly, β cell-specific Chop deletion prevented liver steatosis and hepatomegaly in aged HFD fed mice without affecting basal glucose homeostasis. Third, we provide the first mechanistic evidence that ER remodeling secondary to Chop deletion modulates glucose-induced islet Ca2+ oscillations. Finally, using state-of-the-art GLP1-conjugated Chop AntiSense Oligonucleotides (GLP1-Chop ASO), we demonstrated that the Chop deletion induced GSIS change is a long-term complex event in β cells. In summary, our results demonstrate that Chop depletion in β cells is a new therapeutic strategy to alleviate dysregulated insulin secretion and the consequent fatty liver disease in T2D.

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Identifying novel genes involved in hepatic steatosis through bioinformatics

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The world has crossed a threshold where people suffering from diseases of overnutrition now outnumber those who suffer from undernutrition. The global epidemic of obesity coincides with an increase in non-alcoholic fatty liver disease (NAFLD) – the abnormal accumulation of fat in the liver from causes other than alcohol abuse. The health consequences of the disease can be severe. In many cases, the disease progresses to non-alcoholic steatohepatitis (NASH), which is characterized by an increase in inflammation as well as lipid accumulation.

Large-scale genome-wide studies in a recently-developed outbred mouse model enable the discovery of novel causal genes behind the heterogeneous phenotypes seen in obesity and NAFLD. Modern statistical methods can identify interactions between gene loci and analyze associations within large-scale data sets to determine gene associations that are not readily obvious. These gene loci can then be investigated via traditional hypothesis-driven studies and tested in well-defined mouse models of diet-induced obesity to identify causal genes in NAFLD and NASH.

We developed a novel method of identifying genes involved in lipid accumulation in liver using publicly available data sets. Of 20 gene candidates identified in our initial screen, two genes (*Echs1* and *Nlrp6*) have already been shown to be involved in liver triglyceride accumulation.

We are using mouse and human liver cell models to screen the remaining candidates, and propose to test 2-3 candidate genes in a mouse model system (AAV knockdown of candidates). The results of these experiments will identify novel candidates involved in liver lipid metabolism and may point toward new therapeutic targets for hepatic steatosis and NAFLD.

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Conditional deletion of Pdia1 reveals its multifaceted role in fatty liver disease and its potential as a novel anti-fibrotic therapeutic target

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Lipid accumulation in hepatocytes and augmented collagen production by hepatic stellate cells are the two most important processes that contribute to fatty liver disease. The microsomal triglyceride (TG) transfer protein (MTP) complex is essential for hepatic VLDL secretion and lipid homeostasis whereas the collagen prolyl-4-hydroxylase (P4H) tetramer in conjunction with ascorbate plays an indispensable role in procollagen maturation and folding. Protein disulfide isomerase-A1 (PDIA1) serves as subunits of both MTP and P4H through its chaperone function, which is thought to be independent of its catalytic activity. *Pdia1* global knockout (KO) is embryonically lethal, although PDIA1 function is poorly understood. To elucidate roles of PDIA1 in VLDL secretion and hepatic lipid homeostasis, we created liver-specific *Pdia1*-LKO and inducible global *Pdia1*-iGKO mice. Severe hypolipidemia occurred in both *Pdia1*-KO models due to dramatic decreases in hepatic VLDL secretion. Hepatic TG content was increased by 8-10 fold in *Pdia1*-LKO mice whereas it was only modestly increased in *Pdia1*-iGKO mice. *Pdia1*-iGKO mice also displayed dramatically increased intestinal TG content accompanied with severe growth retardation. *Pdia1* deletion in liver and intestine depleted MTP protein and activity in both tissues without affecting MTP mRNA levels. Suppression of MTP translation, and not accelerated MTP degradation or aggregation, was responsible for the loss of MTP in the *Pdia1*-deleted hepatocytes whereas accelerated MTP degradation appeared to be the key mechanism for MTP loss in *Pdia1*-deleted enterocytes. Significantly, *Pdia1* deletion in collagen-producing tissues depleted the P4H alpha subunit-1 (P4HA1) without affecting its mRNA level. *Pdia1* deletion in mouse embryonic fibroblasts (MEFs) or PDIA1-knock down in immortalized human hepatic stellate cells (hHSCs) destabilized P4HA1 protein and abolished procollagen prolyl-4 hydroxylation and maturation in response to ascorbate. Importantly, mutagenesis and complementary PDIA1 expression studies revealedthat P4HA1 is a direct substrate of PDIA1 and the isomerase activity of PDIA1 is required for the formation of the P4H complex. Moreover, the PDIA1 inhibitor LOC14 prevented ascorbate-stimulated procollagen processing and secretion in MEFs and hHSCs. Thus, PDIA1 is required for hepatic and intestinal lipid transport, provides a novel requirement for P4H function and collagen production and maybe a therapeutic target for liver fibrosis.

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Identification of distinct residues in the carboxy-terminal domain of SREBP2 that control interaction with Scap and degradation by proteasomes

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Lipid homeostasis in animal cells is controlled by sterol regulatory element-binding proteins (SREBPs), membrane-bound transcription factors that are activated in a cholesterol-dependent fashion by Scap, a cholesterol-sensing membrane protein. The amino-terminal domains of SREBPs bind DNA and upregulate lipogenic genes whereas the carboxy-terminal domains (CTDs) bind Scap and perform a regulatory role that is not well understood. Here, we perform a detailed molecular dissection of the CTD of human SREBP2, one of the three isoforms of SREBPs in mammalian cells. We identify key residues that selectively control two distinct functions of SREBP2: i) an arginine residue in exon 18 that controls interaction with Scap; and ii) seven non-contiguous amino acids in exon 19 that control degradation in the absence of Scap. These findings reveal how SREBP2's CTD exerts tight regulation over cholesterol homeostasis and provide a framework for how the CTD of SREBP1s may play a similar regulatory role.

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Large-Scale Multi-Omics Integration Identified Liver Pyruvate Kinase as a Novel Sex-Specific Candidate in NAFLD/NASH

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Background: Non-alcoholic fatty liver disease (NAFLD) ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. An important feature of NAFLD is their differential prevalence and disease phenotypes between males and females. However, the etiology of the disease is not completely understood and there are no available treatments for NAFLD. To address this problem, we recently used multi-omics data, such as genomics, transcriptomics and NAFLD phenotypes, collected from a diverse mouse population and identified several candidate genes driving NAFLD. We followed up on one of these candidates, namely liver pyruvate kinase (L-PK or *Pklr*) to determine its possible sex-specific role using liver tissues from both sexes of mouse and human patients. Our results suggested that L-PK have sex-specific effects in developing NAFLD.

Methods: We used liver-specific loss-of-function and gain-of-function strategies in independent animal models of diet-induced NAFLD and NASH involving both sexes. Following treatment, we measured several metabolic phenotypes associated with obesity, insulin resistance, dyslipidemia, liver steatosis and fibrosis. Furthermore, liver tissues were used for gene expression and western blots, and liver mitochondria for bioenergetics.

Results: In steatosis model, L-PK silencing in male mice improved glucose tolerance, insulin sensitivity and pyruvate/lactate tolerance compared to controls. Further, these animals had reduced plasma cholesterol levels and intrahepatic triglyceride accumulation. Conversely, L-PK overexpression in male mice developed augmented disease phenotypes. In contrast, female mice overexpressing L-PK had no altered phenotypes. Mechanistically, we report that L-PK increased liver triglycerides *via* upregulated *de novo* lipogenesis and increased PNPLA3 levels accompanied by mitochondrial dysfunction. Also, we report that L-PK increased plasma cholesterol levels *via* increased PCSK9 levels.Moreover, we demonstrate that L-PK silencing reduced *de novo* lipogenesis, PNPLA3 and PCSK9 levels and more importantly, improved mitochondrial function. Finally, in fibrosis model, we demonstrate that L-PK silencing in male mice reduced both liver steatosis and fibrosis accompanied by reduced *de novo* lipogenesis and improved mitochondrial function.

Conclusion: Following up on our multi-omics studies, we demonstrate that L-PK acts in a male-specific manner in developing liver steatosis and fibrosis. Given that NAFLD/NASH exhibit sexual dimorphism, our results are crucial for personalized therapeutic implications of L-PK in clinical studies.

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Functional and biophysical investigation of the Scap-SREBP pathway

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Mammalian cells actively sense and regulate the cholesterol concentration of their membranes using the SREBP pathway. When ER membrane cholesterol falls below a threshold concentration of ~5 mol % of total ER lipids, SREBP2 is trafficked from ER to Golgi by the cholesterol sensor Scap. There, SREBP2 undergoes proteolysis to liberate the N-terminal basic helix-loop-helix Leucine zipper (bHLH-Zip) transcription factor from the full-length transmembrane precursor. This transcription factor travels to the nucleus where it binds to sterol response elements (SREs) upstream of lipogenic genes and increases their transcription. As a result, cellular cholesterol levels increase. When ER membrane cholesterol rises above the threshold, SREBP2 transport by Scap is inhibited by the binding of Scap to Insigs, ER retention proteins. SREBP2 transport is also inhibited by oxysterols, which bind Insigs and promote their the binding of Insig to Scap. This feedback loop maintains cholesterol homeostasis. However, despite the biomedical importance of this pathway, we do not have a molecular understanding of how Scap senses cholesterol, responds to changes in cholesterol concentration, or of the protein-protein interactions underpinning the SREBP pathway.

By combining insights from biochemical efforts and cell-based functional assays, we developed systems to express and purify milligram quantities of protein complexes from the SREBP pathway. We can isolate soluble complexes of the interacting domains of Scap and SREBP2; detergent-solubilized complexes of Scap/Insig, Scap/SREBP2, and Scap/Insig/SREBP2; and lipid-reconstituted complexes of Scap/Insig. To facilitate structural efforts, we have generated highly selective Fab antibody fragments and nanobodies using traditional immunization, phage-display, and yeast-display systems. Our current efforts are directed toward obtaining high-resolution structures of these complexes in order to understand how these proteins interact to sense and respond to sterols to ensure cholesterol homeostasis.

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Acute effect of long-chain and medium-chain saturated fatty acids on hepatic energy metabolism.

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Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease, but its progression to the more severe form of nonalcoholic steatohepatitis (NASH) is unpredictable. Excessive steatosis induces hepatocyte inflammation, fibrosis and apoptosis that may later lead to cirrhosis and hepatocellular carcinoma. Despite its significance, the underlying molecular mechanisms that control NAFLD and NASH pathogenesis remain poorly understood. Moreover, there is currently no FDA-approved treatment to inhibit the progression from NAFLD to NASH. Therefore, we developed a protocol to investigate the acute impact of long-chain and medium-chain saturated fatty acids on hepatic energy metabolism. Conscious, unrestrained, overnight fasted 12-wks old male C57Bl/6J mice were given continuous, intravenous infusion of either saline, 20% lard oil or 20% coconut oil emulsions for 5 hrs. Lipid infusion was accompanied by primed, continuous infusion of [6,6-$^{2}H_{2}$]glucose and sodium [$^{13}C_{3}$]lactate intravenously for 4 and 2 hrs, respectively, prior to the end of the experiment. Our analysis showed that by the end of the study, plasma free fatty acids were significantly elevated after both lard and coconut oil infusions, whereas liver fatty acids were increased only in mice treated with coconut oil. What is more, both lard and coconut oil infusions left triglyceride content unchanged in liver. On the other hand, coconut oil treatment significantly decreased plasma glucose level, which was not accompanied by changes in plasma insulin level. ^{13}C and ^{2}H-enrichment of plasma glucose and liver alanine, glutamate, glycerol 3-phosphate, lactate and urea obtained at the end of the study were measured by GC-MS and used to regress liver metabolic fluxes using a flexible modeling platform (INCA). Analysis of liver metabolic fluxes revealed that both lard and coconut oil upregulated fatty acid oxidation flux, however increase triggered by lard oil was significantly higher. Furthermore coconut oil, in contrast to lard oil, significantly downregulated pyruvate cycling in hepatocytes. These data suggest that medium-chain saturated fatty acids contained in coconut oil, in contrast to long-chain fatty acids from lard oil, are metabolized at a slower rate by hepatocytes.

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Reduced Fatty Acid Desaturase 1 Function Alters Glucose Metabolism and Leads to Hepatic Stellate Cell Activation

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Purpose: Polyunsaturated fatty acids (PUFAs) regulate a broad set of physiological processes and have a major impact on human health. Fatty acid desaturase 1 (FADS1, encoding Δ-5 desaturase) is widely recognized as one of the key rate-limiting enzymes required for PUFA metabolism. Genetic alleles at the FADS1 locus lead to reduced FADS1 expression in the liver. Our previous studies have shown that genetic polymorphisms of FADS1 are significantly associated with hepatic fat accumulation. Since hepatic stellate cells (HSCs) play a critical role in liver fibrosis, there is an urgent need to determine the role of FADS1 in modulating HSC function.

Methods: FADS1 expression was knocked down by stably transfecting short hairpin RNA in human immortalized HSC LX2 cells followed by migration assessment using a transwell assay. The expression of genes was examined by qRT-PCR and western blotting. Confocal microscopy was used for evaluation of neutral lipid accumulation and mitochondria membrane potential. FADS1 knock out (KO) mice were also utilized to investigate the underlying role of FADS1 in the development of NASH.

Results: FADS1 knockdown significantly increased cell migration and mRNA expression of some profibrotic markers, such as α-SMA, collagen type I, ET1, and CTGF. In addition, FADS1 knockdown increased the accumulation of neutral lipids as detected by BODIPY staining. Complementary studies found that production of ATP and the lactate/pyruvate ratio were increased after FADS1 was decreased. Meanwhile, the expression of key enzymes that contribute to glycolysis, such as HK2, PKM2 and LDHB, were elevated after inhibiting FADS1. Knockdown of FADS1 also decreased mitochondrial membrane potential and increased intracellular reactive oxygen species (ROS) levels. In vivo, FADS1-KO mice exhibited a higher hepatic triglyceride (TG) level, increased inflammation and fibrosis compared with wild type mice after high fat diet administration for 8 weeks. RNA-Seq data from FADS1-KO mice indicated that PI3K-AKT, MAPK, HIF-1 and Ras signaling pathways may be involved in this process.

Conclusions: Our study shows for the first time that suppression of FADS1 activates hepatic stellate cells, in part, via modulation of lipid and glucose metabolism, which may increase susceptibility to the development and progression of chronic liver disease.

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HDL induces ADAM metallopeptidase domain 17 (ADAM17) to modulate lipid- and inflammatory macrophage phenotypes

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ADAM metallopeptidase domain 17 (ADAM17) is a transmembrane protease critically involved in shedding of many cell surface proteins, including inflammatory cytokines/chemokines and their receptors. A previous study suggested that cholesterol efflux by HDL promotes tumor necrosis factor-α (TNF-α) shedding from endothelial cells by disrupting ADAM17 lipid raft localization. Recently we have shown that HDL can exert both pro-inflammatory and anti-inflammatory effects in macrophages and that both processes are mediated by cholesterol efflux. We therefore hypothesized that HDL-mediated depletion of cellular cholesterol drives the pro-inflammatory and anti-inflammatory effects of HDL and that ADAM17 is required for both effects. Thioglycolate-elicited peritoneal macrophages were isolated and sorted from wild type (WT) and human APOA1 transgenic (hAPOA1^{Tg}) mice. Macrophages from hAPOA1^{Tg} mice and HDL-stimulated macrophages from WT mice showed elevated expression of ADAM17 due to increased cholesterol depletion. Macrophages were stimulated with lipopolysaccharide (LPS) or interferon-ß (IFN-ß), and after 4-20 h, were used to measure expression of inflammatory cytokines/chemokines or analyzed for lipid and lipid raft content. We found that macrophages from hAPOA1^{Tg} mice had reduced lipid content (measured by BODIPY), as compared with WT mice, consistent with the elevated HDL levels in these mice. These macrophages exhibited increased levels of LPS-induced mRNA and secretion of TNF-α, CXCL1 and CCL2, as compared with macrophages from WT mice. Furthermore, macrophages from hAPOA1^{Tg} mice exhibited suppressed levels of LPS- and IFN-ß-induced interferon signaling associated genes, including Mx1 (~60% of LPS and ~30% of IFN-ß alone) and Ifit2 (~30% of LPS or IFN-ß alone). This pro- and anti-inflammatory phenotype of macrophages from hAPOA1^{Tg} mice was mimicked by HDL and was associated with lipid raft disruption, and was restored in cholesterol-loaded macrophages. Strikingly, ADAM17-deficiency prevented the stimulatory effect of HDL on LPS-induced TNF-α and CXCL1 gene and protein expression and the anti-inflammatory effect of HDL on IFN-ß-induced Mx1 and Ifit2 gene expression. Furthermore, ADAM17-deficiency prevented lipid raft disruption, and also increased lipid content in macrophages both in vitro and in vivo in hAPOA1^{Tg} mice. These findings suggest that HDL induces ADAM17, which is required for the pro- and anti-inflammatory effects of HDL through changes in macrophage cholesterol homeostasis.

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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 and binding to EL and investigated the role of ANGPTL8 in EL inhibition by ANGPTL3. We found that inhibition of EL by ANGPTL3 was dose- and temperature-dependent. We found that heparin prevents the inhibition of EL by ANGPTL3, and that when EL is bound to cells by heparan-sulfate proteoglycans, it is partially protected from ANGPTL3 inhibition. Our lab has previously found that ANGPTL3 requires angiopoietin-like 8 (ANGPTL8) to efficiently inhibit LPL. Here we found that ANGPTL8 did not significantly alter the inhibition of EL by ANGPTL3, indicating that ANGPTL8 is necessary for LPL, but not EL inhibition.

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Intestinal Apolipoprotein A1 deletion reduces total and HDL lipoprotein mass and alters bile acid composition in mice

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Apolipoprotein A1 (apoA1) is key to reverse cholesterol transport (RCT) and HDL functionality. Global apoA1 knockout alters HDL composition and impairs HDL function. We wondered whether intestinal apoA1 plays a specific role in dysfunctional HDL biogenesis as 30% of steady-state HDL cholesterol derives from gut and apoA1 couples RCT to innate immune function. To test this possibility, we generated an intestine-specific apoA1 knock-out (iKO) in C57BL/6 mice via CRISPR/Cas9-mediated insertions of the LoxP sites flanking exon 4 of the gene followed by breeding with Vil-Cre mouse strain. apoA1fl/fl mice served as controls for all analyses. Liver, spleen, kidney, and plasma were collected from chow-fed mice (n=4-9/gender/genotype) at 10 and 20 weeks of age. Liver and kidney weights followed expected %BW. Spleens, as %BW, differed by sex and apoA1 status, being 0.50±0.03%, 0.39%±0.07%, 0.33±0.03% and 0.28±0.01% for iKO-F, apoA1fl/fl-F, iKO-M and apoA1fl/fl-M, respectively. Both iKO-F and iKO-M mice had reduced total AUC following isopycnic separation of fluorescently labeled lipoproteins compared to apoA1fl/fl controls. Intestinal apoA1 loss had the greatest impact on F as HDL AUC decreased 38% in iKO-F compared to apoA1fl/fl-F and 60% compared to M of either genotype ($p<0.000$); LDL AUC in iKO-F decreased by 31% compared to all others ($p<0.003$). iKO-M HDL AUC was 19% lower than apoA1fl/fl-M ($p<0.01$). HDL-2b was most reduced in iKO-F, decreasing 39% vs. apoA1fl/fl-F, 52% vs. iKO-M and 59% vs. apoA1fl/fl-M ($p<0.02$ for all). Fecal bile acids, sterols, and fatty acids recovered in a casual 48h sample were measured by GC-MS (n=2-3/gender/genotype) and expressed at ug/mg feces. Bile acid concentrations (ug/mg feces) in F were twice those of M regardless of genotypes ($p<0.04$). Females had higher % primary BAs, while males increased % secondary BAs in feces ($p<0.03$); differences between iKO genders were significant ($p<0.008$). Ursodeoxycholate and a-muricholate (ug/mg feces) were highest in iKO-F ($p<0.006$). Intestinal apoA1 deletion results in loss of lipoprotein mass and altered bile acid composition in both genders, resulting in increased relative spleen weights, significant reduction in total HDL and specifically HDL-2b fractions. The extent to which intestinal apoA1 biology influences health or disease development warrants further study.

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Lipin 1 Regulates Adipocyte Lipogenic Capacity and Systemic Insulin Sensitivity on High Fat Diet.

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Emerging evidence suggests that the capacity of adipocytes to appropriately store lipids and prevent their ectopic accumulation in liver and skeletal muscle may be an important regulator of systemic insulin sensitivity. Lipin 1 is a lipid phosphatase that is highly expressed in adipocytes and catalyzes the conversion of phosphatidic acid (PA) to diacylglycerol (DAG); the penultimate step in triglyceride synthesis. Adipose tissue lipin 1 expression positively correlates with insulin sensitivity in obese humans and mouse models. To test the role of adipose tissue lipin 1 in systemic insulin sensitivity, we generated mice lacking lipin 1 in adipocytes (Adn-Lpin1-/- mice). On normal chow, loss of lipin 1 in adipocytes modestly reduced white and brown adipose tissue mass, but did not affect glucose tolerance or insulin sensitivity. However, in response to 2 weeks high fat diet (HFD; 60% fat) feeding, Adn-Lpin1-/- mice remained lean, but developed hyperglycemia compared to littermate wild-type (WT) mice. Lipidomic analyses demonstrated an increase in liver PA, DAG, and triglyceride in HFD-fed Adn-Lpin1-/- mice compared to WT controls, whereas only PA accumulated in skeletal muscle. Plasma triglyceride concentration was not different, but free fatty acids and glycerol were significantly reduced in Adn-Lpin1-/- vs WT mice fed HFD, suggesting that lipolysis may be decreased. Plasma adiponectin and resistin were significantly reduced in HFD-fed Adn-Lpin1-/- vs WT control mice while leptin concentrations were not different. Lastly, with 5 weeks of HFD feeding, Adn-Lpin1-/- mice became markedly hyperglycemic and glucose intolerant compared to littermate WT controls, despite being leaner. Collectively, these data suggest that loss of lipin 1 in adipocytes leads to insulin resistance on HFD, likely due to an impaired capacity to store lipids appropriately in adipose tissue.

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Monoacylglycerol acyltransferase 1 knockout mice are not protected from obesity-related metabolic disease.

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Monoacylglycerol acyltransferase (MGAT) enzymes catalyze the synthesis of diacylglycerol from monoacylglycerol. In mice, multiple genes encode enzymes with MGAT activity but most work has focused on Mogat1 (expressed in stomach, liver, and adipose tissue) and Mogat2 (enriched in small intestine). MGAT activity is important for dietary fat absorption by intestinal enterocytes and may play a role in the development of obesity-related insulin resistance in hepatocytes. Indeed, antisense oligonucleotide (ASO)-mediated knockdown of Mogat1 expression and hepatic MGAT activity improved glucose tolerance and insulin resistance in obese mice. Using a floxed allele, we have now generated two liver-specific Mogat1-knockout models (LS-Mogat1-KO) by using albumin promoter-driven Cre recombinase (chronic) or adeno-associated virus 8 (AAV8)-TBG-Cre infection (acute). Loss of Mogat1 did not improve hepatic steatosis, glucose tolerance, or insulin sensitivity on a high fat diet in either LS-Mogat1-KO model. Despite having reduced hepatic Mogat 1 expression, liver membranes isolated from both types of KO mice had normal MGAT activity, suggesting compensation by other enzymes with MGAT activity. However, acute depletion of Mogat1 by AAV8-Mogat1 shRNA also did not affect glucose tolerance or insulin sensitivity in obese mice. ASO treatment is also associated with knockdown of target genes in adipose tissue. Therefore, we also generated and characterized mice with fat-specific Mogat1 deletion, but these mice were also not protected from diet-induced obesity or related metabolic derangements. Finally, we generated whole-body Mogat1 KO mice. Though no differences in adiposity were noted on a low fat diet, Mogat1 constitutive KO mice were more obese than wild-type littermate controls and exhibited impairments in glucose and insulin tolerance when challenged with a high-fat diet. Experiments are ongoing to determine the mechanisms by which loss of Mogat1 leads to obesity on a high fat diet, but this could be due to paradoxically enhanced fat absorption and/or compensation by Mogat2. Altogether, these data suggest genetic loss of Mogat 1 does not improve insulin resistance and that constitutive Mogat1 deletion may exacerbate diet-induced obesity by unknown mechanisms.

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Progress Towards Standardizing Metagenomics: Application of Metagenomic Reference Materials to Develop a Reproducible Microbial Lysis Methodology with Minimum Bias

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Metagenomics research has grown exponentially since 2010. This rapid growth has contributed to a lack of reproducibility between different methods and laboratories, which risks limiting our ability to compare between studies and decreases confidence in previous conclusions. To address the diversity of methods available, we sought to compare the performance of many commercially- and academically-sourced lysis protocols. The methods were evaluated using mock microbial community standards with defined composition and known manufacturing tolerances to serve as a ground truth for the measurement. In order to facilitate comparisons, we developed the Measurement Integrity Quotient (MIQ), providing a single, easy to understand numerical score that describes the accuracy of an observed composition relative to a known composition standard. Utilizing this method, we compared the effects of many different variables on lysis efficiency, including differences between thermal, enzymatic, and mechanical (bead) lysis as well as minor changes within a method, including over 40 different bead material/size combinations and cell disruptor type/intensity/run time combinations. Additionally, several replicates of typical sample types (feces, soil, skin, saliva, urine) were tested with different lysis methods with replicates at different laboratories for over 1500 samples tested. Hard, dense ceramic beads of mixed size on an appropriate cell disruptor with a validated protocol created the least biased lysis of all examined methods. The use of the MIQ score provided rapid, easy to understand analysis of accuracy and has been released in a rapidly deployable container image. The use of these data, and methods can allow for the creation of more reproducible metagenomics pipelines with results that better represent the true composition of the sample. These methods were able to achieve minimal amounts of deviation from expected composition and a high degree of run-to-run and interlab reproducibility.

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Deletion of hepatic Ppp1r3b causes dysregulated glucose and lipid metabolism that precedes steatosis in mice.

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Genetic variation near the *PPP1R3B* locus has been associated with multiple cardiometabolic traits including fasting glucose levels and hepatic steatosis. The *PPP1R3B* gene encodes the glycogen regulatory protein Ppp1r3b which is known to regulate glycogen metabolism and plasma glucose homeostasis but lacks a clear connection to liver lipids.

We previously demonstrated that ablation of *Ppp1r3b* in mouse hepatocytes diminishes the conversion of postprandial glucose into hepatic glycogen, causing lower fasting plasma glucose and accelerated activation of gluconeogenic genes. Since defective glucose-to-glycogen synthesis and increased gluconeogenesis are associated with hepatic steatosis, we investigated the impact of *Ppp1r3b* expression on hepatic triglyceride (hepTG) accumulation. In *Ppp1r3b*-floxed mice, we administered adeno-associated viruses to delete (AAV-Cre, “KO”) or overexpress (AAV-Ppp1r3b, “OE”) *Ppp1r3b* in mouse hepatocytes and compared the effects to wildtype expression (AAV-Null, “WT”). Fasted KO mice showed rapid declines in plasma glucose whereas OE mice maintained normal levels even with prolonged fasting. Upon refeeding, KO mice exhibited pronounced induction of *de novo* lipogenesis, demonstrated both by increased lipogenic gene expression and by *in vivo* incorporation of deuterated water into newly synthesized palmitate. Interestingly, steady state hepTG did not differ from WT, which was explained by our discovery that KO mice have both elevated TG secretion and increased ketogenesis during fasting. Primary hepatocytes isolated from KO mice treated with ^{14}C-oleic acid had significantly more labeled lipid oxidized to $^{14}CO_2$ and incorporated into cellular triglycerides than WT hepatocytes, while cells from OE mice had markedly reduced indications of β-oxidation and cellular TG accumulation. These results suggest that although KO mice have increased hepTG synthesis, they also have compensatory changes in hepTG utilization during fasting that confer protection against hepTG accumulation in normal feeding conditions. In KO mice challenged with a high carbohydrate diet or primary hepatocytes supplemented with glucose media, KO hepTG accumulation was drastically increased and KO mice developed histological features of steatosis. These data illustrate a novel role of *Ppp1r3b* in regulating hepatic glucose and lipid metabolism and support the indication that *PPP1R3B* is causal for the human genetic association with hepatic steatosis.

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Fish oil-derived furan fatty acids: new players in the regulation of metabolic syndrome

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Obesity and non-alcoholic fatty liver diseases are multifactorial diseases that affect more than 35% of the world's population. Fish oil (FO) and omega-3 are effective treatments for hypertriglyceridemia with eicosapentaenoic acid shown to reduce cardiovascular and metabolic syndrome-related events. However, the mechanisms involved in these beneficial activities are still unclear. A metabolomic study of healthy volunteers receiving Lovaza, an omega-3-drug, showed a large increase in plasma and urinary CMPF. Moreover, in a high fat diet mouse model, CMPF was protective and reversed steatosis. We identified furan fatty acids (FuFA) present both in fish oil and Lovaza (0.1-1%) as the sources of CMPF. We synthesized the most abundant FuFAs and confirmed their structures by NMR and mass spectrometry and tested their protective effects on a NASH diet mouse model (21 weeks, 40 kcal% fat and 20 kcal% fructose). After 15 weeks, FuFA (25mg/kg/day) was administered for the last 6 weeks. While FuFA treatment did not protect against inflammation, there was a significant increase in cpt1 (1.5-fold, $p<0.01$) and pparα ($p<0.01$) gene expression. This in vivo effect was recapitulated in vitro using freshly isolated mouse primary hepatocytes treated with FuFA (50uM). Expression of Cpt1 and Pparα were increased after 8 and 16 hs (1.6-fold and 1.4-fold respectively) with a decrease in carnitine and acetyl-CoA levels at 3 hours. These findings were consistent with higher rates of oxidation and further supported by primary hepatocyte Seahorse data showing an increase in basal respiration and maximal rates of oxygen consumption after uncoupling and hepatocytes and liver increased AMPK and ACC phosphorylation. At the biochemical level, we have demonstrated that FuFA-CoA are potent allosteric inhibitors of ACC-1 and -2, further promoting fatty acid b-oxidation. Finally, an MS-based metabolic analysis indicates that mice metabolize FuFA with no evidence of CMPF formation, precluding a complete evaluation of the metabolic effects of FuFA-derived CMPF. In conclusion, the increased FAs β-oxidation and decrease in hypertriglyceridemia observed in ?-3 and FO treatment might be related to the presence of FuFA in fish oil and omega-3 preparations.

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Identification of Mitochondrial Pyruvate Carrier Inhibitors to Treat Nonalcoholic Fatty Liver Disease

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Nonalcoholic liver disease (NAFLD) is a common comorbidity in patients characterized as having metabolic syndrome. Recently, the mitochondrial pyruvate carrier (MPC) has emerged as an innovative target for the treatment of NAFLD with beneficial effects on intermediary metabolism. The goal of the present study was to identify small molecules that interact and inhibit the MPC and to test their effects on metabolism, insulin sensitivity, and NAFLD endpoints in mouse models of obesity. The use of a BRET-based reporter system for MPC interaction confirmed previous evidence of small molecules that act as potent MPC inhibitors. These included thiazolidinediones (pioglitazone, rosiglitazone, and MSDC-0160), zaprinast, and 7ACC2. Zaprinast and 7ACC2 interact with the MPC and inhibit mitochondrial pyruvate metabolism with EC50s of nM concentrations. Zaprinast and 7ACC2 also potently inhibited pyruvate-mediated respiration in isolated wild-type, but not MPC-deficient mitochondria, demonstrating a direct effect for inhibition of mitochondrial pyruvate metabolism that is MPC-dependent. MPC inhibitors also suppressed glucose production in isolated hepatocytes in an MPC-dependent manner; consistent with a suppression of gluconeogenic flux. Administration of zaprinast, 7ACC2, or MSDC-0160 improved glucose tolerance, insulin sensitivity, and reduced the expression of genes associated with stellate cell activation in the liver of diet-induced obese mice. MPC inhibitors also directly suppressed stellate cell activation in vitro and genetic MPC deletion in stellate cells reduced the induction of stellate cell activation markers in mice fed a high fat, high cholesterol diet. Mechanistically, MPC inhibition seems to increase the catabolism of fatty acids and branched-chain amino acids in hepatocytes, which is related to improvements in glucose metabolism and insulin sensitivity. Ongoing studies will elucidate the precise mechanisms leading to enhanced fatty acid and amino acid catabolism and overall metabolic improvements. In conclusion, these data provide proof of concept evidence in mice for the efficacy of novel MPC modulators as insulin-sensitizing agents and NASH therapeutics. Moreover, chemically-diverse MPC inhibitors were shown to have similar effects on insulin sensitivity and NASH endpoints demonstrating a generalization of the MPC inhibitor mechanism of action.

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Elucidating the physiological function of FIT2 in murine ER homeostasis and lipid storage

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The endoplasmic reticulum (ER) is the largest membrane organelle and packages excess lipids into lipid droplets (LD), which serve as reservoirs of membrane precursors and metabolic energy. It is incompletely understood how ER morphology and composition support LD formation. FIT2 is a member of an evolutionarily conserved family of ER-resident proteins. Demonstrating the importance of FIT2 function in cells and organisms, human mutations in FIT2 cause childhood deafness-dystonia, and FIT2-deficient mice die from catastrophic intestinal failure. Initially, FIT2 was thought to function specifically in lipid droplet (LD) biogenesis, but recent cellular studies from our laboratory suggests a fundamental role in ER function. We next sought to determine the physiological consequences of this function and investigated the effect of FIT2 deficiency in murine liver—a tissue with ER enrichment and dynamic LD content. Our characterization of FIT2 liver-specific knockout mice (FIT2LKO) demonstrates that FIT2 exerts a critical role in preserving ER homeostasis in liver. However, in contrast to other FIT2 knockout systems, hepatic FIT2 deficiency does not reduce hepatic lipid content. Collectively, our data suggest that in the liver, FIT2 is essential for ER function but not required for LD formation.

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Adipose tissue lipolysis provides signals that modulate hepatic gene expression program

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Hepatic steatosis can result from multiple mechanisms, including excess lipolysis from adipocytes, impaired b-oxidation, and increased de novo fatty acid synthesis. In addition to providing substrate for triglyceride biosynthesis in the liver, the adipose tissue plays an essential role in communicating with distal tissues through secreted signals. In this study we will investigate lipids that are secreted during adipose tissue lipolysis and the transcriptional changes that occur in response to adipose tissue lipolysis. In this study we will be using in vitro and in vivo models to characterize the adipocyte secreted lipidome. Using LC-MS analysis, we have analyzed conditioned media from adipocytes stimulated with vehicle or a b3-adrenergic receptor agonist CL-316,243. We found that activation of lipolysis leads to changes in multiple lipid classes, including an increase in free fatty acids, phosphatidylserines, ceramides, sphingomyelin, and Lyso-phosphatidylcholines. Notably, the conditioned media from adipocytes treated with CL-316,243, promotes lipid droplet biogenesis and induction in expression of genes involved in fatty acid catabolism and lipid droplet biogenesis. Using littermate controls and mice lacking ATGL in adipocytes, we completed targeted LC-MS analysis of serum from mice treated with vehicle or CL-316,243. We found that free fatty acids were significantly upregulated upon activation of lipolysis, but found additional lipids changing in vivo, such as phospholipids, ceramides, and triglycerides. Transcriptional profiling of livers from mice treated with vehicle or CL-316,243 showed that pathways involved in lipid, sulfur, nitrogen and nucleic acid metabolism were upregulated. However, livers from mice lacking ATGL in adipocytes did not show these changes. To identify putative sensors of lipolysis in the liver, we used Ingenuity Pathway Analysis (IPA), and found over 200 putative upstream regulators that are predicted to drive changes in hepatic gene expression, including PPARa, NRF2, TP53, HNF4a, SREBP, and many others. We suspect that PPARa and HNF4a are not the only fatty acid sensors that respond to lipolysis from adipocytes. Together these studies provide a detailed view of adipose secreted lipids and the transcriptional response in the liver. These studies highlight the importance of studying cell-cell communication to understand mechanisms that regulate hepatic lipid balance and cellular homeostasis.

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Insulin signaling in the liver promotes lipogenesis but suppresses inflammation

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Insulin resistance is one of the few factors consistently associated with the development and progression of NAFLD, but the underlying mechanisms remain unclear. It is generally assumed that the hyperinsulinemia that evolves with insulin resistance drives insulin signaling and de novo lipogenesis to excess. The resulting steatosis is thought to lead, ultimately, to hepatocyte injury and inflammation. Here, to dissect the role of hepatic insulin signaling in the development of NAFLD, we deleted the insulin receptor in the hepatocytes of mice fed a Western diet (15.2% sucrose, 42.7% anhydrous milkfat, and 0.2% cholesterol) for 3-5 weeks. We found that Liver Insulin Receptor Knockout (LIRKO) mice indeed showed reduced expression of the following lipogenic genes: Sterol Regulatory Element Binding Protein (SREBP)-1c was reduced by 53% ($p<.01$); fatty acid synthase was reduced 72% ($p=.07$); and stearoyl CoA desaturase 1 was reduced 84% ($p<.001$). In parallel, hepatic triglycerides were reduced 58% ($p<.01$). Surprisingly, despite this reduction in triglycerides, LIRKO mice showed a marked increase in hepatocyte injury: ballooning and lobular infiltrates were observed in LIRKO but not control livers upon histological examination; the inflammatory markers Tnfa and Mcp-1 were increased 2.52- and 3.64-fold ($p<0.01$); and expression of the fibrogenic markers Col3a1 and Col1a1 were increased 2.05- and 3.4-fold ($p<0.05$). Insulin exerts many of its metabolic effects by suppressing the transcription factor FoxO1. Interestingly, we found that the deletion of FoxO1 in LIRKO livers prevented the development of ballooning or inflammation, and restored inflammatory gene expression towards normal. Taken together, these data show that hepatic insulin signaling has a dual effect on the development of NAFLD. On the one hand, insulin promotes steatosis by inducing SREBP-1c and lipogenesis. On the other hand, it prevents hepatocyte injury and inflammation by suppressing FoxO1. These data support a new model of NAFLD, in which hepatocyte injury and inflammation are uncoupled from steatosis.

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An AMPK-Caspase-6 Axis Controls Liver Damage in Nonalcoholic Steatohepatitis

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Liver cell death has an essential role in nonalcoholic steatohepatitis (NASH). The activity of the energy sensor adenosine monophosphate (AMP)-activated kinase (AMPK) is repressed in NASH. Liver-specific AMPK knockout aggravated liver damage in mouse NASH models. AMPK phosphorylated pro-apoptotic caspase-6 protein to inhibit its activation, keeping hepatocyte apoptosis in check. Suppression of AMPK activity relieved this inhibition, rendering caspase-6 activated in human and murine NASH. AMPK activation or caspase-6 inhibition, even after the onset of NASH, improved liver damage and fibrosis. Once phosphorylation was decreased, caspase-6 was activated by caspase-3/-7. Active caspase-6 cleaved Bid to induce cytochrome c release, generating a feedforward loop that leads to hepatocyte death. Thus, the AMPK-caspase-6 axis regulates liver damage in NASH, implicating AMPK and caspase-6 as therapeutic targets.

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The nuclear receptor FXR regulates hepatic lipids via a reduction in lipid absorption

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Non-alcoholic fatty liver disease (NAFLD), a disease characterized by hepatic triglyceride (TAG) accumulation, is a leading cause of liver disease. Farnesoid X receptor (FXR) agonists are currently being tested to treat NAFLD for their ability to reduce hepatic triglyceride accumulation. Previous studies have proposed that FXR activation decreases hepatic lipogenesis through an FXR-SHP-SREBP1C pathway. Here we propose a novel mechanism by which FXR activation also decreases hepatic triglyceride accumulation, through a reduction in intestinal lipid absorption, likely through changes in bile acids. Using a potent synthetic FXR agonist (GSK2324) we show that FXR activation reduces liver TAG in wildtype (WT) mice, but not in FXR-/- mice. Hepatic lipidomic analysis showed that GSK2324 reduces both mono- and poly-unsaturated fatty acids (MUFA/PUFA). While reductions in MUFAs are due to reduced lipogenesis, decreased PUFAs suggested a reduction in dietary TAG absorption. Using radiolabeled TAG (C18:1) we show a reduction in absorption of fatty acids in WT mice treated with GSK2324 in response to a lipid challenge. Similarly, using a fluorescent BODIPY-labelled C:12 fatty acid, we find that WT mice have decreased lipid accumulation in enterocytes in response to FXR activation. Lastly, GC-MS analysis of feces shows increased fatty acids, consistent with decreased absorption. Since FXR is the master regulator of bile acid metabolism, we investigated whether bile acid levels were altered in response to FXR activation. Analysis of bile acid species reveal that the biliary bile acid levels are decreased in GSK2324 treated WT, but not FXR-/- mice. Taurine-(T-) conjugated bile acids synthesized by the liver (T-cholic acid, T-β-muricholic acid, and T-chenodeoxycholic acid) are decreased in GSK2324 treated WT mice. Reductions in bile acid levels are known to alter intestinal function, both through their effects as signaling molecules and as detergents to facilitate intestinal absorption of nonpolar nutrients, i.e. lipids, cholesterol, and vitamins. Thus, our data suggest that decreased bile acids may underlie the reduction in lipid absorption upon FXR activation in vivo. Together, our studies identify a novel pathway by which FXR activation alters lipid metabolism, which involves changes in biliary bile acid levels and reduced absorption of lipids.

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Interrogating Liver Macrophage LXR Signaling in Health and Non-Alcoholic Fatty Liver Disease

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The mechanisms that drive the behavior of hepatic macrophages in homeostasis and disease are poorly understood. The liver X receptor (LXR) transcription factors are highly expressed in hepatic macrophages and hepatocytes. While in hepatocytes LXR signaling controls cholesterol metabolism, macrophage LXR signaling regulates both cholesterol metabolism and inflammation, processes that are central to the pathogenesis of non-alcoholic steatohepatitis (NASH). Activation of LXRs represses inflammation by interfering with nuclear factor κb (NFκb) activation. NFκb is a downstream target of multiple NASH signaling pathways, including toll-like-receptors, interleukin-1, and tumor-necrosis factor a. LXRa is highly expressed in Kupffer cells (the resident macrophages of the liver) and rapidly upregulated in bone marrow derived macrophages upon entry into both healthy and NASH livers. This led to the hypothesis that loss of LXRa in hepatic myeloid cells would worsen NASH phenotypes in mice with metabolic disease via increased NFκb signaling. Mice lacking myeloid LXRa (LysMΔLXRa) and Cre-negative controls were fed the Amylin (AMLN) NASH-model diet for 20 weeks. LysMΔLXRa demonstrated increased hepatic fibrosis compared to Cre-negative littermate controls. Whole liver transcriptomic analysis revealed over 900 genes associated with loss of myeloid LXRa. Pathway analysis of differential genes suggests that the differences in gene expression are in part due to increased activity of TGFb, Il1b, and TNFa, and decreased activity of Ppara. Kupffer cells from LysMΔLXRa mice expressed increased levels of Trem2, Atf3, and Spp1 compared to littermate controls. RNA-seq analysis of Kupffer cells showed that loss of myeloid LXRa augments the transcriptional response of Kupffer cells to the AMLN diet. Together, these experiments suggest that myeloid LXRa may control a protective gene program during NASH pathogenesis.

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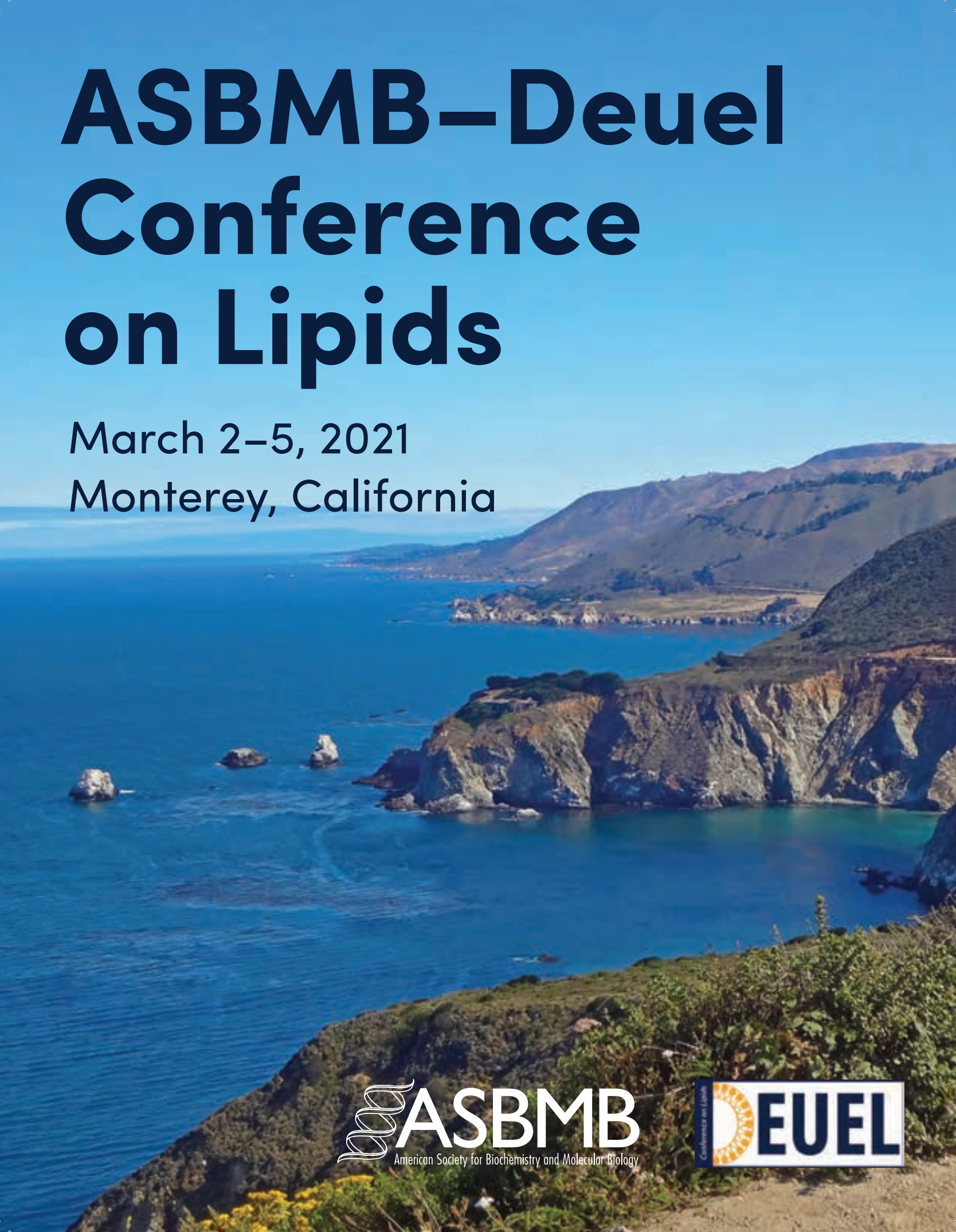

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