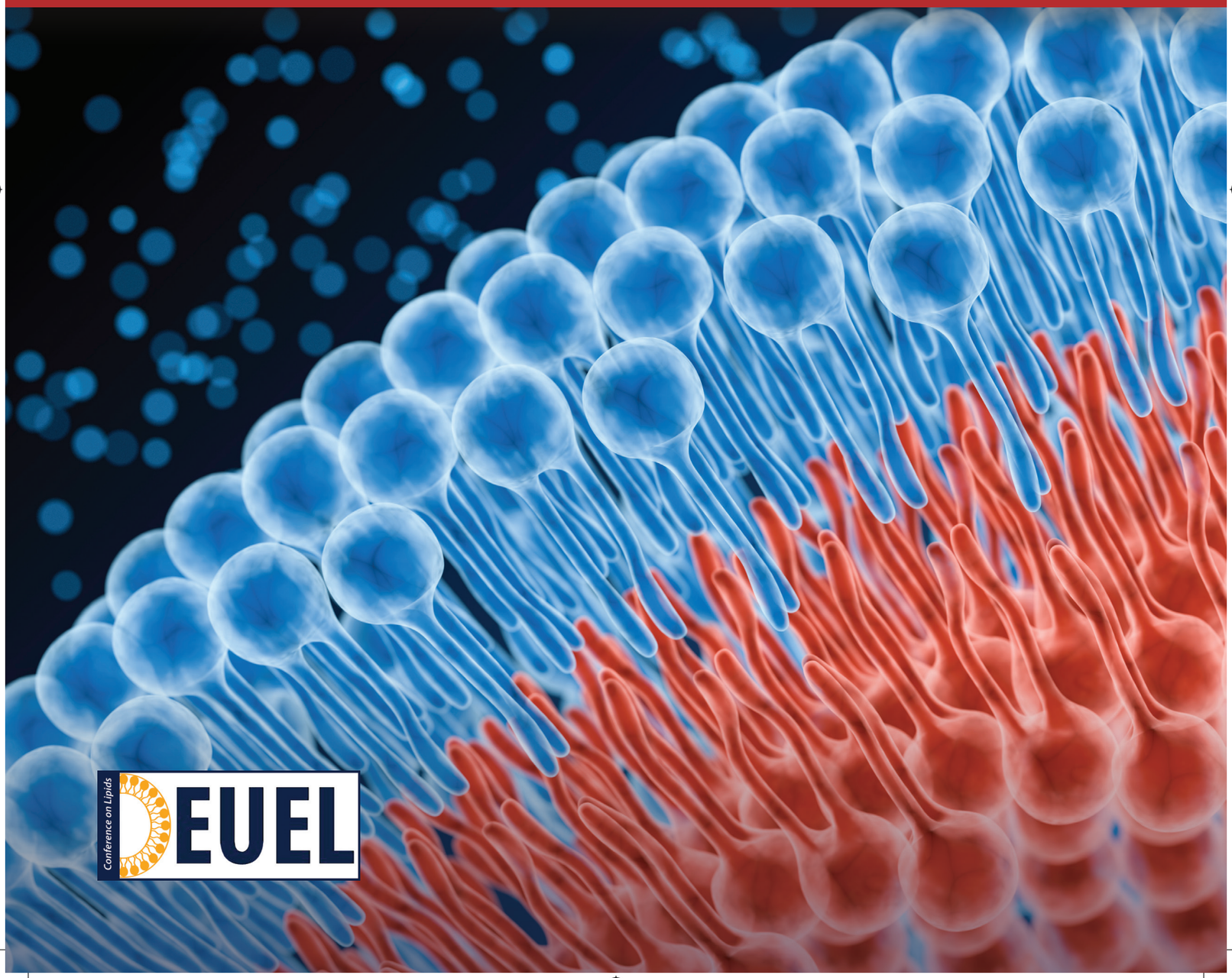
DEUEL CONFERENCE ON LIPIDS

March 7 – 10, 2017
Monterey, Calif.

More than any meeting in the lipid field, the Deuel Conference on Lipids provides a collegial and informal setting for close interactions between scientists from industry and academia.



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ASBMB
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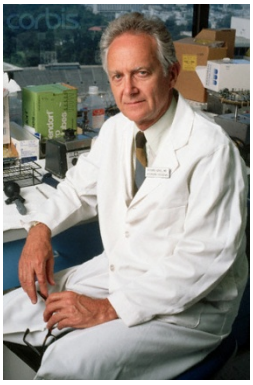
2017 DEUEL Conference on Lipids

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 video-taping 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



The Havel Lecture was named after 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.

Richard J. Havel is known to many as "Mr. Lipoprotein, USA." Havel 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.

Richard 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 AHA Council on Arteriosclerosis.

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

2017 HAVEL AWARD LECTURE

Peter Tontonoz,
University of California, Los Angeles
"Transcriptional control of lipid metabolism in physiology and disease"

PAST HAVEL AWARDEES

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"

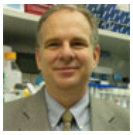
2010

David J. Mangelsdorf,
University of Texas Southwestern Medical Center
"Nuclear receptor control of lipid metabolism"

2002

Michael S. Brown and Joseph L. Goldstein,
University of Texas Southwestern Medical Center
"SREBPs: Master regulators 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
"The enzymes of cholesterol breakdown"

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"

Schedule of Events

	Tuesday, March 7	Wednesday, March 8	Thursday, March 9		Friday, March 10
7AM		Breakfast 7-8:30	Breakfast 7-8:30	Board Meeting 7-8:30	Breakfast 7-8:30
8AM					
9AM		Session 1 8:30-10:10	Session 3 8:30-10:10		Session 5 8:30-11:15
10AM		Coffee Break 10:10	Coffee Break 10:10		
11AM		Session 1, Cont. 10:25 – 12:05	Session 3, Cont. 10:25 – 12:10		Closing 11:15
12PM		Free Time 12:05-5:00	Free Time 12:10-5:00		
1PM					
2PM					
3PM	Registration 3-5:00				
4PM					
5PM	Opening Reception and Dinner 5:00-7:30	5-6:30 Poster Session 1	5-6:30 Poster Session 2		
6PM		6:30-7:45 Dinner	6:30-7:45 Dinner		
7PM		7:45-9:35 Session 2	7:45-9:30 Session 4		
8PM	The Havel Lecture 8:00 – 9:05				
9PM					
10PM					

Meeting Program

The Deuel Conference on Lipids, March 7 – 10, 2017

The Intercontinental Clement Monterey, Monterey, Calif.

Chair: Jean Schaffer, Washington University School of Medicine

Tuesday, March 7

3:00 – 5:30 Meeting Registration

5:00 – 7:30 Opening Reception and Dinner

8:00 – 8:05 Welcome: Jean Schaffer, Washington University School of Medicine

8:05 - 8:10 The Havel Lecture Introduction
Karin Bornfeldt, University of Washington

8:10 – 8:55 The Havel Lecture
"Transcriptional control of lipid metabolism in physiology and disease"
Peter Tontonoz, University of California, Los Angeles

Wednesday, March 8

8:30am– 12:05pm
Session 1: Lipid Trafficking and Organelle Homeostasis, Pacific Ballroom
Session Chair: **Joe Witztum, University of California, San Diego**

8:30 – 9:05 **"Mechanisms and physiology of fat synthesis and storage"**
Robert Farese, Harvard School of Public Health

9:05 – 9:40 **"Lipid droplet budding from the ER"**
William Prinz, NIDDK

9:40 – 9:55 **"A genetically encoded Apolipoprotein B reporter to illuminate LDL dynamics in larval zebrafish"**
Steven Farber, Johns Hopkins University

9:55 – 10:10 **"Brown adipose tissue hyperactivity in lysosomal storage disorders due to impaired mitophagy"**
Philip Gordts, University of California

10:10 – 10:25 Coffee break

10:25 – 11:00 **"Macrophage catabolism of lipids in extracellular lysosomal synapses"**
Frederick Maxfield, Weill Cornell Medical College

11:00 – 11:15 **"A way of LDL-cholesterol out of lysosome through membrane contacts"**
Bao-Liang Song, Wuhan University

11:15 – 11:30 **"Targeting lipid-droplet associated protein, FSP27, in the presence of fenofibrate ameliorates diet-induced steatohepatitis"**
Ananthi Rajamoorthi, Saint Louis University

11:30 – 12:05 "**Selective autophagy and lipid metabolism: a two side coin"**
Ana Maria Cuervo, Albert Einstein Medical College

12:05 – 5:00 Free time

5:00 – 6:30 Poster Session 1, Ocean Terrace Ballroom

6:30 – 7:45 Dinner, Ocean Terrace Ballroom

7:45 – 9:35pm

Session 2: Mitochondrial Metabolism, Pacific Ballroom

Session Chair: **Brandon Davies, University of Iowa**

7:45 – 8:20 **"Mitochondrial protein acylation and muscle bioenergetics in health and disease "**
Debbie Muoio, Duke University School of Medicine

8:20 – 8:55 **"Making mitochondria's quintessential lipid—coenzyme Q"**
David Pagliarini, The Morgridge Institute at UW-Madison

8:55 – 9:00 Journal Lipid Research Lectureship Award Introduction
William Smith, University of Michigan Medical School

9:00 – 9:35 Journal of Lipid Research Lectureship Award Lecture
"Genetic dissection of mitochondrial function"
Johan Auwerx, École Polytechnique Fédérale in Lausanne, Switzerland

Thursday, March 9

8:45– 11:45am

Session 3: Targeting Cardiometabolic Disease, Pacific Ballroom

Session Chair: **Richard Lehner, University of Alberta**

8:30 – 9:05 **"Lp(a): Science and therapy "**
Sotirios (Sam) Tsimikas, University of California, San Diego

9:05 – 9:40 **"The new biology of endoplasmic reticulumin lipid metabolism "**
Gökhan Hotamisligil, Harvard School of Public Health

9:40 – 9:55 "**Artery wall inflammatory processes, rather than systemic inflammation, are required for arterial macrophage accumulation in diabetes"**
Jenny Kanter, University of Washington School of Medicine

9:55 – 10:10 **"Targeting ire1 with small molecules counteracts progression of atherosclerosis"**
Ebru Erbay, Bilkent University

10:10 – 10:25 Coffee break

10:25 – 10:30 Journal of Clinical Investigation Lectureship Award Introduction
Sarah Jackson, Executive Editor, JCI

10:30 – 11:05 Journal of Clinical Investigation Lectureship Lecture
"Mining the blood for new cardiometabolic hormones"
Robert Gerszten, Beth Israel Deaconess Medical Center

11:05 – 11:20 **"Emerging role of acylcarnitines in adaptive thermogenesis and the impact on lipid metabolism"**
Claudio Villanueva, University of Utah

11:20 – 11:35 **"A haploid mammalian genetic screen identifies key determinants of metabolically-regulated ERAD of HMGCR and cholesterol biosynthesis"**
Noam Zelcer, Academic Medical Center of the University of Amsterdam

11:35 – 12:10 **"Nature and nurture of tissue resident macrophages"**
Christopher Glass, University of California, San Diego

12:10 – 5:00 Free time

5:00 – 6:30 Poster Session 2, Ocean Terrace Ballroom

6:30 – 7:45 Dinner, Ocean Terrace Ballroom

7:45 – 9:35pm
Session 4: Regulators of Metabolism, Pacific Ballroom
Session Chair: **Jay Horton, University of Texas Southwestern Medical Center**

7:45 – 8:20 **"Translational control by RNA binding proteins: A new layer of regulation influencing lipid metabolism secretion"**
Markus Stoffel, ETH Zurich

8:20 – 8:55 **"Beneficial metabolic and anti-inflammatory effects of the novel lipid class, branched fatty acid esters of hydroxy fatty acids"**
Barbara Kahn, Beth Israel Deaconess Medical Center

8:55 – 9:30 **"Adipose tissue and systemic lipid homeostasis: More than meets the eye"**
Phillip Scherer, University of Texas Southwestern

Friday, March 10

8:30 – 11:15am
Session 5: Clinical Studies in Cardiometabolic and Lipid Disorders, Pacific Ballroom
Session Chair: **Fred Kraemer, Stanford University School of Medicine**

8:30 – 9:05 **"DiscovEHRing novel drug targets for lipid disorders "**
Alan Shuldiner, University of Maryland School of Medicine

9:05 – 9:40 **"Development of cyclodextrin as a treatment for Niemann-Pick C1 disease "**
Daniel Ory, Washington University in St. Louis

9:40 – 9:55 **"Afamin is associated with prevalent and incident type 2 diabetes in the general population and predictive for gestational diabetes in pregnancy"**
Hans Dieplinger, Medical University of Innsbruck

9:55 – 10:10 Coffee Break

10:10 – 10: 25 **"ANGPTL8 requires ANGPTL3 to inhibit lipoprotein lipase and plasma triglyceride clearance"**
Jorge Haller, Regeneron Pharmaceuticals

10:25 – 10:40 **"Genetic association of waist-to-hip ratio with cardiometabolic traits, type 2 diabetes and coronary heart disease"**
Connor Emdin, Broad Institute of Harvard and MIT

10:40 – 11:15 "**Why Perform the CANTOS, CIRT, and PROMINENT trials?**"
Paul Ridker, University of Maryland School of Medicine

11:15 Closing

Poster Presentations

= Abstract/Board Number

Even number boards present Wednesday, 5:00pm–6:30pm

Odd number boards present Thursday, 5:00pm-6:30pm

1	Adipose-specific Knockout of Trib1 Reduces Plasma Lipids and Diet-induced Insulin Resistance, and Increases Circulating Adiponectin	**16**	Somatic Genome Editing with AAV Vectors Generates and Corrects a Metabolic Disease
2	Long-term epigenetic re-programming of myeloid precursor cells in a hyperlipidemic environment	**17**	Mapping Endolysosomal Lipid Accumulation In Vitro and In Vivo
3	Hepatic Sortilin Regulates Apolipoprotein B Secretion only Under Conditions of Secretory Stress	**18**	Brown Adipose Tissue mTORC2 couples Metabolic Fuel Selection and Thermogenesis in part through Controlling FoxO1-acetylation
4	Afamin is associated with prevalent and incident type 2 diabetes in the general population and predictive for gestational diabetes in pregnancy	**19**	Artery wall inflammatory processes, rather than systemic inflammation, are required for arterial macrophage accumulation in diabetes
5	Transcriptional integration of scavenger receptor class B type I gene expression by the nuclear receptors FXR and LXR via intron binding	**20**	Essential role of sphingolipid pathway in the maintenance of mammalian skin and energy homeostasis
6	Genetic association of waist-to-hip ratio with cardiometabolic traits, type 2 diabetes and coronary heart disease	**21**	Ces1d/TGH deficiency protects against high-sucrose diet-induced hepatic steatosis
7	Targeting IRE1 with Small Molecules Counteracts Progression of Atherosclerosis	**22**	The Golgi-Associated Retrograde Protein (GARP) Complex Regulates Intracellular Cholesterol Transport via Targeting NPC2 Localization to Lysosomes
8	A genetically encoded Apolipoprotein B reporter to illuminate LDL dynamics in larval zebrafish	**23**	A novel role for TTC39B in obesity and white/beige adipose tissue
9	Nat1 deficiency causes insulin resistance and impaired hepatic amino acid metabolism	**24**	Hepatic LRH-1, A Key Regulator of Lipid Storage And Phospholipid Diversity.
10	Aerobic exercise training selectively modulate oxysterols and gene expression reducing aortic cholesterol accumulation in apo E KO mice	**25**	Expression of a natural antibody (E06) targeted to oxidized phospholipids (OxPL) attenuates the progression of TLR2-mediated abdominal aortic atherosclerosis
11	The E3 ubiquitin ligase Idol controls expression of neuronal lipoprotein receptors and synaptic plasticity	**26**	Targeting lipid-droplet associated protein, FSP27, in the presence of fenofibrate ameliorates diet-induced steatohepatitis
12	Brown Adipose Tissue Hyperactivity in Lysosomal Storage Disorders Due To Impaired Mitophagy	**27**	Spatial control of cholesterol efflux and atherosclerosis by MeXis, a novel non-coding RNA
13	N-acyl taurines: novel bile components that inhibit food intake	**28**	CD36 regulates the PI3K pathway and controls glucose metabolism. Low CD36 expression strongly correlates with type-2 diabetes and complications.
14	ANGPTL8 requires ANGPTL3 to inhibit lipoprotein lipase and plasma triglyceride clearance	**29**	CD36 is required for enhanced hepatic de-novo lipogenesis (DNL) in response to high-sucrose feeding.
15	In vivo reporter of lysosomal lipid accumulation	**30**	Advanced glycated albumin chronically induces macrophage infiltration in rat Periepididimal Adipose Tissue Leading to Insulin Resistance

31	Liver-specific knockdown of long-chain acyl-CoA synthetase 4 disturbed glucose and phospholipid metabolic pathways leading to insulin resistance in mice fed a high-fat diet	**40**	Antisense oligonucleotide-mediated knockdown of MPZL3 protects from the metabolic consequences of a high-fat, energy-dense diet in mice
32	A way of LDL-cholesterol out of lysosome through membrane contacts	**41**	The role of abhydrolase domain-containing 15 (Abhd15) in insulin-regulated lipolysis in white adipocytes.
33	Lanosterol modulates TLR4-mediated innate immune responses in macrophages	**42**	Disabled-2 Determines Commitment of A Pre-adipocyte Population
34	Regulation of hepatic LDLR receptor by a novel membrane-bound E3 ubiquitin ligase controls plasma LDL cholesterol levels	**43**	Investigation of the role of ANGPTL3 in regulating the plasma level of low-density lipoprotein
35	Post-transcriptional regulation of bile acid synthesis mediated by ZFP36L1	**44**	Oxygen-dependent regulation of fatty acid metabolism mediates adipocyte function
36	Emerging role of acylcarnitines in adaptive thermogenesis and the impact on lipid metabolism	**45**	Analyzing the full spectrum of genomic variation with Lp(a) Cholesterol: Novel insights from deep, whole genome sequence data in 5,192 Europeans and African Americans
37	Phospholipid remodeling regulates intestinal stemness by controlling cellular cholesterol availability	**46**	A haploid mammalian genetic screen identifies key determinants of metabolically-regulated ERAD of HMGCR and cholesterol biosynthesis
38	A New ANGPTL Protein that Directs Triglyceride Partitioning with ANGPTL3	**47**	Lipin-2 and lipin-3 coordinate the compartmentalization of lipid droplets and ApoB48 lipidation in enterocytes
39	Apolipoprotein A1 Post-Translational Modifications and High-Density Lipoprotein Efflux Capacity: Preliminary Data from The Chicago Healthy Aging Study	**48**	Defining the Crosstalk between Cholesterol Homeostasis and Type I Interferon Signaling

1

Adipose-specific Knockout of Trib1 Reduces Plasma Lipids and Diet-induced Insulin Resistance and Increases Circulating Adiponectin

Mikhaila A. Smith[1], Jian Cui[2], Sumeet A. Kheterpal[1], Daniel J. Rader[1], Robert C. Bauer[2]

[1]University of Pennsylvania, Philadelphia, PA; [2]Columbia University, New York, NY

Tribbles-1 (TRIB1) was recently identified through genome-wide association studies as a novel mediator of plasma lipids and coronary artery disease in humans. Although subsequent *in vivo* mouse work confirmed a role for hepatic TRIB1 in these associations, little is known about metabolic roles for extrahepatic Trib1. Interestingly, SNPs near the TRIB1 gene are significantly associated with circulating adiponectin levels in humans, suggesting a metabolic role for adipose TRIB1. To further investigate this, we generated adipose-specific Trib1 KO mice (Trib1_ASKO) by crossing Trib1 cKO mice to transgenic adiponectin-Cre mice. Chow-fed Trib1_ASKO mice exhibited no differences in adipose tissue mass and overall body mass as compared with control littermates (n = 8/group). However, Trib1_ASKO mice had reduced total (−16.9%, $p < 0.01$), HDL (−16.7%, $p < 0.01$), and non-HDL cholesterol (−17.3%, $p = 0.068$), as well as plasma triglycerides (−28.6%, $p < 0.001$) as compared with WT mice. Trib1_ASKO mice also had increased plasma adiponectin levels, a finding more pronounced in female mice (+33.3%, $p < 0.001$) than in males (+16.4%, $p = 0.072$). Despite this increase, transcript levels of adipoQ were moderately decreased in Trib1_ASKO mice, suggesting a post-transcriptional mode of regulation. Transcript and protein levels of C/EBPa, the best described target of Trib1 and a key regulator of adipogenesis, remained unchanged. To further investigate the metabolic consequences of adipose-specific KO of Trib1, WT and Trib1_ASKO mice were fed a high-fat diet (HFD; 45% kcal fat) for 12 weeks to induce obesity. HFD-fed Trib1_ASKO mice had reduced fasting plasma glucose (−22.3%, $p < 0.05$), insulin (−38.2%, $p < 0.05$), and glucose tolerance (−19.8% AUC, $p < 0.05$) compared with control mice. Body mass and fat mass of HFD-fed Trib1_ASKO mice remained unchanged from WT, and the reductions in plasma lipids and increase in plasma adiponectin persisted in the HFD-fed state. In summary, we present here the first *in vivo* validation of the human genetic association between TRIB1 and plasma adiponectin and provide evidence suggesting that adipose TRIB1 contributes to the genetic associations observed in humans between TRIB1 and multiple metabolic parameters.

2

Long Term Epigenetic Reprogramming of Myeloid Precursor Cells in a Hyperlipidemic Environment

Anette Christ[1,2], Patrick Guenther[3], Mario Lauterbach[2], Minhi Park[4], Michael Fitzgerald[4], Andreas Schlitzer[3], Joachim Schultze[3], Eicke Latz[1,2]

[1]University of Massachusetts Medical School, Department of Medicine, Worcester, MA; [2]Institute of Innate Immunity, University Hospital Bonn, Bonn, Germany; [3]Life and Medical Science Institute, Genomics and Immunoregulation, University of Bonn, Bonn, Germany; [4]Massachusetts General Hospital, Boston, MA

Efforts to reverse the pathologic consequences of vulnerable atherosclerotic plaques are often impeded by the complex pro-inflammatory environment within the plaque, which might be provoked by a long term reprogramming of myeloid cells. Perseverative epigenetic modifications in myeloid cells probably evolved as an ancient mechanism to protect against pathogens because they confer nonspecific protection from secondary infections but may lead to uncontrolled immune responses in the context of sterile inflammation. Given the above hypothesis, we sought to investigate whether a hyperlipidemic environment can considerably modify myeloid (precursor) subsets in atherosclerosis-prone mouse models, which in turn could evoke a condition of continuous immune cell activation. Therefore, *Ldlr*$^{-/-}$ mice were fed 1) a standard chow diet, 2) a high fat/high cholesterol diet (HFD) for 4 weeks, or 3) an HFD for 4 weeks and than given a 4-week resting period (to study a long term training effect). To test for evidence of HFD-induced memory build-up, bone marrow myeloid cells were extracted from the three feeding groups and stimulated *ex vivo* with a panel of Toll-like receptor ligands. In contrast to the chow group, stimulated cells from the HFD/rested animals gave responses as great as or even greater than stimulated cells from the 4-week HFD-fed animals. Interestingly, sequencing of mRNA isolated from flow-sorted bone marrow myeloid precursors revealed a more pronounced induction of genes involved in stem cell proliferation and myeloid lineage differentiation as well as many pro-inflammatory genes involved in innate immune signaling in both the HFD and the HFD/rested animals. In line with this, ATAC sequencing data imposed on sorted myeloid precursors from HFD and HFD/rested animals revealed more open chromatin structures in certain promoter and enhancer regions crucially involved in inflammatory signaling pathways. The strongly activated cell phenotype was reflected on a functional level by markedly enhanced *in vivo* proliferation and increased TNFα production upon *in vivo* LPS injection 6 h before cell isolation. These data strongly support the concept of a diet-induced (epi)genetic reprogramming already on the level of myeloid precursors, which may induce a long term hyperactive state of diverse peripheral myeloid cell subsets.

3

Hepatic Sortilin Regulates Apolipoprotein B Secretion Only under Conditions of Secretory Stress

Donna M. Conlon[1], Amrith Rodrigues[1], Kathy Guo[1], Lilly E. Wilson[1], Daniel Rader[1]

[1]University of Pennsylvania, Philadelphia, PA

Sortilin is a multiligand-sorting receptor involved in trafficking of proteins from the Golgi apparatus to the lysosome and has been widely shown to be associated with plasma lipid traits and coronary artery disease. Whereas overexpression of sortilin in the liver reduces VLDL production, the reported effects of the genetic loss of sortilin on apolipoprotein B100 (apoB) and VLDL secretion have been contradictory and perplexing; loss of sortilin has been shown in different studies to result in both increased and decreased apoB/VLDL secretion. These conflicting studies were carried out in a variety of different models and methods of knocking down sortilin expression. We measured VLDL secretion in both Sort1$^{-/-}$ mice and Sort1 liver-specific KO mice on a chow diet and did not observe a difference in either apoB or TG secretion. The previously reported increases in VLDL secretion occurred on either the background of apoB overexpression or in mice on a high fat diet, suggesting the requirement for a metabolic stress. Therefore, we placed Sort1$^{-/-}$ mice on a 45% HFD for 12 weeks and observed a significant increase in VLDL secretion as compared with wild-type mice. To attempt to further clarify the role of sortilin, we explored the role of sortilin deficiency in apoB secretion in siRNA-treated McA-RH7777 cells. Similar to what was observed in the mouse, loss of sortilin alone was not associated with any change in apoB secretion in a basal state. However, when we lipid-loaded the cells with oleic acid or palmitic acid, there was an observed increase in apoB secretion with Sort1 knockdown as compared with control. This increase was also observed when cells were stressed by the addition of tunicamycin and proteasomal inhibitors, suggesting that sortilin regulates apoB secretion only when both apoB secretion is increased and the cell is stressed. Based on these data, we propose that hepatic sortilin regulates the post-endoplasmic reticulum (ER) fate of apoB for degradation and export and acts to coordinate intracellular apoB metabolism in response to the number and quality of apoB particles that reach the Golgi and the level of post-ER presecretory proteolysis activity.

4

Afamin Is Associated with Prevalent and Incident Type 2 Diabetes in the General Population and Predictive for Gestational Diabetes in Pregnancy

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The human vitamin E-binding glycoprotein afamin is primarily expressed in the liver and secreted into the bloodstream. Recently, we could demonstrate that afamin concentrations are associated with prevalent and incident metabolic syndrome in the general population. In pregnant women, afamin concentrations linearly increase 2-fold during uncomplicated pregnancies. We therefore investigated in the present study the association between afamin and the prevalence and incidence of type 2 diabetes mellitus (T2DM) in the general population and in pregnancy. Individual-level baseline (20,094) and follow-up data (n = 13,427) of six population-based studies (Bruneck, n = 826; KORA F3, n = 3,136; KORA F4, n = 3,050; CoLaus, n = 4,773; YFS, n = 2,270; NHLBI Family Heart Study, n = 1,877), one prospective study with data collected from general medical practices (NPHS-II, n = 2,674), and one healthy working population (SAPHIR, n = 1,488) were investigated. Study-level data were combined using random effects meta-analyses. In a further (case control) study, women with gestational diabetes mellitus (GDM, n = 209) were compared with women with uncomplicated pregnancies (n = 209) matched for age and body mass index (BMI). Blood samples were taken between the 11th and 13th gestational week. Differences between group medians were analyzed by Mann-Whitney U test. In all study groups, afamin concentrations were measured with ELISA. Each increase of afamin by 10 mg/liter was associated with a higher probability for prevalent T2DM (n = 1,398): odds ratio (OR) = 1.19 (95% confidence interval (CI) 1.12–1.26), $p = 5.96 \times 10^{-8}$ (extended adjustment for age, sex, BMI, HDL cholesterol, ln-triglycerides, hypertension). Increasing afamin concentrations (increment 10 mg/liter) were a significant predictor for incident T2DM (n = 585): OR = 1.30 (95% CI 1.23–1.38), $p = 3.53 \times 10^{-19}$. Afamin serum concentrations increase faster during pregnancy and were therefore significantly higher in first-trimester pregnant women developing GDM as compared with those with an uncomplicated pregnancy. This meta-analysis in more than 20,000 individuals showed that afamin is strongly associated with prevalence and incidence of T2DM independent of metabolic risk factors. Because treatment timing in women with pregnancy complications, such as GDM, is essential and a preventive effect is best observed when starting early during pregnancy, afamin may serve as an invaluable early predictive marker with far reaching therapeutic consequences. Afamin might thus be a promising prognostic marker for the early diagnosis of T2DM and GDM.

5

Transcriptional Integration of Scavenger Receptor Class B Type I Gene Expression by the Nuclear Receptors FXR and LXR via Intron Binding

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The farnesoid X receptor (FXR) plays critical roles in hepatic and plasma cholesterol metabolism, in particular HDL-C homeostasis. Obeticholic acid (OCA) is an FXR agonist being developed for treating various chronic liver diseases. The scavenger receptor class B type I (SR-BI) is the major receptor for HDL-C and SR-BI transcription in mice has been shown to be activated by FXR via its binding to three FXR-responsive elements (FXREs) embedded in the first intron. Our previous studies showed that OCA treatment effectively lowered plasma HDL-C levels and increased hepatic SR-BI mRNA and protein expressions in hypercholesterolemic hamsters but not in normolipidemic hamsters, suggesting that hepatic cholesterol might play a role in OCA-induced SR-BI transcription. In this current study, by conducting genomic sequence analysis, reporter assays, and direct DNA binding assays, we have identified a regulatory region in the first intron of the hamster SR-BI gene that contains a functional FXRE motif and an LXRE site separated by 57 base pairs. The nucleotide sequence within this regulatory region is highly conserved between hamsters and mice, with identical binding sequences and the spacing between FXRE and LXRE. The hamster promoter reporter activity was increased 15-fold by OCA, 5-fold by LXR agonist GW3965, and 30-fold by OCA and GW3965 cotreatment, indicating a synergistic activation of SR-BI gene transcription by the two nuclear receptors. Moreover, individual mutations of FXRE or LXRE abolished transcriptional activation exerted by both agonists. To demonstrate this synergistic activation of SR-BI gene transcription *in vivo*, hamsters fed a normal diet were administered GW3965 (30 mg/kg), OCA (10 mg/kg), or a combination for 10 days. Examination of liver SR-BI mRNA and protein levels confirmed that SR-BI gene expression was not activated by individual treatments, but the combination of OCA and GW3965 increased hepatic SR-BI mRNA levels 1.7-fold ($p < 0.001$) and protein levels 1.8-fold ($p < 0.001$) compared with the vehicle control group. These new findings provide a molecular explanation for the strong inducing effect of OCA on SR-BI expression in hypercholesterolemic hamsters. Of great significance, we have identified a novel transcriptional mechanism for SR-BI expression through an integrated activation by FXR and LXR via intron bindings.

6

Genetic Association of Waist/Hip Ratio with Cardiometabolic Traits, Type 2 Diabetes, and Coronary Heart Disease

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Abdominal adiposity has been associated with adverse cardiometabolic traits and coronary heart disease (CHD), independent of overall body mass index (BMI). However, whether abdominal adiposity is a causal risk factor for CHD is unclear. Waist/hip ratio adjusted for BMI (WHRadjBMI), a marker of abdominal adiposity, has been strongly associated with direct imaging assessments of abdominal fat, type 2 diabetes, and CHD in observational studies. We constructed a genetic instrument using 48 single nucleotide polymorphisms associated with WHRadjBMI and performed Mendelian randomization analyses to test whether abdominal adiposity causally relates to cardiometabolic quantitative traits, type 2 diabetes, and/or CHD. We pooled summary statistics from a study of 149,821 individuals for type 2 diabetes, 184 305 individuals for CHD, and individual-level data for 111,986 individuals in the UK Biobank. In 111,986 individuals in the UK Biobank, the mean age was 57 years, 58,845 participants (52.5%) were women, and the mean WHR was 0.875. Genetically elevated WHRadjBMI (a 1-S.D. value increase) was associated with 28-mg/dl higher triglyceride levels (n = 188,578), 4.0-mg/dl higher 2-h glucose levels (n = 133,010), and 2.0-mm Hg higher systolic blood pressure (n = 104 350; each $p <$ 0.001). Genetically elevated WHRadJBMI (a 1-S.D. value increase) was associated with higher risk of type 2 diabetes (odds ratio = 1.77, confidence interval (CI) 1.57–2.00; n = 261,807) and CHD (OR = 1.46, CI 1.32–1.62; n = 296–291). In a phenome-wide association study of 36 additional diseases, genetically elevated WHRadjBMI did not associate with any other disorder (all $p > 0.03$). In mediation analysis, adjustment for genetically elevated triglyceride levels attenuated the association of genetically elevated WHRadjBMI with CHD (OR = 1.23, CI 1.11–1.36 after adjustment). In conclusion, abdominal adiposity, independent of BMI, appears to be a causal risk factor for adverse cardiometabolic traits, type 2 diabetes, and CHD. Triglyceride-rich lipoproteins appear to mediate part of the excess risk for CHD associated with elevated abdominal adiposity.

7

Targeting IRE1 with Small Molecules Counteracts Progression of Atherosclerosis

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Metaflammation, an atypical, metabolically induced, chronic low-grade inflammation, plays an important role in the development of obesity, diabetes, and atherosclerosis. An important primer for metaflammation is the persistent metabolic overloading of the endoplasmic reticulum (ER), leading to its functional impairment. Activation of the unfolded protein response (UPR), a homeostatic regulatory network that responds to ER stress, is a hallmark of all stages of atherosclerotic plaque formation. The most conserved ER-resident UPR regulator, the kinase/endoribonuclease IRE1, is activated in lipid-laden macrophages that infiltrate the atherosclerotic lesions. Using RNA sequencing in macrophages, we discovered that IRE1 regulates the expression of many pro-atherogenic genes, including several important cytokines and chemokines. We show that IRE1 inhibitors uncouple lipid-induced ER stress from inflammasome activation in both mouse and human macrophages. *In vivo*, these IRE1 inhibitors led to a significant decrease in hyperlipidemia-induced IL-1β and IL-18 production, lowered T helper type-1 immune responses, and reduced atherosclerotic plaque size without altering the plasma lipid profiles in apolipoprotein E-deficient mice. These results demonstrate that pharmacologic modulation of IRE1 counteracts metaflammation and alleviates atherosclerosis.

8

A Genetically Encoded Apolipoprotein B Reporter to Illuminate LDL Dynamics in Larval Zebrafish

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The larval zebrafish is a powerful model system to study vertebrate metabolism, exhibiting striking similarity to human metabolic processes and disease phenotypes. Furthermore, it is the only vertebrate system conducive to high-throughput whole animal screening. β-Lipoprotein phenotypes, including elevated levels of apolipoprotein-B (apoB), a preponderance of small dense low-density lipoprotein (sdLDL) particles, and elevated levels of LDL cholesterol (LDL-C), have all been identified as important risk factors for the world's most prevalent metabolic diseases: cardiovascular disease, diabetes, and metabolic syndrome. Using state-of-the-art genome engineering approaches, we developed transgenic zebrafish carrying an engineered luciferase tag fused to apolipoprotein-B in the endogenous gene locus. To our knowledge, this is the first successful transgenic apoB fusion protein generated in a whole animal context. This genetically encoded reporter is also remarkably sensitive and enables detection of the lipoprotein size distribution from the vanishingly small quantities of plasma present in individual larvae (approximately 1,000 times less than is required for traditional approaches). We have demonstrated that the tagged isoform of apoB is present in the expected lipoprotein fractions separated by ultracentrifugation and native polyacrylamide gel electrophoresis. We have also used these assays to characterize β-lipoprotein phenotypes in response to numerous genetic and pharmacological stimuli, ranging from dietary manipulation to microsomal triglyceride transfer protein inhibition. Specifically, lomitapide produced a 56 ± 9% ($p < 1e{-}06$) decrease in β-lipoprotein particle number and shifted the size distribution toward smaller particles. Crisper targeting of the zebrafish apoC-II gene locus produced the expected major shift in β-lipoprotein particle size. An ongoing forward genetic screen identified a novel phospholipase that profoundly reduces β-lipoprotein size. The speed, sensitivity, and single-larval resolution of these assays will allow us to execute a truly high-throughput pharmacogenetic screen to identify novel regulators of β-lipoprotein biology in the context of a live vertebrate organism. This approach 1) requires no prior knowledge of drug targets, 2) is completely unbiased and therefore sensitive to targets in every cell and tissue type, 3) accounts for the compensatory effects on *in vivo* physiology, and 4) has been designed to facilitate rapid characterization of the drug target and mechanism of action for each hit.

9

Nat1 Deficiency Causes Insulin Resistance and Impaired Hepatic Amino Acid Metabolism

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Genome-wide association studies have linked *NAT2* (*N*-arylamine acetyltransferase 2) variants to insulin resistance (IR) and plasma triglyceride (TG) levels, and knocking out the orthologous gene in mice (*Nat1*) causes IR (Knowles *et al.* (2015) *J. Clin. Invest.* **125,** 1739-1751) and mitochondrial dysfunction *in vivo* (Chennamsetty *et al.* (2016) *Cell Rep*. **17,** 527-540). *Nat1* knockout mice (*Nat1* KO) also demonstrate a decreased ability to utilize fat for energy and decreased exercise capacity. Since human *NAT2* and mouse *Nat1* are mainly expressed in liver and intestine, but insulin-mediated glucose uptake occurs mostly in skeletal muscle and adipose tissue, we hypothesized that hepatic *Nat1* may play a role in altering systemic energy metabolism. Comprehensive profiling of metabolome and lipidome of *Nat1* KO and control mice demonstrated marked changes in acylcarnitines, phospholipids, glycerolipids that were decreased in plasma of *Nat1* KO mice but elevated in the liver *versus* control mice. Moreover, *Nat1* KO mice have significantly lower plasma levels of amino acids. Consistent with these changes, hepatic transcriptomic and proteomic analyses of *Nat1* KO mice have shown marked changes of genes involved in amino acid metabolism. We further pursued the correlation of *Nat1* expression with genes among hepatic transcriptomes of nearly 100 mice species from the Hybrid Mice Diversity Panel, and pathway analysis of the enriched genes verified a robust correlation of *Nat1* expression with genes involved in amino acid metabolism. Our data support a role of hepatic *Nat1* in systemic energy homeostasis and modifying amino acid metabolism.

10

Aerobic Exercise Training Selectively Modulates Oxysterols and Gene Expression, Reducing Aortic Cholesterol Accumulation in ApoE KO Mice

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Oxysterols modulate the development of atherosclerosis by mediating lipid synthesis, uptake, and exportation as well as inflammation and cytotoxicity in the arterial wall. Regular physical exercise prevents atherosclerosis; improves lipid metabolism, reverse cholesterol transport (RCT), and antioxidant defenses; and reduces inflammation. We investigated the role of a 6-week aerobic exercise training (AET) program in the accumulation of cholesterol and oxysterols in arterial wall and plasma of dyslipidemic mice. Sixteen-week-old male apoE KO mice fed a chow diet were included in the protocol. Animals were trained in a treadmill running (15 meters/min) 5 days/week for 30 (T30; $n = 13$) or 60 min (T60; $n = 29$). A sedentary control group was assigned for each trained group (S30; $n = 14$ and S60; $n = 32$). Plasma lipids and glucose were determined by enzymatic techniques. Plasma and aortic arch oxysterols were measured by GC-MS. The expression of genes involved in lipid metabolism was determined by real-time quantitative RT-PCR. Results (mean ± S.D.) were compared by one-way analysis of variance with Newman-Keuls post-test or Student's *t* test. Body weight and plasma total cholesterol, triglyceride, HDL-C, glucose, and oxysterols were similar among groups. As compared with their respective S groups, AET enhanced 7α-hydroxycholesterol (T30; 44%) and 7β-hydroxycholesterol (T60; 70%) and reduced cholesterol (T60; 68%) in aorta. 7-Ketocholesterol levels were unchanged by AET. 27-Hydroxycholesterol was undetectable in all samples. In addition, AET increased Cyp27a1 (54%), Cd36 (75%), and catalase (70%) and decreased Abcg1 (31%), Olr1 (65%), Cyp7b1 (35%), and Ch25h (48%) mRNA. No changes were observed in the expression of Abca1, Nr1h3, and Nr1h2. In conclusion, AET increases 7α/7 β-hydroxycholesterol that is related to the elevated expression of Cd36 in aorta of these hypercholesterolemic mice. Apart from the cytotoxic effect of these compounds, they may also improve RCT. In addition, the increase and reduction of Cyp27a1 and Cyp7b1, respectively, may contribute as an alternative pathway of lipid diffusion from the arterial wall. Finally, the reduction of cholesterol in aorta may be a consequence of systemic actions of AET that increase cholesterol flow to the liver and feces by the RCT.

11

The E3 Ubiquitin Ligase Idol Controls Expression of Neuronal Lipoprotein Receptors and Synaptic Plasticity

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Neuronal lipoprotein receptors are linked to learning and memory, but the pathways governing their abundance and the mechanisms by which they affect the function of synapses are incompletely understood. Here we demonstrate that control of neuronal lipoprotein receptor levels by the E3 ubiquitin ligase Idol regulates dendritic morphogenesis, and mediates structural remodeling of synapses upon neuronal activities. Loss of Idol in neurons results in constitutive overexpression of lipoprotein receptors, dysregulated spine morphogenesis, and defects in long term potentiation. Idol-deficient mice show profound impairment in experience-dependent reorganization of synaptic circuits in the barrel cortex as well as diminished spatial and associative learning. Our results identify regulation of neuronal lipoprotein receptor abundance as a critical post-transcriptional mechanism underlying experience-dependent structural and functional plasticity of synapses.

12

Brown Adipose Tissue Hyperactivity in Lysosomal Storage Disorders Due to Impaired Mitophagy

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We assessed the impact of lysosomal dysfunction on energy homeostasis and metabolism in a mouse model for type IIIa mucopolysaccharidoses (MPS IIIa). MPS IIIa is caused by a deficiency in sulfamidase, a lysosomal enzyme important for heparan sulfate (HS) catabolism. Loss of sulfamidase results in lysosomal HS storage, which impairs autophagosome-lysosome fusion and reutilization of substrates for growth and energy metabolism. At 10 weeks of age, MPS IIIa mice demonstrate fasting and postprandial hypertriglyceridemia compared with wild-type mice, with a reduction of white adipose tissue depots. Partitioning of dietary [^{3}H]triolein showed a marked increase in intestinal uptake and utilization in brown adipose tissue (BAT). Both hepatic production and clearance of triglyceride-rich lipoproteins did not differ from wild-type controls. BAT hyperactivity was associated with increased beige adipose tissue, hyperthermia, hyperphagia, and hyperdipsia and increased energy expenditure. Fasted MPS IIIa mice maintained hyperthermia when subjected to low temperature but became cachexic and profoundly hypothermic when treated with a lipolytic inhibitor. Impaired autophagy in MPS IIIa was associated with increased mitochondria content in beige adipose tissue and BAT, indicating impaired mitophagy and explaining the BAT hyperactivity. The reliance on increased lipid fueling was driven by a reduced ability to generate energy from glycogenolysis via impaired glycophagy, which was supported by the elevated liver and skeletal muscle glycogen content in MPS IIIa mice. The increased mitochondria content in beige and BAT and postprandial dyslipidemia partially reversed upon a 5-week treatment with recombinant sulfamidase. In conclusion, we hypothesize that the mechanism behind the increased BAT activity and its resulting persistent increase in energy demand seen in MPS models might be tied to the cachexia observed in MPS IIIa patients.

13

N-Acyl Taurines: Novel Bile Components That Inhibit Food Intake

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N-Acyl taurines (NATs) are endogenous, circulating molecules that are highly regulated, but with little known about the biological function. NATs are amphipathic molecules, with a hydrophobic acyl chain and hydrophilic taurine region, and are highly concentrated in bile. NATs are excellent detergents that are able to solubilize lipids in aqueous solutions at up to 12-fold lower concentrations than classic bile acids. Oral administration of *N*-oleoyl taurine (NOT) increased early intestinal triacylglycerol absorption by 32%, demonstrating one important function of NATs in bile. In an *in vitro* supersaturated simulated bile assay, NOT decreased formation of cholesterol crystals by 20% and dissolved 28% of preformed crystals, indicating a use in prevention of cholesterol-based gallstones. In addition to detergent functions, NATs are biologically active molecules that are synthesized and degraded or excreted rapidly. After injection of NOT, plasma NOT concentration peaked after 30 min and returned to baseline by 60 min, with the majority of NOT being degraded or excreted into bile or urine. Similarly, within 3 h of inhibition of FAAH, the enzyme that degrades NATs, some NAT species increase up to 100-fold in liver and plasma. When administered daily to HFD-fed mice, low-dose NOT (10 mg/kg) prevented weight gain, and high-dose NOT (25 mg/kg) decreased body weight by 7% within 14 days. NOT-treated mice gained less fat mass than controls, without altering lean mass. Along with lower adiposity, NOT-treated mice displayed improved glucose tolerance and lower liver triglyceride and plasma cholesterol. The lack of weight gain is probably due to diminished food intake with NOT treatment because mice ate 17 and 27% less with low- and high-dose treatment, respectively, than before treatment. No difference in energy expenditure was observed with treatment. In conclusion, NATs are a previously overlooked component of bile that can improve classic digestion-related functions of bile as well as altering whole-body metabolism.

14

ANGPTL8 Requires ANGPTL3 to Inhibit Lipoprotein Lipase and Plasma Triglyceride Clearance

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Angiopoietin-like protein 3 (ANGPTL3) and ANGPTL8 are secreted proteins and inhibitors of lipoprotein lipase (LPL)-mediated plasma triglyceride clearance. It remains a topic of interest to understand how these ANGPTL proteins interact to regulate LPL activity to supply appropriate amounts of fatty acids to tissues for storage or oxidation. ANGPTL3 inhibits LPL activity and increases serum triglycerides (TG) in mice independent of ANGPTL8. The effects on LPL activity and serum TG could be reversed with an ANGPTL3-blocking antibody. We now show that ANGPTL8 has a functional LPL-inhibitory motif but requires ANGPTL3 to inhibit LPL and increase serum TG in mice. Site-directed mutagenesis revealed that the ability of ANGPTL8 to block LPL activity and increase serum TG did not require ANGPTL3 with a functional LPL inhibitory motif. An antibody to the C terminus of ANGPTL8 reversed LPL inhibition by ANGPTL8 in the presence of ANGPTL3. The antibody did not disrupt the ANGPTL8-ANGPTL3 complex but comes in close proximity to the LPL-inhibitory motif in the N terminus of ANGPTL8. Collectively, these data show that ANGPTL8 has a functional LPL-inhibitory motif but can only inhibit LPL in the presence of ANGPTL3.

15

In Vivo Reporter of Lysosomal Lipid Accumulation

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Existing technologies do not allow for the measurement of lipid accumulation in the lumen of late endosomes/lysosomes within living tissues or organisms. Current assessments typically involve electron microscopy of fixed tissue sections. We present an optical reporter that non-invasively measures endolysosomal lipid accumulation and flux *in vivo*. We developed a nanoscale sensor that enters cells by endocytic processes and quantitatively reports lipids within the lumen of lysosomes *in vivo*. The reporter uses the intrinsic near-infrared fluorescence emitted by carbon nanotubes, which can be excited and detected through several millimeters to centimeters of live tissue. Upon intravenous injection, the reporter localized specifically to resident macrophages in the liver. The reporter responded to lipid content within the lysosomal lumen of hepatic macrophages via a distinct blue-shifting response upon lipid accumulation. We conclude that the nanosensor responds to lipids in its immediate vicinity via a solvatochromic mechanism. The reporter can be interrogated via a non-penetrating fiber probe or via whole-animal spectral imaging, and the signal was also imaged within tissue sections to provide quantitative histological measurements. We used the reporter to quantify lysosomal accumulation non-invasively in disease models, including Niemann-Pick type A/B. This technology portends rapid and non-invasive assessments of lipid accumulation within living tissues and organisms for research and drug development applications.

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Somatic Genome Editing with Adeno-associated Viral (AAV) Vectors Generates and Corrects a Metabolic Disease

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Current methods to query the impact of single genes on the development of metabolic disease can require years of gene targeting and mouse breeding to generate a model in which these phenotypes can be studied. Thus, more rapid and efficient methods are required to investigate the ever-growing list of candidate genes identified through human genetic studies. By packaging the CRISPR/Cas9 system into adeno-associated viral (AAV) vectors, we aimed to achieve targeted delivery and efficient gene editing in adult mice to model a metabolic disease, atherosclerosis. The *Ldlr* knockout mouse model is commonly used to study this disease, so we hypothesized that somatic disruption of *Ldlr* in the liver could generate atherosclerosis in adult mice. AAV vectors based on serotype 8 were used to deliver small guide RNAs (gRNAs) to adult Cas9 transgenic mice. The mice received 1) a nontargeting gRNA, 2) a gRNA targeting *Ldlr*, or 3) gRNAs to both *Ldlr* and *Apob*. Mice were placed on a Western diet and followed for 20 weeks for changes in plasma cholesterol and susceptibility to atherosclerosis. Disruption of *Ldlr* with AAV-CRISPR was robust, resulting in severe hypercholesterolemia (*Ldlr* (728 $\pm$ 174 mg/dl) *versus* control (350 $\pm$ 18.7 mg/dl)) and atherosclerotic lesions in the aorta (*Ldlr* (2.21 $\pm$ 2.10% lesion area) *versus* control (0.0% lesion area)) when maintained on a Western diet. Mice receiving gRNAs to both *Ldlr* and *Apob* had an identical degree of *Ldlr* disruption but dramatically lower cholesterol (125 $\pm$ 27.3 mg/dl), profound hepatic steatosis, and a complete prevention of Western diet-induced atherosclerosis. These data show that the AAV-CRISPR system is a valuable and immediately useful time-saving tool for metabolic disease studies that can also be used to test the involvement of liver-expressed candidate genes in combination.

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Mapping Endolysosomal Lipid Accumulation *in Vitro* and *in Vivo*

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Lysosomal lipid accumulation directly results in lysosomal storage disorders and has been implicated in cancer, neurological disorders, and metabolic diseases, including atherosclerosis and non-alcoholic fatty liver disorder. We have developed a nanoscale tool, composed of an intrinsically fluorescent single-walled carbon nanotube and single-strand DNA, that functions as a carbon nanotube optical reporter (CNOR) for lipids. Specifically, the near-infrared emission of the reporter undergoes a wavelength shift in the presence of lipids. Unlike other probes that are limited to specific lipids in biological membranes, this reporter localizes to the lysosomal lumen and reports on the overall lipid content of its environment. Using hyperspectral microscopy, we generate endolysosomal lipid maps to spatially and temporally map lipid dynamics in live cells and live animals. For fast non-invasive optical detection of the near-infrared reporter signal from live animals, we developed and use an *in vivo* hand-held spectrometer. Additionally, a novel high-throughput screening-compatible instrument can rapidly acquire the emission spectra from the reporter present in the endolysosomal lumen of live cells.
In live cells, the reporter is endocytosed and localizes to the lysosome. Following tail vein injection into a mouse, the reporter localizes to the liver and remains in the late endosomes and lysosomes of Kupffer cells. The reporter could detect Niemann-Pick type C, in live patient-derived fibroblasts, and reversal of the disease phenotype following the action of 2-hydroxybetacyclodextrin. In a mouse model for Niemann-Pick type A, lysosomal lipid accumulation in Kupffer cells was detected, for the first time, in a live animal. In conclusion, the CNOR for lipids presents a versatile nanoscale optical probe for investigating lysosomal lipid accumulation *in vivo* and *in vitro*, in the context of metabolic diseases, lysosomal storage disorders, and other pathologies. The nanoscale reporter, when coupled with the appropriate experimental platform, can non-invasively identify general lipid accumulation in the lysosomal lumen, with single-lysosome, single-cell, or single-animal resolution.

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Brown Adipose Tissue mTORC2 Couples Metabolic Fuel Selection and Thermogenesis in Part through Controlling FoxO1 Acetylation

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Brown adipose tissue (BAT) is a remarkable metabolic tissue. In mice, BAT is capable of switching between energy storing and energy burning states in response to only mild temperature shifts, and when active, it engages many metabolic pathways simultaneously (in some cases paradoxically) to fuel thermogenesis. Now that the presence of BAT in humans is widely appreciated, understanding BAT metabolism is not only an interesting model of metabolic regulation but a goal with physiological relevance to understanding human metabolic homeostasis. Whereas the transcriptional regulation of BAT development and function has been extensively described, the signaling mechanisms linking nutrient availability and thermogenesis with the BAT metabolic program are less understood. We investigated the role of the nutrient/hormone-sensing mTOR signaling pathway in BAT by deleting Rictor, an essential component of mTOR complex 2 (mTORC2), using the UCP1-Cre/CreER drivers, which selectively target mature brown adipocytes. BAT-specific ablation of mTORC2 reprograms BAT metabolism by reducing glucose uptake and *de novo* lipogenesis and increasing lipid uptake and oxidation. This fuel switching is associated with increased expression of several thermogenic genes, suggesting enhanced thermogenesis. In support, mice with mTORC2 loss in BAT are protected from HFD-induced obesity when living at thermoneutrality, a temperature condition more relevant to humans in which there is no thermal stress. Mechanistically, our data suggest that mTORC2 normally suppresses BAT activity at least in part by inhibiting FoxO1 transcriptional activity, which it does by promoting FoxO1 acetylation independently of AKT-mediated FoxO1 phosphorylation. Moreover, treating mice with a β_3-adrenergic agonist normally induces FoxO1 deacetylation in BAT, suggesting that regulating FoxO1 acetylation may be part of the normal thermogenic response. These data uncover a novel mechanism by which mTORC2 controls metabolism, and when mice are living under more human-like conditions, inhibiting this pathway in BAT is protective against nutrient overload and metabolic disease.

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Artery Wall Inflammatory Processes, Rather than Systemic Inflammation, Are Required for Arterial Macrophage Accumulation in Diabetes

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Diabetes accelerates atherosclerosis; however, the mechanisms that drive the increased cardiovascular disease risk are incompletely understood. We have previously shown in mouse models that diabetes results in increased systemic inflammation and increased expression of inflammatory mediators (including the damage-associated molecular pattern protein S100A9) in circulating monocytes, macrophages in the peritoneal cavity, and atherosclerotic lesion macrophages. The goal of this study was to investigate the relative contribution of systemic inflammation *versus* inflammatory processes in the lesion to arterial macrophage accumulation, using streptozotocin-induced diabetes in LDL receptor-deficient mice fed a low-fat diet. Diabetes augmented systemic inflammation, myeloid cell accumulation in the artery wall (1.8-fold increase, $n = 8–10$, $p < 0.05$), monocyte adhesion (1.2-fold increase, $p < 0.05$), and aortic endothelial cell expression of Icam1 (1.8-fold), Vcam1 (1.5-fold), and Ccl2 (2.5-fold, $n = 9–10$, $p < 0.05$), without affecting plasma cholesterol or monocytosis. To modulate diabetes-promoted inflammatory processes in different locations/compartments, we used three models: bone marrow S100A9-deficient chimeras, bone marrow TLR4-deficient chimeras, and treatment with an anti-inflammatory agent that primarily targets intestinal inflammation (5-aminosalicylic acid; 5-ASA). No model affected the severity of diabetes or plasma cholesterol. Hematopoietic S100A9 deficiency, but not hematopoietic TLR4 deficiency, reduced systemic inflammation to levels observed in non-diabetic mice (*e.g.* diabetes-induced a 2–2.5-fold increase in leukocyte Il1b mRNA, which was normalized by S100A9 deficiency ($p < 0.01$, $n = 7–10$) but was not significantly reduced by hematopoietic TLR4 deficiency ($n = 11–14$)). Conversely, hematopoietic TLR4 deficiency, but not hematopoietic S100A9 deficiency, reduced diabetes-accelerated myeloid cell accumulation in the artery wall (determined by the presence of fatty streaks and aortic en face Sudan IV staining; non-diabetics 0.24 ± 0.04-mm^2 lesion, wild-type diabetics 0.54 ± 0.09 mm^2, and diabetic hematopoietic TLR4 deficiency 0.30 ± 0.07-mm^2 lesion, $n = 16–21$, $p < 0.05$; whereas hematopoietic S100A9 deficiency resulted in a 0.79 ± 0.16-mm^2 lesion in diabetic mice, $n = 18$). Furthermore, 5-ASA reduced both diabetes-induced intestinal inflammation and aspects of systemic inflammation, but not arterial macrophage accumulation. Finally, laser capture microdissection of CD68-positive lesional macrophages demonstrated that hematopoietic TLR4 deficiency prevents inflammatory processes in the artery wall. Together, our data strongly suggest that although systemic inflammation is increased in diabetes, inhibition of inflammatory processes in the artery wall is required to prevent lesional macrophage accumulation.

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Essential Role of Sphingolipid Pathway in the Maintenance of Mammalian Skin and Energy Homeostasis

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The epidermis is the outermost layer of the skin, which acts as a barrier to protect the body from the external environment and control water and heat loss. This barrier function is established through the multistage differentiation of stem cells in the epidermis and the presence of bioactive lipids, such as ceramides, the levels of which are tightly regulated by a balance of ceramide synthase and ceramidase activities. Ceramics and their enzymes ceramidases are a crucial component of the epithelial sphingolipid pathway. Here we reveal the essential role of alkaline ceramidase 1 (Acer1) in the skin, because Acer1-deficient (Acer$^{-/-}$) mice showed elevated levels of different ceramide species in the skin, aberrant hair shaft cuticle formation, and cyclic alopecia. We show that Acer1 is specifically expressed in differentiated interfollicular epidermis, infundibulum, and sebaceous glands, and consequently Acer$^{-/-}$ mice showed significant alterations in infundibulum and sebaceous gland architecture. Acer$^{-/-}$ skin also showed perturbed hair follicle stem cell compartments. These alterations in the epidermis led to Acer$^{-/-}$ mice showing increased transepidermal water loss and a hypermetabolism phenotype with associated reduction of fat content with age. Our study collectively suggests that Acer1 is indispensable for mammalian skin and energy homeostasis and reveals a new *in vivo* role of the sphingolipid pathway. (Liakath-Ali *et al.* (2016) *J. Pathol.* **239,** 374–383).

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Ces1d/TGH Deficiency Protects against High-sucrose Diet-induced Hepatic Steatosis

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Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease. Triacylglycerol (TG) accumulation in liver is a hallmark of NAFLD. In humans, increased *de novo* lipogenesis is an important contributor to ectopic lipid accumulation in the liver and to NAFLD progression. Murine carboxylesterase 1d (Ces1d), which was also previously annotated as Ces3 or triacylglycerol hydrolase (TGH), is the ortholog of human carboxylesterase 1 (CES1). Ces1d/TGH exhibits lipolytic activity and participates in hepatic lipid metabolism. Ces1d/TGH-deficient mice are protected from high-fat diet-induced liver steatosis and present with attenuated hepatic *de novo* lipogenesis. To investigate whether Ces1d/TGH can mitigate steatosis induced by overactivated *de novo* lipogenesis, wild-type and Ces1d/TGH-deficient mice were fed with a high-sucrose diet. We found that Ces1d/TGH-deficient mice were protected from high-sucrose diet-induced hepatic lipid accumulation. Effects of Ces1d/TGH deficiency on genes involved in hepatic lipid synthesis and lipid droplet regulation have been observed. Changes in metabolic balance were observed in the Ces1d/TGH-deficient mice, which also contributed to the attenuated hepatic lipid accumulation in the high-sucrose diet-induced steatotic model. These studies suggest that inhibition of Ces1d/TGH exerts a protective effect against NAFLD, making CES1 a novel pharmacological target for NAFLD management.

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The Golgi-associated Retrograde Protein (GARP) Complex Regulates Intracellular Cholesterol Transport via Targeting NPC2 Localization to Lysosomes

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Proper intracellular cholesterol trafficking is critical for maintaining cholesterol homeostasis. Two lysosome-resident proteins, Niemann-Pick type C1 (NPC1) and NPC2, mediate the exit of LDL-derived cholesterol from lysosomes. However, whether other participants are also involved in this process remains largely unknown. Here, we defined an essential role of the Golgi-associated retrograde protein (GARP) complex in mobilization of lysosomal cholesterol. By combining a chemical mutagenesis and amphotericin B-based selection, we isolated two cholesterol trafficking-defective cell lines carrying the same mutation in the vacuolar protein sorting (*VPS*) gene. Depletion of VPS and VPS-containing GARP complex subunits impaired the biosynthetic sorting of NPC2 protein to the lysosomal compartment, and consequently, caused cholesterol accumulation. The mistrafficking of NPC2 in GARP-deficient cells was attributable to attenuated retrieval of cation-independent mannose 6-phosphate receptor (CI-MPR) to the *trans*-Golgi network. Strikingly, cholesterol overload was also detected in *VPS*-deficient mice, further validating the physiological role of the GARP complex in cholesterol homeostasis. We conclude that the GARP complex orchestrates intracellular trafficking of LDL-derived cholesterol via regulating CI-MPR-dependent sorting of NPC2 to lysosomes.

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A Novel Role for TTC39B in Obesity and White/Beige Adipose Tissue

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Obesity and hypercholesterolemia are major risk factors for atherosclerosis. SNPs in TTC39B (T39) have been associated with alterations in HDL cholesterol levels as well as waist/hip ratio. T39 is a scaffolding protein that has recently been shown to promote the ubiquitination and degradation of LXR, a transcription factor that rids the body of excess cholesterol. *T39*$^{-/-}$*Ldlr*$^{-/-}$ mice on a Western diet (WTD) showed reduced atherosclerosis and reduced hepatic steatosis compared with *Ldlr*$^{-/-}$ controls. Others have shown that *LXR*α$^{-/-}$ mice display reduced adiposity on high-fat, high-cholesterol diets in association with increased formation of thermogenic white adipose tissue (WAT; beige adipose). In a mouse adipocyte model, LXR binds to the promoter of *Ucp1* and blocks PPARγ binding, inhibiting *Ucp1* transcription. To investigate its therapeutic potential, mice were treated with a *T39* antisense oligonucleotide (ASO), reducing *T39* expression in the liver and adipose tissue. *Ldlr*$^{-/-}$ mice treated with the T39 ASO fed a WTD had decreased serum cholesterol and triglycerides and decreased liver triglycerides and lipogenic gene expression as predicted. They also exhibited significantly lower body weight and a decrease in gonadal adipose (gWAT) weight in male and female mice; adipose lipogenic gene expression was not altered. Further analysis provided evidence of increased expression of beige/brown adipose tissue genes in gWAT, including *Ucp1*, *Pgc1α*, and *Pparγ1* and *β2 AR*. gWAT also had increased PPARγ1 protein levels with no changes in PPARγ2. The subcutaneous adipose tissue (iWAT) had increased expression of *Ucp1* and another beige/brown adipose gene, *Cidea*. iWAT immunohistochemistry stained with UCP1 primary antibody also revealed increased UCP1 staining in T39 ASO-treated mice. *Ldlr*$^{-/-}$ mice treated with the T39 ASO placed on a high-fat, sucrose, cholesterol diet were also resistant to weight gain compared with control and had significantly improved glucose and insulin tolerance. Intriguingly, these preliminary data suggest that T39 may be involved in facilitating or balancing the interaction between LXR and PPARγ in regulating fat storage in white adipose tissue. Taken together, these results indicate that T39 may be a novel therapeutic target in the treatment of metabolic disease.

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Hepatic LRH-1, a Key Regulator of Lipid Storage and Phospholipid Diversity

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Non-alcoholic fatty liver disease (NAFLD) affects 30% of the adult population of the United States. Aside from lifestyle modification, there is currently no treatment for NAFLD. Activation of liver receptor homolog-1 (Lrh-1), known to bind phospholipid ligands, has been shown to effectively reduce liver triglyceride (TG) in DIO mice, raising Lrh-1 as a possible target for treating NAFLD. Despite this finding, hepatic TGs are equivalent in controls and liver-specific Lrh-1 knockout (LKO or Lrh1AlbCre) mice, regardless of diet. Given this discrepancy, we sought to characterize the role of Lrh-1 in hepatic lipid metabolism by acutely deleting Lrh-1 in the adult liver, thus eliminating potential compensatory developmental effects associated with LKO. To acutely eliminate Lrh-1 in hepatocytes, 6-week-old Lrh-1fl/fl male mice were infected with AVV8-TBG-eGFP (control) or AAV8-TBG-Cre (LKOAAVCre) via retro-orbital injection and fed chow or a high fat diet. LKOAAVCre mice developed hepatic steatosis after 6 weeks on standard chow or a high fat diet. This increased TG phenotype was specific to LKOAAVCre, but not to the LKO model. Furthermore, LKOAAVCre hepatocytes exhibited large lipid droplets, which were visible as early as 2 weeks post-infection, thus suggesting that lipid handling is significantly altered in LKOAAVCre hepatocytes. Despite similar rates of fatty acid transport, SeaHorse analyses of LKOAAVCre hepatocytes showed impaired fatty acid oxidation and increased lipid storage. Consistent with other studies showing that perturbations in phospholipid pools affect lipid storage, lipidomic analyses revealed a significant reduction in phospholipid species containing arachidonic acid, thus reducing the overall diversity of key membrane phospholipids. RNA-Seq analyses from LKOAAVCre livers confirmed that factors promoting lipid droplet size (Cidec, Plin4) were greatly increased, whereas key enzymes in the biosynthesis of unsaturated fatty acids were reduced (Fads1, Fads2, and Elovl5). Collectively, our data establish a novel role for Lrh-1 as a key regulator of lipid storage, thereby providing the first *in vivo* evidence as to why phospholipids serve as Lrh-1 ligands.

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Expression of a Natural Antibody (E06) Targeted to Oxidized Phospholipids (OxPLs) Attenuates the Progression of TLR2-mediated Abdominal Aortic Atherosclerosis

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Intraperitoneal administration of the TLR2 agonist Pam3CSK4 to HFHC-fed LDLr$^{-/-}$ mice promotes atherosclerosis characterized predominantly by severe lesions in the abdominal aorta. In this study, we investigated the role of oxidized phospholipids (OxPLs) in the pathogenesis of abdominal atherosclerosis by comparing the impact of TLR2 agonism in LDLr$^{-/-}$ mice *versus* LDLr$^{-/-}$ mice expressing a single chain variant (scFv) of the E06 IgM natural antibody. E06 binds to the phosphocholine of OxPL present on OxLDL as well as on apoptotic cells and debris. The E06-scFv transgene is under control of the apoE promoter and is expressed in liver and macrophages. Biweekly i.p. injection of Pam3CSK4 for 12 weeks resulted in severe abdominal atherosclerosis, which was greatly reduced by the E06-scFv (76% *versus* 38% abdominal lesions). A similar experiment conducted in LDLr$^{-/-}$ × Rag1$^{-/-}$ mice *versus* E06-scFv/LDLr$^{-/-}$ Rag1$^{-/-}$ mice revealed that although the absolute extent of abdominal lesion was reduced by the absence of T/B cells, lesions were almost prevented in the E06-scFv mice (24% *versus* 5%), indicating that most of the macrophage contribution to abdominal atherosclerosis was mediated by OxPL. Because the E06-scFv is also expressed in macrophages in culture, we prepared bone marrow-derived macrophages and stimulated them with Pam3CSK4 in culture. In contrast to strong pro-inflammatory gene expression of macrophages from LDLr$^{-/-}$ mice, inflammatory gene expression from E06-scFv macrophages was greatly attenuated. In addition, adipose tissue around the abdominal aorta was enhanced in LDLr$^{-/-}$ mice, and adipose tissue macrophages (ATMs) displayed an M1 phenotype, in contrast to an M2 phenotype from ATMs of E06-scFv mice.

Our study demonstrates that Pam3CysK4 mediated abdominal atherosclerosis by promoting outside-in inflammation mediated in part by TLR2 stimulation of ATMs surrounding the abdominal aorta. That this was greatly attenuated by E06 indicates that OxPL are dominant mediators of this TLR2-induced inflammation. These data help to explain why IgM in general, which are greatly enriched in oxidation-specific antibodies, and E06 in particular provide protection against inflammation and atherosclerosis. Taken together, these studies also support the feasibility of using E06, or an equivalent anti-OxPL antibody, as a therapeutic intervention to prevent inflammation and the progression of atherosclerosis.

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Targeting Lipid Droplet-associated Protein, FSP27, in the Presence of Fenofibrate Ameliorates Diet-induced Steatohepatitis

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Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the Western world. NAFLD progresses from benign steatosis to non-alcoholic steatohepatitis (NASH) to cirrhosis and is also related to hepatocellular carcinoma. Despite decades of extensive research, no targeted treatments are approved for NAFLD/NASH. We have shown previously that FSP27 (fat-specific protein 27), a lipid droplet-associated protein that facilitates the fusion of small lipid droplets, promotes both fasting- and diet-induced hepatosteatosis. However, long term silencing of Fsp27 did not reduce liver triglyceride accumulation in either high-fat diet-fed C57BL/6 mice or chow-fed ob/ob mice. Here, we show that silencing Fsp27 in combination with the PPAR-α agonist, fenofibrate, not only promotes resistance to obesity, but also ameliorates steatosis, inflammation, and fibrosis in a diet-induced mouse model of NASH. These data suggest that a combination therapy that includes both knockdown of Fsp27 and activation of PPAR-α could be used therapeutically to manage NAFLD/NASH.

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Spatial Control of Cholesterol Efflux and Atherosclerosis by MeXis, a Novel Non-coding RNA

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The nuclear receptor LXR regulates the expression of genes involved in cellular responses to cholesterol overload, including Abca1. Macrophage-specific cholesterol efflux driven by Abca1 has been causally linked to the prevention and reversal of atherosclerosis. In this work, we identify the lncRNA MeXis as an amplifier of LXR-dependent Abca1 gene transcription in macrophages. MeXis interacts with and influences the promoter binding of nuclear receptor transcriptional coactivators. Loss of MeXis in immune cells has a marked impact on chromatin accessibility at the Abca1 locus, impairs cellular responses to cholesterol overload, and accelerates the development of atherosclerosis. Our findings identify MeXis as a transcriptional gatekeeper that modifies the actions of LXR in lipid-dependent control of macrophage gene expression.

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CD36 Regulates the PI3K Pathway and Controls Glucose Metabolism: Low CD36 Expression Strongly Correlates with Type 2 Diabetes and Complications

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Onset and progression of obesity-associated complications are linked to dysfunction in pathways of fatty acid (FA) and glucose metabolism. We identified a novel molecular pathway that links FA supply to energy regulation by insulin and 5[prime]-AMP-activated protein kinase (AMPK). The mechanism involves the direct impact of CD36 *versus* CD36 + FA on protein interactions within the multiprotein signaling complexes of both pathways. CD36 signaling enhances PI3K and suppresses AMPK, whereas CD36 + FA signaling is differentially modulated by the FA type. Our studies indicate that saturated FAs (such as palmitic or myristic acid), but not unsaturated FAs (such as oleic or linoleic acid), are capable via CD36 of simultaneously suppressing PI3K/Akt activation while enhancing AMPK activation. These effects result in rapid and opposite regulation of the FA and glucose metabolism. The operation of this mechanism would serve to coordinate utilization of glucose and FA, but its dysfunction would result in deleterious metabolic effects. In line with this, findings from a human RNA-seq and GWAS database showed that reduced CD36 expression in various tissues strongly correlates ($p < 10^{-7}$) with the presence of type 2 diabetes and diabetes-associated renal, cardiovascular, and ophthalmic complications.

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CD36 Is Required for Enhanced Hepatic *de Novo* Lipogenesis (DNL) in Response to High-sucrose Feeding

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The development of nonalcoholic fatty liver disease (NAFLD) precedes and contributes to the etiology of insulin resistance. Several pathways determine triglyceride accumulation in the liver, including *de novo* lipogenesis (DNL), fatty acid (FA) uptake, FA oxidation, and lipoprotein secretion. DNL is highly responsive to glucose and insulin, and its contribution becomes significant with intake of a high carbohydrate diet. We recently found using CHO cells that express both CD36 and the insulin receptor (IR) that CD36 enhances insulin signaling through interaction with IR and recruitment of the Src kinase Fyn to phosphorylate IR. The addition of insulin to cells expressing CD36 resulted in robust phosphorylation of AKT and of the enzyme ATP citrate lyase (ACLy), whereas these effects were blunted in cells lacking CD36. ACLy is the primary enzyme responsible for converting citrate to acetyl-CoA, which can be used for fatty acid biosynthesis. We examined the response of hepatic lipogenesis in CD36-deficient mice maintained on a high carbohydrate (60% sucrose), low fat (10%) diet. CD36-deficient mice did not gain weight or fat mass on the diet, whereas the WT mice showed a 30% weight increase and more than doubled their fat mass. In WT mice, hepatic levels of the two key lipogenic enzymes FA synthase (FAS) and ACLy and those of phospho-ACLy were significantly increased, whereas no increase was observed in livers of CD36-deficient mice. Plasma FA composition measurements showed severalfold increases in the DNL products, palmitic and palmitoleic acids, in WT mice fed the sucrose diet, and no increases were observed in the CD36-deficient mice. Thus, CD36 appears to be essential for hepatic lipogenesis, and its deficiency prevents the increase in DNL induced by high carbohydrate diets.

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Advanced Glycated Albumin Chronically Induces Macrophage Infiltration in Rat Periepididimal Adipose Tissue, Leading to Insulin Resistance

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Advanced glycated end products (AGE) relate to the development of diabetes mellitus (DM) and its long term complications. We investigated the effect of chronic administration of AGE-albumin, associated or not with *N*-acetylcysteine (NAC), on rat insulin sensitivity, periepididimal adipose tissue transcriptome, macrophage infiltration, and polarization.

One-month-old healthy male Wistar rats ($n = 7–8$) received a daily i.p. injection of homologous control (C) or AGE-albumin (20 mg/kg/day) alone or together with NAC (600 mg/liter of drinking water) for 90 consecutive days. AGE was determined by ELISA; carboxymethyllysine and pyrraline were determined by CG-MS/MS. Biochemical parameters were determined by enzymatic techniques; urinary thiobarbituric acid-reactive substances were determined by spectrophotometry; glucose disappearance constant was determined by the insulin tolerance test; macrophage infiltration and gene expression were determined by immunohistochemistry and real-time quantitative RT-PCR, respectively. Results (mean ± S.E. or median ± range) were compared by one-way analysis of variance or Kruskal-Wallis test. Total AGE, CML, and PYR were 9.2, 7000, and 235 times higher in AGE-albumin as compared with C. Food consumption, body weight, systolic blood pressure, plasma lipids, glucose, hepatic and renal function, and adipose tissue relative weight, area, and volume were similar among groups. TBARS was reduced in AGE + NAC as compared with AGE (1.4×) and C + NAC (1.6×), respectively. AGE albumin reduced insulin sensitivity as compared with C (1.4×); this was prevented by NAC. Immunostaining for F4/80 increased 1.3× in AGE-albumin as compared with C and was reduced in AGE + NAC. CD11b, MRC, Ager, Ddost, Cd36, Nfkb1, Il6, Il10, Tnf, Nos2, and Il12 expressions were unchanged. Slc2a4 and Ppara were increased in AGE + NAC in comparison with C + NAC (Slc2a4, 1.6×; Ppara, 2.2×) and AGE (Slc2a4, 2.3×; Ppara, 3.3×), whereas Itgam and Mrc were decreased in AGE + NAC in comparison with AGE (1.8× and 1.5×, respectively) and C + NAC animals (2× and 1.9×, respectively). Validation of the transcriptome analysis demonstrated that AGE-albumin increased Col12a1 (1.3×) as compared with C. AGE-albumin sensitizes the adipose tissue to inflammation due to macrophage infiltration contributing to insulin resistance. NAC antagonizes AGE-albumin and may be a useful tool in the prevention of AGE action on insulin resistance and long term complications of DM.

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Liver-specific Knockdown of Long Chain Acyl-CoA Synthetase 4 Disturbed Glucose and Phospholipid Metabolic Pathways, Leading to Insulin Resistance in Mice Fed a High-fat Diet

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The liver plays a central role in whole-body lipid metabolism by regulating the uptake, synthesis, oxidation, and export of lipids. Dysfunction of lipid metabolism in liver underlies the development of obesity, diabetes, non-alcoholic fatty liver disorder (NAFLD), and cardiovascular disease. The enzyme family long chain acyl-CoA synthetase (ACSL) is known to play key roles in nearly all pathways of fatty acid metabolism. Within ACSL family members, ACSL4 has unique substrate specificity for arachidonic acid, and hepatic ACSL4 was reported to be abnormally expressed in pathological conditions, such as hepatocarcinoma and NAFLD. However, currently, little is known about the specific roles played by ACSL4 in liver lipid metabolism. In this current study, we utilized an adenoviral shRNA delivery approach to generate liver-specific knockdown of ACSL4. Injection of Ad-shACSL4 in adult mice fed a high fat diet (HFD) substantially reduced liver ACSL4 mRNA and protein expression. Knockdown of hepatic ACSL4 resulted in significant increases in serum glucose and insulin levels. A glucose intolerance test further indicated an insulin-resistant phenotype upon knockdown of ACSL4. Hepatic gene expression analysis revealed that several lipogenic genes (SREBP1c, fatty acid synthetase, and SCD1) were up-regulated in the liver of Ad-shACSL4-infected mice compared with control mice infected with Ad-shLacZ. Utilizing a lipidomic approach, we examined lipid species in liver tissues. Knocking down ACSL4 did not affect hepatic triacylglycerol, diacylglycerol, phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol levels. However, several abundant lyso-PC species (LPC (16:0) and LPC (18:0)) and lyso-PE species (LPE (16:0) and LPE (18:0)) were significantly increased. In addition, ceramide levels were elevated in Ad-shACSL4-infected livers. Ceramide and lyso-PCs have been linked to insulin resistance, and ACSL4 is the key enzyme to generate arachidonoyl-CoAs as the substrate for LPCAT3 to synthesize PC from saturated lyso-PCs and polyunsaturated fatty acids at the *sn*-2 position. Our study results provide the first *in vivo* evidence demonstrating that ACSL4 has an important function in glucose and PL metabolism in liver tissue. Collectively, the increased lipogenesis and disturbed glucose and PL metabolism could be causal factors for the observed insulin resistance in ACSL4-depleted livers of mice under a hyperlipidemic state.

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A Way of LDL Cholesterol Out of Lysosome through Membrane Contacts

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Cholesterol is dynamically transported among organelles and is essential for multiple cellular functions. To explore the mechanism of LDL-derived cholesterol transport, we established an amphotericin B-based assay enabling a genome-wide shRNA screen for delayed LDL cholesterol transport and identified 341 hits with particular enrichment of peroxisome genes, suggesting a previously unappreciated pathway for cholesterol transport. We show dynamic membrane contacts between peroxisome and lysosome, which are mediated by lysosomal synaptotagmin VII binding to the lipid phosphatidylinositol 4,5-bisphosphate on the peroxisomal membrane. LDL cholesterol enhances such contacts, and cholesterol is transported from lysosome to peroxisome. Disruption of critical peroxisome genes leads to cholesterol accumulation in the lysosome. Together, these findings reveal an unexpected role of peroxisome in intracellular cholesterol transport. We further demonstrate massive cholesterol accumulation in human patient cells and a mouse model of peroxisomal disorders, suggesting a contribution of abnormal cholesterol accumulation to these diseases. I will also present our latest progress regarding cholesterol transport.

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Lanosterol Modulates TLR4-mediated Innate Immune Responses in Macrophages

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The cross-talk between innate immune responses and cholesterol homeostasis is instrumental for proper macrophage (MΦ) function. However, the consequences of the regulation of the cholesterol synthetic pathway in classically activated MΦs are not fully understood. Here we show that Toll-like receptor 4 (TLR4) activation of MΦs promotes the accumulation of lanosterol, the first sterol of cholesterol synthesis, due to the HDAC1-mediated transcriptional inhibition of lanosterol-14α-demethylase (Cyp51A1). Pharmacologically mediated accumulation of lanosterol through administration of ketoconazole to mice increases their survival to endotoxemic shock by reducing cytokine secretion. Importantly, similar effects were observed after inducible deletion of Cyp51A1 in MΦs. This effect was associated with the modulation of STAT1 activation. In conclusion, our data show that lanosterol accumulation in MΦs promotes antimicrobial activity and favors anti-inflammatory proresolving response in MΦs. This finding is of critical importance because it identifies, for the first time, lanosterol as an endogenous mediator of innate immune responses of MΦs.

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Regulation of Hepatic LDL Receptor (LDLR) by a Novel Membrane-bound E3 Ubiquitin Ligase Controls Plasma LDL Cholesterol Levels

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Hepatic low-density lipoprotein receptor (LDLR) levels are inversely correlated to, and are the major determinant of, plasma LDL cholesterol (LDL-C) levels. Hepatic expression of LDLR is essential for the clearance of circulating LDL-C from the blood stream. Therapeutic strategies that reduce circulating plasma LDL levels, including statins and, most recently, proprotein convertase subtilisin/kexin 9 (PCSK9) monoclonal antibodies, have shown clear benefits in reducing cardiovascular disease (CVD) and CVD risk. These therapies reduce LDL-C levels by increasing hepatic expression of the LDLR. Here, we identify a novel membrane-bound E3 ubiquitin ligase as an important regulator of plasma LDL-C levels that is also a locus for LDL-C levels in human genome-wide association studies. In mice, silencing with antisense oligonucleotides decreased plasma LDL-C concentrations by 30% and reciprocally regulated hepatic LDLR protein levels. In contrast, overexpression studies resulted in decreased hepatic LDLR and increased LDL-C levels, suggesting that the mechanism of action is through regulation of LDLR. These studies identify a novel regulator of circulating LDL-C levels via direct modulation of hepatic LDLR levels and demonstrate the therapeutic potential to lower LDL-C levels, a major risk factor for cardiovascular disease.

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Post-transcriptional Regulation of Bile Acid Synthesis Mediated by ZFP36L1

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Bile acids are detergents and important signaling molecules that activate the nuclear receptor FXR to control key metabolic processes, including feedback mechanisms to maintain bile acid homeostasis. Activation of FXR decreases the mRNA levels of several bile acid synthetic genes, including the rate-limiting enzyme *Cyp7a1*. We show that *Cyp7a1* mRNA levels are very rapidly reduced following FXR activation, suggesting a post-transcriptional mechanism. We identify the RNA-binding protein *Zfp36l1* as an FXR target gene and show that hepatic overexpression of ZFP36L1 in mice decreases *Cyp7a1* mRNA levels. In contrast, *Zfp36l1*$^{L\text{-}KO}$ mice have increased levels of *Cyp7a1* mRNA and biliary bile acids as well as reduced plasma cholesterol levels. When fed a Western diet, *Zfp36l1*$^{L\text{-}KO}$ mice exhibit reduced adiposity and steatosis as well as reduced lipid absorption, consistent with increased bile acid levels. Thus, we have identified a novel pathway that controls *Cyp7a1* and bile acid metabolism but may also have wider implications in diseases such as obesity and hepatosteatosis.

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Emerging Role of Acylcarnitines in Adaptive Thermogenesis and the Impact on Lipid Metabolism

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Cold-induced thermogenesis is a highly energy-demanding process that protects endotherms against a reduction in ambient temperature. The metabolic fuel that drives thermogenesis in brown fat relies in part on internal and peripheral sources of energy. However, the molecular mechanisms that allow endotherms to adapt to the cold are not well understood. Using non-targeted LC-MS-based lipidomics, we identified plasma acyl-carnitines as the most significantly changed lipid class in response to cold exposure. We found that the liver undergoes a metabolic switch to provide fuel for brown fat thermogenesis by producing acyl-carnitines. Plasma long chain acyl-carnitines (LCAC) are elevated in response to the cold and with β_3-adrenergic receptor activation. In response to the cold, FFAs increase in the plasma, activating the nuclear receptor HNF4α to stimulate Cpt1b and Octn2 expression in the liver, genes that encode for enzymes involved in LCAC synthesis and release, respectively. Conditional deletion of HNF4α in the liver blocks the cold-induced changes in expression of genes involved in LCAC metabolism and lowers circulating LCAC levels, and mice exhibit sensitivity to cold. Inhibiting the rise in free fatty acids in response to a cold stimulus prevents the increase in expression of genes involved in acyl-carnitine metabolism and blocks the rise in plasma acyl-carnitines. In response to the cold, brown adipose tissue increases uptake of LCAC. Furthermore, stable isotope labeling of palmitoyl-carnitine confirms that brown adipocytes are able to utilize LCAC as a fuel source for thermogenesis. Finally, a bolus of L-carnitine, which leads to elevated LCACs, is able to rescue the cold sensitivity of 2-year-old mice. Our data highlight an elegant mechanism where white adipose tissue provides FFAs for hepatic carnitilation to produce plasma LCAC as a fuel source for brown adipose tissue thermogenesis.

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Phospholipid Remodeling Regulates Intestinal Stemness by Controlling Cellular Cholesterol Availability

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Phospholipid remodeling is a critical determinant of membrane composition and function. How membrane phospholipid composition affects tissue stem cell function is unknown. Here we demonstrate that Lpcat3-dependent phospholipid remodeling regulates intestinal stem cell (ISC) proliferation and differentiation. Loss of Lpcat3 in mouse intestine enhances stem cell proliferation *in vivo* and promotes crypt organoid growth *ex vivo*. The basis of this phenotype is a marked induction of cholesterol biosynthesis and cellular cholesterol content in the absence of Lpcat3. Exogenous delivery of polyunsaturated phospholipids, or inhibition of cholesterol synthesis, rescues the effect of Lpcat3 deficiency on organoid growth *ex vivo*. Pharmacologic inhibition of cholesterol synthesis *in vivo* normalizes crypt hyperproliferation in Lpcat3-deficient mice. Conversely, provision of excess cholesterol is sufficient to drive crypt organoid growth in culture, and high cholesterol diet feeding promotes ISC proliferation *in vivo*. Finally, we show that the increased cholesterol biosynthesis in Lpcat3-deficient crypts is mediated by activation of PI3K/AKT pathway in response to phospholipid remodeling. These findings identify membrane phospholipid composition and cellular cholesterol content as important modulators of intestinal stem cell proliferation and differentiation.

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New ANGPTL Protein That Directs Triglyceride Partitioning with ANGPTL3

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Energy is stored primarily in triglycerides (TG), which are partitioned between oxidative tissues and adipose tissue in accordance with nutritional cues. The reciprocal regulation of lipoprotein lipase (LPL) activity in oxidative tissues and white adipose tissue (WAT) directs tissue TG uptake according to nutritional status. Angiopoietin-like protein 3 (ANGPTL3) is a secreted glycoprotein that is made exclusively in the liver and has been shown inhibits LPL activity. By searching for conservative LPL binding domain, we identified another ANGPTL family member, ANGPTL8. ANGPTL8 is expressed in the liver and adipose tissue and circulates in plasma of humans. Expression of ANGPTL8 was reduced by fasting and increased by refeeding in both mice and humans. ANGPTL8 interacts with N terminal domain of ANGPTL3 and potentiates its inhibitory effects on LPL activity. By generating knockout mice, we showed that both ANGPTL3 and ANGPTL8 are required for directing TG to WAT in the fed state. In wild-type mice, feeding increased TG uptake into WAT 8-fold. In contrast, in the WAT of *Angptl3*$^{-/-}$ or *Angptl8*$^{-/-}$ mice, VLDL uptake failed to increase. *Angptl3*$^{-/-}$ mice have a decreased circulating TG level and improved insulin sensitivity, which make them favorable drug targets.

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Apolipoprotein A1 Post-translational Modifications and High-density Lipoprotein Efflux Capacity: Preliminary Data from the Chicago Healthy Aging Study

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HDL efflux capacity has robust associations with coronary heart disease risk. HDL efflux is at least partially mediated by apolipoprotein A1 (apoA1). It is unknown whether inter-individual differences in apoA1 structure are associated with differences in HDL efflux. The objective of this work was to compare apoA1 post-translational modifications (PTMs) between individuals with high and low HDL efflux. We used serum from eight Chicago Healthy Aging Study participants (four with high and four with low HDL efflux). HDL efflux was measured using radiolabeled cholesterol in a validated *ex vivo* tissue culture assay. Serum samples were enriched for apoA1 by anti-apoB immunoprecipitation and methanol-chloroform precipitation before analysis by liquid chromatography mass spectrometry (LC-MS) of intact proteins (*i.e.* no protease). Samples were analyzed via top-down proteomics, which utilizes whole protein mass and fragmentation data to identify and achieve complete chemical characterization of apoA1 (including PTM and polymorphisms). ApoA1 PTM relative intensities by high and low efflux were compared using analysis of variance. Participants' mean $\pm$ S.D. age was 72 $\pm$ 3.8 years, total cholesterol 178 $\pm$ 53 mg/dl, LDL-C 91 $\pm$ 33 mg/dl, triglycerides 131 $\pm$ 69 mg/dl, and HDL-C 61 $\pm$ 38 mg/dl. The normalized efflux was 1.7 $\pm$ 0.05 in the high and 0.65 $\pm$ 0.05) in the low HDL efflux groups. Sixteen different PTMs of apoA1 were observed across the eight participants. Unmodified apoA1 had a higher intensity (-fold difference (fd) = 1.18, $p < 0.001$) in the four participants with high HDL efflux. Three apoA1 PTMs had significantly higher normalized intensities in high HDL efflux participants: palmitic acid addition (fd = 2.17, $p < 0.0001$), oleic acid addition (fd = 2.08, $p < 0.0001$), and arachidonic acid addition (fd = 2.32, $p < 0.001$). These preliminary data suggest that palmitic acid, oleic acid, and arachidonic acid apoA1 PTMs may be important markers and/or mediators of HDL efflux in humans.

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Antisense Oligonucleotide-mediated Knockdown of MPZL3 Protects from the Metabolic Consequences of a High-fat, Energy-dense Diet in Mice

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The development of pharmacotherapies for long term weight loss has been hindered by lack of knowledge regarding body-weight regulatory mechanisms. Because global obesity rates continue to rise, there is an unmet need to identify novel pathways that regulate energy homeostasis. We recently have demonstrated that mice deficient in the gene encoding myelin protein zero-like 3 (MPZL3) have reduced body weight and fat mass, increased energy expenditure, and reduced hepatic lipid synthesis (Czyzyk *et al.* (2013) *AJPEM* **305,** E282–E292). The goals of the current study were to determine whether acute and peripherally restricted knockdown of MPZL3 gene expression could 1) recapitulate the lean and hypermetabolic phenotypes observed in mice with global MPZL3 knockout and 2) ameliorate the negative metabolic effects of long term exposure to a high-fat diet. Six-week-old male C57BL/6N mice were placed on a high-fat, energy-dense (HED) diet for 7 weeks. The mice were then treated for 8 weeks systemically (i.p., twice weekly) with either an antisense oligonucleotide that targeted MPZL3 (MPZL3 ASO) or a scrambled control (n = 8/group). Treatment with MPZL3 ASO significantly reduced MPZL3 expression ≥80%, as quantified by RT-PCR, in liver, skeletal muscle, and brown and white adipose tissue, but not in hypothalamic brain tissue. Significant decreases in body weight ($p < 0.05$), fat mass ($p < 0.01$), and circulating lipid levels ($p < 0.001$) resulted from treatment with MPZL3 ASO in as little as 3 weeks without reducing lean mass or food intake. These reductions were significantly greater after 8 weeks. Expression of several genes encoding lipogenic enzymes was found to be significantly down-regulated in both liver and white adipose tissues. The metabolic rate was measured using indirect calorimetry methods. Although there was no change in oxygen consumption levels, an increase in fat oxidation was observed, as indicated by a significant decrease in respiratory exchange ratios with MPZL3 ASO treatment ($p < 0.001$). These data demonstrate that the resistance to the negative metabolic effects of an HED results from MPZL3 knockdown and is independent of the compensatory changes associated with a global knockout approach in mice. Reduction of MPZL3 could be a potential therapeutic approach for the treatment of obesity and associated lipidemia.

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The Role of Abhydrolase Domain-containing 15 (Abhd15) in Insulin-regulated Lipolysis in White Adipocytes

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Adipose tissue (AT) is a multifunctional organ that plays an important role in lipid and glucose homeostasis. Dysfunction of lipid and glucose metabolism in AT leads to insulin resistance and contributes to the development of obesity and type II diabetes. In 3T3L1 adipocytes, ABHD15 has been identified as a phosphorylation substrate of Akt/PKB in insulin signaling. Moreover, it interacts with phosphodiesterase 3B (PDE3B) by protein-protein interaction. PDE3B itself plays an important role in insulin-mediated suppression of lipolysis in adipocytes. Thereby, we hypothesize that ABHD15 plays a role in insulin-regulated lipolysis in AT. ABHD15 is highly expressed in white adipose tissue (WAT) and to a lower extent in liver. In mice, fasting strongly decreased ABHD15 expression, whereas refeeding increased its expression in WAT. We generated whole body ABHD15-knockout (KO) mice and characterized them with regard to lipid metabolism. Interestingly, after an insulin bolus, ABHD15-KO mice did not suppress lipolysis because they showed higher plasma free fatty acid (FFA) levels in comparison with WT mice. Accordingly, the phosphorylation of Akt and the dephosphorylation of protein kinase A (PKA) and HSL by insulin were strongly impaired in ABHD15-KO mice. Increased FFA release often leads to insulin resistance. Young ABHD15-KO mice showed similar insulin sensitivity as WT mice. However, ABHD15-KO mice became insulin-resistant upon aging. Further, ABHD15-KO mice showed decreased postprandial plasma adiponectin levels on a chow or high glucose diet (HGD) at a young age. In addition, HGD-challenged ABHD15-KO mice had higher postprandial plasma insulin and glucose levels. Consistently, insulin-suppressed FFA release from WAT was impaired in HGD-fed ABHD15-KO mice. We explored the mechanism behind this phenotype and found that ABHD15 decreased the activity and expression of its interaction partner PDE3B *in vitro*. Interestingly, PDE3B expression was substantially decreased in WAT of ABHD15-KO mice. In WAT, ABHD15 is required for insulin-mediated suppression of lipolysis. The mechanism is still under investigation; however, our data reveal that ABHD15 binds PDE3B and regulates its expression *in vitro* and that ablation of ABHD15 *in vivo* strongly decreases PDE3B mRNA and protein expression and might thereby decrease insulin-mediated suppression of lipolysis by increasing pHSL and pPKA levels.

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Disabled-2 Determines Commitment of a Preadipocyte Population

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Disabled-2 (Dab2) is a widely expressed clathrin-binding endocytic adaptor protein and is known for the endocytosis of the low-density lipoprotein (LDL) family receptors. Dab2 also modulates endosomal Ras/MAPK (Erk1/2) activity by regulating the disassembly of Grb2-Sos1 complexes associated with clathrin-coated vesicles. We found that the most prominent phenotype of Dab2 knockout mice was their striking lean body composition under a high fat and high caloric diet, although the weight of the mutant mice was indistinguishable from wild-type littermates on a regular chow. The remarkable difference in resistance to high caloric diet-induced weight gain of the *dab2*-deleted mice was presented only in juvenile and not in mature mice. Investigation using Dab2-deficient embryonic fibroblasts and mesenchymal stromal cells indicated that Dab2 promoted adipogenic differentiation by modulation of MAPK (Erk1/2) activity, which otherwise suppresses adipogenesis through the phosphorylation of PPARγ. The results suggest that Dab2 is required for the excessive calorie-induced differentiation of an adipocyte progenitor cell population that is present in juvenile but depleted in mature animals. The finding provides evidence for a limited preadipocyte population in juvenile mammals and the requirement of Dab2 in the regulation of Ras/MAPK signal in the commitment of the precursor cells to adipose tissues.

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Investigation of the Role of ANGPTL3 in Regulating the Plasma Level of Low-density Lipoprotein

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ANGPTL3 has emerged as a key regulator of lipoprotein metabolism in humans. Loss-of-function mutations in both ANGPTL3 alleles cause familial combined hypolipidemia characterized by low plasma levels of triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). Whereas known effects of ANGPTL3 in inhibiting lipoprotein lipase and endothelial lipase contribute to the low TG and HDL-C, respectively, the basis of the low LDL-C remains unclear. Here we show that RNAi-mediated ANGPTL3 silencing in mouse liver resulted in very low TG, HDL-C, and LDL-C, similar to the human phenotype. The effect was observed in wild-type and obese mice, whereas in hCETP-apolipoprotein B-100 (apoB-100) double transgenic mice, the silencing decreased LDL-C and TG, but not HDL-C. In a humanized mice model (Apobec1(–/–) carrying the human apoB-100 transgene) deficient in LDL receptor (LDLR), ANGPTL3 silencing had a modest effect on LDL-C, suggesting that the effect was linked to LDLR. This observation is supported by an additive effect on LDL-C between ANGPTL3 and PCSK9 siRNAs. Both ANGPTL3 silencing and gene deletion using the CRISPR/Cas9 genome editing system in human hepatocytes validate that ANGPTL3 deficiency reduced nascent apoB-100 secretion and increased LDL uptake. Thus, our study implies that reduced secretion and increased uptake of apoB-containing lipoproteins may contribute to the low LDL-C observed in mice and humans with genetic ANGPTL3 deficiency.

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Oxygen-dependent Regulation of Fatty Acid Metabolism Mediates Adipocyte Function

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Obesity is associated with pathological alterations in adipose tissue, including the suppression of endogenous synthesis of fatty acids (*de novo* lipogenesis (DNL)). Decreased DNL in adipose tissue is associated with insulin resistance and adverse systemic metabolic effects. Interestingly, our group and others have shown that increased adipose tissue DNL confers beneficial systemic metabolic effects in mouse models of obesity and diabetes, mediated in part by the actions of the DNL product monounsaturated fatty acid palmitoleate. However, the mechanisms of decreased DNL in obese adipose tissue have not been well characterized. Here, we show that hypoxia is a key factor regulating the level of the key DNL enzyme stearoyl-CoA desaturase-1 (SCD1) in adipocytes, and its product palmitoleate protects adipocytes against hypoxia-induced endoplasmic reticulum stress. Furthermore, we investigated the potential mediators of oxygen-dependent DNL regulation in adipocytes and show that oxygen sensor HIF prolyl-4-hydroxylase 3 (PHD3) is a key regulator of SCD1 protein level and hypoxia-induced endoplasmic reticulum dysfunction in adipocytes. Altogether, this study highlights the importance of SCD1 and PHD3 as potential mediators of obesity-induced pathologies in adipocytes and underscores the role of hypoxia in driving adipose tissue dysfunction.

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Analyzing the Full Spectrum of Genomic Variation with Lp(a) Cholesterol: Novel Insights from Deep, Whole Genome Sequence Data in 5,192 Europeans and African Americans

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Lipoprotein(a) (Lp(a)) is a heritable, independent risk factor for coronary heart disease; however, the factors influencing Lp(a) are poorly understood. Deep (30×) whole genome sequencing (WGS) permits an assessment of the full spectrum of genomic variation contributing to Lp(a) variation. Here, we associate variation in 30× WGS data from 2,255 Estonians from the Estonian Biobank and 2,937 African Americans from the Jackson Heart Study (JHS), with Lp(a) cholesterol (Lp(a)-C). We replicate prior single variant associations at the LPA locus (p = 1.2e−74) and observe two novel loci: SORT1 (p = 4.7e−23) and CETP (p = 3.0e−9). Adjustment for HDL cholesterol mitigates the association at the CETP locus (p = 0.1); however, the Lp(a)-C association persists at SORT1 despite adjusting for LDL cholesterol (p = 3.4e−18) or apoB (p = 1.0e−17). Copy number variants (KIV2-CN), as ascertained by quantitative PCR, have been previously associated with Lp(a) levels. We now directly genotype KIV2-CN (mean = 40; S.D. = 7) from WGS. We observe a strong negative association between Lp(a)-C and KIV2-CN in JHS (−0.05 S.D./CN, p = 9.3e−84) and Estonia (−0.05 S.D./CN, p = 1.5e−70). Furthermore, we find that 69.6% (JHS) and 89.6% (EST) of individuals with high Lp(a)-C (top 5th percentile) have a low KIV2-CN count (bottom 25th percentile) or a high LPA genetic risk score (top 25th percentile). Stepwise conditional analysis at LPA identifies four (Estonia) and three (JHS) SNPs separately associated with Lp(a)-C, independent of KIV2-CN count. Separately, via interaction analysis, we discovered three genome-wide significant SNPs influencing the effect of KIV2-CN count on Lp(a)-C levels in both cohorts.

An aggregate of rare (minor allele frequency <1%), nonsynonymous variants in LPA (p = 4.9e−4) and SORT1 (p = 0.02) showed evidence of association with Lp(a)-C, independent of common LPA variants and KIV2-CN. Additionally, an aggregate of rare, non-coding liver enhancer variants near the LPA transcription start site was associated with Lp(a)-C (p = 1.8e−5). In conclusion, deep whole genome sequencing provides new insights on the full spectrum of genomic variation in Lp(a)-C through single variant association analysis across the genome, direct KIV2-CN estimation from the sequence itself, detailed fine mapping, interaction analyses, and rare variant burden analyses in coding and non-coding regions.

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A Haploid Mammalian Genetic Screen Identifies Key Determinants of Metabolically Regulated ERAD of HMGCR and Cholesterol Biosynthesis

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The cellular demand for cholesterol requires tight control of its biosynthesis by the mevalonate pathway. Regulation of HMGCR, a rate-limiting enzyme in this pathway and the target of the statin class of cholesterol-lowering drugs, is a key control point herein. HMGCR is subject to transcriptional and post-transcriptional regulation. However, our understanding of the factors involved in metabolically regulated degradation of HMGCR is far from complete. To identify genes required for this process, we used CRISPR/Cas9 to engineer mammalian haploid cells with the fluorescent protein mNeon fused in-frame to the endogenous HMGCR. We used Hap1-HMGCR-mNeon cells in a haploid genetic screen and identified positive and negative determinants of metabolically regulated HMGCR degradation. Among these, we characterized the involvement of an ERAD component, previously not implicated in regulation of HMGCR and cholesterol biosynthesis, and provide mechanistic insight into its involvement in this process. Our study also highlights the feasibility of applying mammalian haploid genetic screens as a tool to study complex metabolic traits.

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Lipin-2 and Lipin-3 Coordinate the Compartmentalization of Lipid Droplets and ApoB48 Lipidation in Enterocytes

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The small intestine absorbs more than 95% of dietary triglycerides (TGs), which are hydrolyzed to monoacylglycerols and free fatty acids in the intestinal lumen. Enterocytes rapidly synthesize TGs using primarily monoacylglycerol acyltransferase rather than enzymes of the glycerol 3-phosphate pathway, raising the question of why the latter enzymes are present in the intestine. The resulting TGs may be incorporated into chylomicron particles or stored as lipid droplets (LDs), which may occur in distinct subpopulations in the cytosol, connected to the ER membrane, or within the ER lumen for the massive lipidation of apoB48 during chylomicron assembly. The molecular mechanisms underlying the partitioning of LDs to different compartments and the related role in apoB48 lipidation during postprandial chylomicron assembly are poorly understood. We identified the phosphatidic acid phosphatase enzymes lipin-2 and lipin-3 (components of the glycerol 3-phosphate pathway) as critical for the coordination of LD compartmentalization and apoB48 lipidation during chylomicron assembly. Lipin-2 and -3 influence the levels of CTP:phosphocholine cytidylyltransferase α and membrane phosphatidylcholine (PC) homeostasis, which may influence LD connections with the ER membrane and LD entry into the ER lumen. Lipin-2 and -3 deficiency in the mouse raises membrane PC levels and destroys LD-ER connections, preventing the appearance of lumenal LDs. This leads to impaired apoB48 lipidation, blocking chylomicron maturation and secretion. Our findings reveal a role for the lipin-2 and -3 enzymes of the glycerol 3-phosphate pathway in membrane phospholipid homeostasis in the enterocyte, which is required for apoB48 lipidation and chylomicron assembly.

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Defining the Cross-talk between Cholesterol Homeostasis and Type I Interferon Signaling

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Perturbations in host lipid homeostasis are observed in response to both viral and microbial infections. Recent studies indicate that reprogramming lipid metabolism in immune cells is a fundamental and key component of generating productive inflammatory and immunity responses. However, the molecular mechanisms underlying the cross-talk between lipid metabolic reprogramming and host defense are still poorly understood. Our previous work identified an unanticipated circuit, where cholesterol biosynthesis and type I interferon (IFN) signaling reciprocally regulate each other. Mechanistic studies indicate that limiting cholesterol biosynthetic flux in macrophages spontaneously up-regulates type I IFN production and IFN-mediated inflammation, resulting in heightened immunity. Mechanistic studies revealed that induction of type I IFN-mediated inflammation dependent on the endoplasmic reticulum (ER) membrane-associated protein STING (stimulator of interferon genes). Our work expands on these observations and tests the hypothesis that cholesterol, in the ER membrane, regulates STING activity via direct binding. We will also test the hypothesis that type I IFNs redistribute subcellular cholesterol distribution to facilitate type I IFN-mediated immune responses and entrain specific forms of host defense. Current work is focused on developing model systems and techniques to test these hypotheses. It is our expectation that these studies will mechanistically advance our understanding of how lipid reprogramming and inflammation are intertwined.

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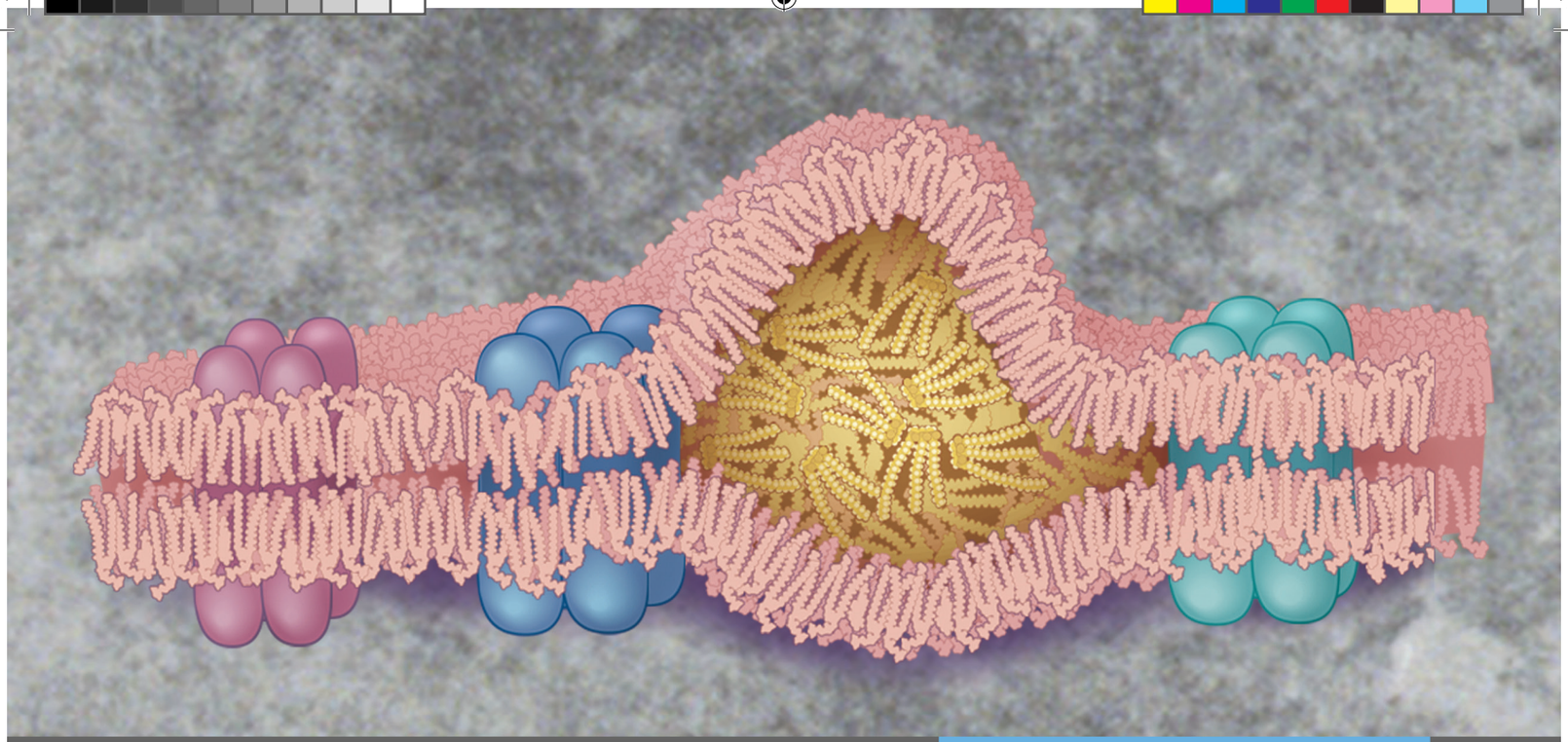

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