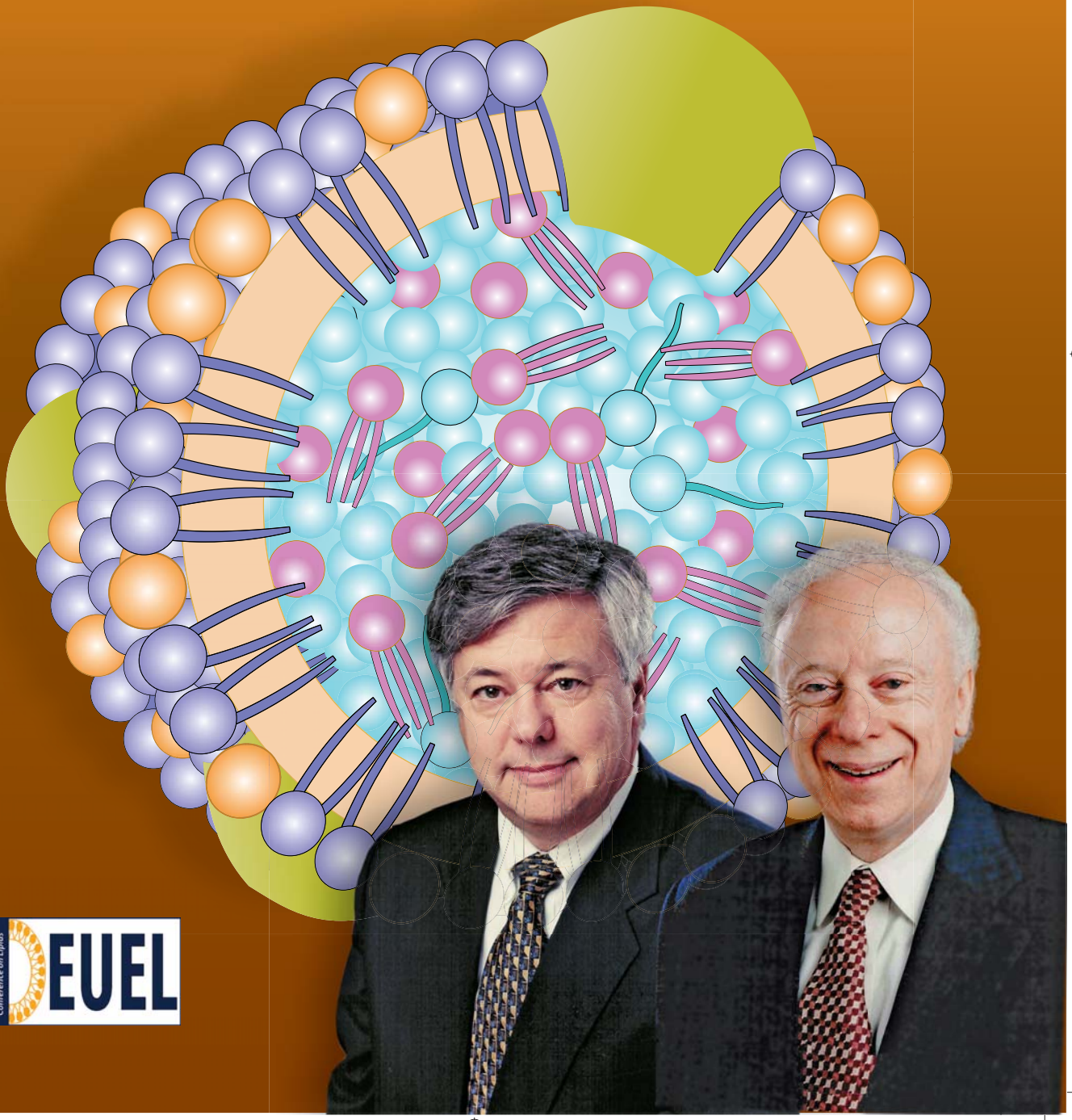
DEUEL CONFERENCE ON LIPIDS

March 3 – 6, 2015
Monterey, CA



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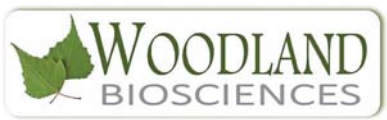
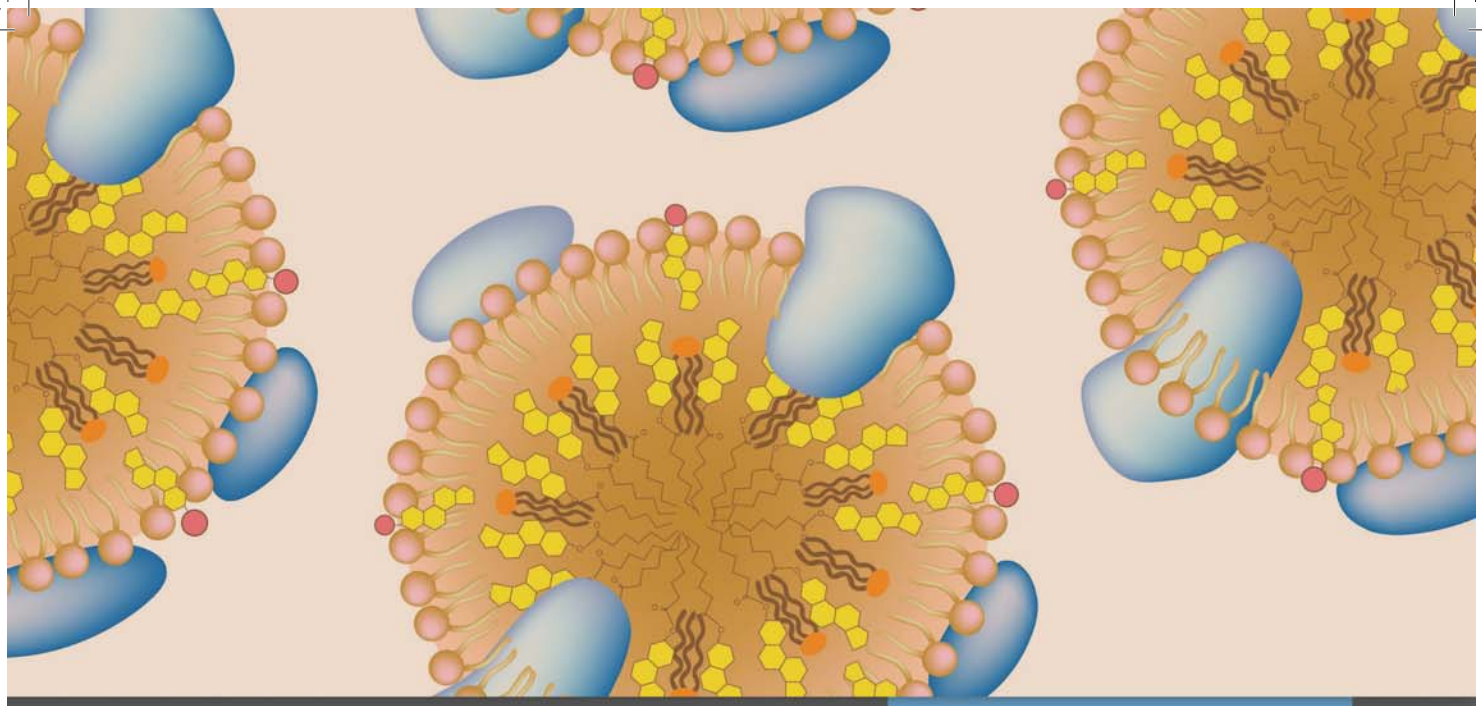


Table of Contents

2015 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.



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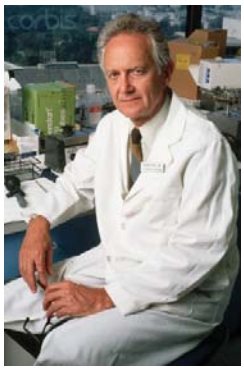
THE HAVEL LECTURE



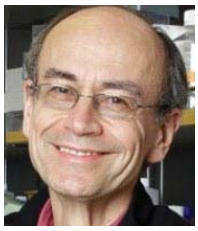
The Havel Lecture was named after Richard J. Havel because he has done more than anyone else to keep the Conference vibrant. For many years, he organized the meeting, and each year he has energized the scientific discussions.

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

2015 AWARD LECTURE



THOMAS SUDHOF, STANFORD UNIVERSITY

"Brown & Goldstein-inspired science off field:
lipid membrane fusion at the synapse"

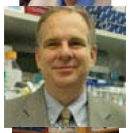
2014 
Rudolf Zechner, University of Graz
"Lipolysis - more than just the breakdown of fat"

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
"Nuclear receptor control of lipid metabolism"

2009 
Stephen G. Young, University of California, Los Angeles
"Adventures in lipid metabolism"

2008 
Helen H. Hobbs, University of Texas Southwestern
"Going to extremes to identify genetic variations contributing to cardiovascular risk"

2007 
Ronald Evans, Stalk Institute
"PPARdelta and the marathon mouse: running around physiology"

2006 
David Russell, University of Texas Southwestern
"The enzymes of cholesterol breakdown"

2005 
Johann Deisenhofer, HHMI/University of Texas Southwestern
"Structure of the LDL receptor"

2004 
Jeffrey M. Friedman, Rockefeller University
"Oxysterol regulation of macrophage gene expression"

2003 
Bruce Spiegelman, Harvard Medical School
"Transcriptional control of energy and glucose metabolism"

2002 
Michael S. Brown and Joseph L. Goldstein, University of Texas Southwestern
"SREBPs: Master regulators of lipid metabolism"

Schedule of Events

	Tuesday, March 3		Wednesday, March 4	Thursday, March 5		Friday, March 6
7AM			Breakfast 7-8:30	Breakfast 7-8:45	Board Meeting 7-8:45	Breakfast 7-8:45
8AM			Session 1 8:30-10:10			
9AM				Session 3 8:45-10:10		Session 5 8:45-12:00
10AM			Coffee Break 10:10 – 10:30	Coffee Break 10:10 – 10:30		
11AM			Session 1, Cont. 10:30 – 11:50	Session 3, Cont. 10:30-11:50		
12PM			12-1:30 Lunch	12-1:30 Lunch		
1PM			Session 2 1:30-3:10	Session 4 1:30-2:50		
2PM				Break 2:50-3:10		
3PM	Registration 3-5:30		Break 3:10-3:30	Session 4, Cont. 3:10-5:10		
4PM			Session 2, Cont. 3:30-5:10			
5PM		Welcome Dinner and Reception 5-7:00	Free Time	Free Time		
6PM			Wine Reception and Trainees' Poster Session #2	Reception and Dinner 6:30-9:00		
7PM	7-8:00 The Havel Lecture					
8PM	Trainees' Poster Session #1					
9PM						

Meeting Program

The Deuel Conference on Lipids, March 3–6, 2015

The Clement Monterey Hotel, Monterey, California

"CELEBRATING THE SCIENTIFIC PARTNERSHIP OF MIKE BROWN AND JOE GOLDSTEIN"

Co-Chairs: Tim Osborne (Sanford/Burnham MRI) and Helen Hobbs (UT Southwestern)

Tuesday, March 3

3:00 – 5:30 pm Meeting Registration

5:00 – 7:00 pm Welcome Reception and Dinner

7:00 – 8:00pm The Havel Lecture
"Brown & Goldstein-Inspired Science off Field: Lipid Membrane Fusion at the Synapse"
Thomas Südhof, Stanford University

Trainees' Poster Session #1

Wednesday, March 4

Wednesday, March 4, 8:30 AM – 11:50 PM

Session Chair: Helen H. Hobbs, HHMI-University of Texas Southwestern

Session 1 Genetics and Genomics

8:30 – 9:10 **"CRISPR RNA-guided Surveillance Complexes"**
Jennifer Doudna, HHMI-University of California, Berkeley

9:10 – 9:50 **"Gene Regulation and Disease: From Maps to Mechanisms"**
John Stamatoyannopoulos, University of Washington

9:50 – 10:10 **"Identification of miR-148a as a Novel Regulator of Cholesterol Metabolism"**
Leigh Goedeke, Yale & New York University

10:10 - 10:30 Coffee Break

10:30 - 11:10 **"Mitochondrial Parts, Pathways, and Pathogenesis"**
Vamsi Mootha, HHMI-Harvard University

11:10 - 11:50 **"Kidney Disease in African Americans"**
Martin Pollak, Harvard University

Wednesday, March 4, 1:30 – 5:10 PM

Session Chair: Tim Osborne, Sanford Burnham

Session 2 Energy Homeostasis and Adipose Tissue

1:30 – 2:10 **"Neural Circuits Regulating Food Intake and Metabolism"**
Jeffrey Friedman, HHMI-Rockefeller University

2:10 – 2:50 **"Brown and Beige Fat: Basic Biology and a Novel Therapeutic Pathway"**
Bruce Spiegelman, Harvard University

2:50 – 3:10 **"Reduced PPARγ-driven Lipogenisis Protects ATGL-deficient Mice from Diet-induced Obesity"**
Renate Schreiber, University of Graz

3:10 – 3:30 Break

3:30 – 4:10 **"X Marks the Spot: Sex Differences in Obesity"**
Karen Reue, University of California, Los Angeles

4:10 – 4:50 **"Fuel, Fire, and FGF21"**
David Mangelsdorf, HHMI-University of Texas Southwestern

4:50 – 5:10 The eLife Lecture
"Lpcat3-dependent Production of Arachidonyl Phospholipids Is a Key Determinant of Triglyeride-rich Lipoprotien Production"
Xin Rong, HHMI-University of California, Los Angeles

Wine Reception and Trainees' Poster Session #2

Thursday, March 5

Thursday, March 5, 8:45 AM to 11:50 PM

Session Chair: Eric Olson, University of Texas Southwestern

Session 3 Myocyte and Myocardial Development and Metabolism

8:45 – 9:10 **"Mechanisms of Muscle Development, Disease and Regeneration"**
Eric Olson, University of Texas Southwestern

9:10 – 9:50 **"Drilling Down a la B&G: Probing Transcription by Single Molecule Imaging"**
Robert Tjian, HHMI-University of California, Berkeley

9:50 – 10:10 **"ApoC-III Inhibition Increases Hepatic Uptake of Tryglyceride-rich Lipoprotiens via Members of the Low-density Lipoprotien Receptor Family"**
Jeffery D. Esko, University of California, San Diego

10:10 – 10:30 Coffee Break

10:30 – 11:10 **"TGFbeta in the Pathogenesis of Disease: A Matter of Aneurysmic Proportions"**
Hal Dietz, HHMI-Johns Hopkins University

11:10 – 11:50 **"Genes, Genomes and the Future of Medicine"**
Richard Lifton, HHMI-Yale University

Thursday, March 5, 1:30-5:10 PM

Session Chair: Monty Krieger, Massachusetts Institute of Technology

Session 4 Receptor Biology and Protein Trafficking

1:30 – 2:10 **"The Phase of Fat: The Cell Biology of Lipid Droplets"**
Tobias Walther, Yale University

2:10 – 2:50 The Journal of Lipid Research Lecture – Introduction: Edward Dennis, University of California, San Diego
"G Protein Coupled Receptor (GPCRs) and Arrestin Coupled Receptors (ACRs): A Tale of Two Transducers"
Robert Lefkowitz, HHMI-Duke University

2:50 –3:10 Break

3:10 – 3:50 **"Protein and RNA Sorting in the Secretory Pathway"**
Randy Schekman, HHMI-University of California, Berkeley

3:50 – 4:10 **“Receptor-medicated ER Exit: Two Tails Are Better than One?”**
Xiao-Wei Chen, IMM & PKU-THU Center for Life Sciences

4:10 – 4:50 **“Membrane disruption, a unified theme for apoptotic and necrotic cell death"**
Xiaodong Wang, National Institute of Biological Sciences

4:50 – 5:10 **“Characterization of the Effects of a Novel DGAT2 Inhibitor on Hepatic Lipid Metabolism”**
Julie J. Purkal, Cardiovascular and Metabolic Diseases Research Unit

6:30 – 9:00 **Reception/Dinner at Monterey Bay Aquarium**
Host for the Roast: Robert Lefkowitz

Friday, March 6

Friday, March 6, 8:45 AM to 12:00 Noon

Session Chair: Jay Horton

Session 5 From Bench to Bedside and Back Again

8:45 – 9:25 **"Immune and Autoimmune Responses to Cytosolic DNA"**
James Chen, HHMI-University of Texas Southwestern

9:25 – 11:00 **“SCAP: Centerpiece for Cholesterol Control”**
Michael Brown, University of Texas Southwestern
Joseph Goldstein, University of Texas Southwestern

Poster Presentations

1

The miR-199/DNM Regulatory Axis Controls Receptor-mediated Endocytosis

Juan Aranda[1], Alberto Canfrán-Duque[1], Leigh Goedeke[1], Yajaira Suárez[1], Carlos Fernández-Hernando[1]

[1]Yale University School of Medicine, New Haven, CT

Small non-coding RNAs (microRNAs) are important regulators of gene expression that modulate many physiological processes; however, their role in regulating intracellular transport remains largely unknown. Intriguingly, we found that the dynamin (DNM) genes, a GTPase family of proteins responsible for endocytosis in eukaryotic cells, encode the conserved miR-199a/b family of miRNAs within their intronic sequences. Here, we demonstrate that miR-199a/b regulates endocytic transport by controlling the expression of important mediators of endocytosis, such as clathrin heavy chain (CLTC), Rab5A, low-density lipoprotein receptor (LDLR), and caveolin-1 (Cav-1). Importantly, miR-199a/b-5p overexpression markedly inhibits CLTC, Rab5A, LDLR, and Cav-1 expression, thus preventing receptor-mediated endocytosis in human cell lines (Huh7 and HeLa). Of note, miR-199a-5p inhibition increases target gene expression and receptor-mediated endocytosis. Altogether, our work identifies a novel mechanism by which miRNAs regulate intracellular trafficking. In particular, we demonstrate that the DNM/miR-199a/b-5p genes act as a bifunctional locus that regulates endocytosis, thus adding an unexpected layer of complexity in the regulation of intracellular trafficking.

2

Identification of miR-148a as a Novel Regulator of Cholesterol Metabolism

Leigh Goedeke[1,2], Juan F. Aranda[1,2], Alberto Canfrán-Duque[1], Noemi Rotllan[1], Cristina M. Ramírez[1], Chin-Sheng Lin[2], Elisa Araldi[1,2], Norma N. Anderson[3], Miguel A. Lasunción[4], Yajaira Suárez[1], Carlos Fernández-Hernando[1]

[1]Yale University School of Medicine, New Haven, CT; [2]New York University School of Medicine, New York, NY; [3]University of Texas Southwestern Medical Center, Dallas, TX; [4]Hospital Ramón y Cajal, Madrid, Spain

The hepatic low-density lipoprotein receptor (LDLR) pathway is essential for clearing circulating LDL cholesterol (LDL-C). Although the transcriptional regulation of LDLR is well-characterized, the post-transcriptional mechanisms that govern LDLR expression are just beginning to emerge. Here, we developed a high-throughput genome-wide screening assay to systematically identify microRNAs (miRNAs) that regulate LDLR activity in human hepatic cells. From this screen, we characterize miR-148a as a negative regulator of LDLR expression and activity and define a novel LXR/SREBP1-mediated pathway by which miR-148a regulates LDL-C uptake. Importantly, inhibition of miR-148a increases hepatic LDLR expression and decreases plasma LDL-C *in vivo*. We also provide evidence that miR-148a regulates hepatic ABCA1 expression and circulating high-density lipoprotein cholesterol (HDL-C) levels. Collectively, these studies uncover miR-148a as an important regulator of hepatic LDL-C clearance through direct regulation of LDLR expression and demonstrate the therapeutic potential of inhibiting miR-148a to ameliorate the elevated LDL-C/HDL-C ratio, a prominent risk factor for cardiovascular disease.

3

Innate Immune Response Regulates Cholesterol Metabolism

Elisa Araldi[1,2], Alberto Canfran-Duque[1], Marta Fernandez-Fuertes[1], Aranzazu Chamorro-Jorganes[1], Julio Madrigal-Matute[2], Miguel Angel Lasuncion[3], Carlos Fernandez-Hernando[1], Yajaira Suarez[1]

[1]Yale University School of Medicine, New Haven, CT; [2]New York University School of Medicine, New York, NY; [3]Hospital Ramon y Cajal and Universidad de Alcala de Henáres, Alcala de Henáres, Spain

Cholesterol is an essential molecule for physiological processes, like bile acid or steroid hormone production, cell membrane fluidity, and proliferation. However, elevated levels of cholesterol are also linked to life-threatening diseases like the metabolic syndrome, cardiovascular diseases, and atherosclerosis. During the atherosclerotic process, cholesterol present in low-density lipoproteins (LDL) penetrates the intimal layer of the arterial wall. Here, LDL particles are oxidized (oxLDL) and promoting inflammation by recruiting and activating immune cells. Monocyte-derived macrophages ingest oxLDL to become the lipid-rich foam cell, which is a hallmark feature of atherosclerosis and leads to lesion expansion and chronic inflammation. Although the detrimental role of cholesterol during the inflammatory process is well established, it is still unclear how inflammation in turn may affect cholesterol homeostasis. In order to gain a better understanding of this process, we performed whole genome expression analysis of classically activated (LPS/IFN-γ-treated) mouse bone marrow-derived macrophages. Interestingly, we found that genes involved in cholesterol synthesis and homeostasis were differentially regulated upon stimulation with LPS and IFN-γ. In particular, dehydrocholesterol-24-reductase (Dhcr24) and lanosterol 14-demethylase (Cyp51) were significantly down-regulated upon LPS and IFN-γ stimulation. Importantly, similar results were observed in human peripheral blood monocyte-derived macrophages. Cyp51 demethylates lanosterol into the following sterol intermediates, whereas Dhcr24 is the enzyme that catalyzes the conversion of desmosterol to cholesterol in the last steps of cholesterol biosynthesis. A block in those enzymes inhibits cholesterol synthesis and results in the accumulation of lanosterol and desmosterol, respectively. Consistently, reduced levels of Cyp51 and Dhcr24 in LPS/IFN-γ-activated macrophages lead to the accumulation of both lanosterol and desmosterol. Interestingly, accumulation of these intermediates counter-regulates inflammation in macrophages *in vitro* by decreasing cytokine secretion, reactive oxygen species production, and phagocytosis. Additionally, in a mouse model of lipopolysaccharide-induced endotoxemic shock, pharmacological accumulation of lanosterol (through ketoconazole-induced inhibition of Cyp51) or desmosterol (using triparanol to block Dhcr24) results in increased survival and decreased inflammation compared with control animals. In conclusion, inflammation negatively regulates cholesterol synthesis by decreasing the activity of key cholesterol synthesis genes. In turn, accumulation of cholesterol synthesis intermediates negatively regulates inflammation.

4

Mechanism of Intestinal Cholesterol Absorption and Intracellular Cholesterol Transport

Bao-Liang Song[1,2]

[1]College of Life Sciences, Wuhan University, Wuhan, China; [2]Shanghai Institutes for Biological Sciences, Shanghai, China

Niemann-Pick C1-like 1 (NPC1L1) is a polytopic transmembrane protein responsible for intestinal cholesterol absorption. We have shown that NPC1L1 mediates cholesterol uptake via vesicular endocytosis. The mechanism of sterol-stimulated NPC1L1 internalization is still mysterious. Recently, we identified an endocytic peptide signal, YVN*XX*F (where *X* represents any amino acid), in the cytoplasmic C-terminal tail of NPC1L1. Cholesterol binding on the N-terminal domain of NPC1L1 released the YVN*XX*F-containing region of NPC1L1 from association with the plasma membrane and enabled Numb binding. We also found that Numb, a clathrin adaptor, specifically recognized this motif and recruited clathrin for internalization. Disrupting the NPC1L1-Numb interaction decreased cholesterol uptake. Ablation of Numb in mouse intestine significantly reduced dietary cholesterol absorption and plasma cholesterol level. Together, these data show that Numb is a pivotal protein for intestinal cholesterol absorption and may provide a therapeutic target for hypercholesterolemia. Other progress regarding intracellular cholesterol transport in this laboratory will be discussed as well.

5

The Role of Seipin in Lipid Droplet Expansion and Adipogenesis

Hongyuan Yang[1]

[1]School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, Australia

Obesity is characterized by accumulation of adipocytes loaded with lipid droplets (LDs). By genetic screening in yeast, we have identified a large number of gene products that regulate the size and number of LDs. In particular, we demonstrate that deletion of a previously uncharacterized gene, *FLD1*, results in the formation of "supersized" LDs (>50 times the volume of normal ones). Interestingly, null mutations of Seipin (the human orthologue of Fld1p), are associated with human Berardinelli-Seip congenital lipodystrophy 2 (BSCL2). We use mouse and fly models to confirm an essential role of Seipin in adipogenesis. Therefore, Seipin regulates two important aspects of lipid storage: adipocyte differentiation (systemic lipid storage) and lipid droplet formation (cellular lipid storage). Our recent results suggest that Seipin functions in the metabolism of phospholipids and that Seipin deficiency causes accumulation of certain lipid species, such as phosphatidic acid. These accumulated lipids may interfere with PPARγ function during adipocyte differentiation, causing severe lipodystrophy. These lipid species may also cause morphological changes of LDs (*e.g.* the formation of "supersized" LDs) in other cell types.

6

Lipid Droplet Proteins and Metabolic Diseases

Pingsheng Liu[1]

[1]Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Lipid droplets (LDs) are a cellular organelle that consists of a neutral lipid core, a monolayer phospholipid membrane, and proteins. Accumulated studies suggest that LDs are involved in the synthesis, storage, transportation, modification, and hydrolysis of lipids as well as in protein storage and degradation. The excessive storage of lipids, especially ectopic lipid accumulation, is a major risk factor for metabolic syndromes. Therefore, LD research plays an important role in understanding metabolic diseases. To study how ectopic lipid storage mediates metabolic syndromes, LDs from tissues other than white adipose, including muscle, liver, heart, adrenal gland, and brown adipose tissue, were isolated and subjected to proteomic studies. Among the recent findings, the liver LD enzyme 17βHSD13 was found to be highly expressed in non-alcohol-induced fatty liver relative to healthy liver. This enzyme is also highly overexpressed in liver LDs of diabetic db/db mice as well as high fat diet-induced diabetic mice. Furthermore, experimental overexpression of 17βHSD13 stimulates fatty liver formation in mice. Overexpression also results in increased maturation of SREBP-1, suggesting that the effects of 17βHSD13 may be mediated through SREBP-1. These results indicate that an alteration in LD proteins is one of the causative factors of aberrant and ectopic lipid storage, with implications for our understanding of metabolic diseases.

7

Examining the Role of miR-33b in Regulation of Preadipocyte Proliferation and Differentiation

Nathan L. Price[1], Carlos Fernandez-Hernando[1]

[1]Yale University School of Medicine, New Haven, CT

Impaired WAT function results in an inability to properly remove and store circulating lipids, thereby promoting the accumulation of lipids in non-adipose tissues and the development of diseases such as type II diabetes and atherosclerosis. *De novo* lipid biosynthesis is controlled by sterol regulatory element-binding proteins (SREBPs). The SREBP family of transcription factors consists of the SREBP-1a, SREBP-1c, and SREBP-2 proteins, which are encoded by the genes *SREBP-1* and *SREBP-2*. In addition to classical transcription regulators, a class of non-coding RNAs, termed microRNAs (miRNAs), has emerged as critical mediators of a variety of cellular functions, including lipid metabolism and adipogenesis. Work done by our laboratory and others has established miR-33 as an important regulator of cholesterol, fatty acid, and glucose metabolism. In humans, two isoforms of miR-33 exist: miR-33b, which is encoded by the *SREBP-1* gene, and miR-33a, which is located within the *SREBP-2* gene. In the liver, miR-33a and miR-33b work synergistically with their host genes to maintain lipid homeostasis. SREBP1 is highly induced during adipocyte differentiation and is a key transcription factor for the induction of adipogenic genes. Although the role of miR-33 in the liver has been established, it is unknown whether miR-33b is induced along with SREBP1 during adipogenesis or what role it may play in the regulation of adipocyte differentiation and function. Our preliminary findings show that adipocyte differentiation induces a dramatic increase in the expression of miR-33b along with SREBP-1, whereas SREBP-2 and miR-33a remain largely unaltered. Overexpression of miR-33b in preadipocytes prior to differentiation reduces their capacity to differentiate into mature adipocytes, as evidenced by reduced lipid accumulation and expression of adipogenic genes. Overexpression of miR-33b reduces proliferation of preadipocytes possibly due to its targeting of HMGA2, which is important for the clonal expansion phase of adipocyte differentiation. Alternatively, inhibition of miR-33b increases lipid content of differentiated adipocytes and elevates the expression of some adipogenic genes. Together, these data indicate that, similar to its host gene *SREBP-1*, miR-33b is an important regulator of human adipogenesis and may serve as a novel therapeutic target for treatment of obesity and other metabolic diseases.

8

ApoC-III Inhibition Increases Hepatic Uptake of Triglyceride-rich Lipoproteins via Members of the Low-density Lipoprotein Receptor Family

Jeffrey D. Esko[1], Philip L. Gordts[1]

[1]University of California, San Diego, La Jolla, CA

Hypertriglyceridemia is an independent risk factor for cardiovascular disease (CVD). Apolipoprotein C-III (apoC-III) strongly correlates with plasma triglyceride (TG) levels, and human apoC-III mutations correlate with reduced CVD. Inhibiting hepatic apoC-III synthesis with anti-sense oligonucleotides (ASO) reduces circulating levels of plasma apoC-III and TGs in animals and humans, including patients with lipoprotein lipase (LpL) deficiency. This latter result was surprising, because apoC-III was thought to modulate TGs primarily by inhibiting LpL. We now report similar results in apoC-III ASO-treated mice with induced depletion of LpL. Increased clearance of TG was associated with greater uptake in liver but not in peripheral LpL-expressing tissues, such as heart and skeletal muscle. To determine the mechanism for reduced plasma TG, we determined the roles of hepatic heparan sulfate proteoglycan (HSPG) receptors and members of the LDL receptor family in ASO-mediated clearance of TG-rich lipoprotein (TRL). Administration of the apoC-III ASO reduced TG levels in mice lacking hepatic expression of the enzyme heparan sulfate *N*-deacetylase-*N*-sulfotransferase (Ndst1), the LDL-related protein 1 (Lrp1), and the LDL receptor (Ldlr) and in animals with combined deletions of Ndst1 and Lrp1 or Ldlr. However, administration of apoC-III ASO to mice lacking *both* Lrp1 and Ldlr had no effect on plasma TG. Furthermore, apoC-III-depleted particles cleared more rapidly when injected into apoC-III ASO-treated mice lacking Ndst1 compared with wild-type mice, whereas no difference in clearance rates of TRL was observed in mice lacking Ldlr and Lrp1 and treated with apoC-III ASO. Thus, apoC-III inhibits hepatic clearance of TRL mediated by the LDLR/LRP1 axis, an effect that is independent of modulating LpL activity.

9

Regulatory Systems of Acyl-CoA Thioesterase 12 (ACOT12) That Control Cytosolic Acetyl-CoA Degradation and the Lipid Biosynthesis

Yasuhiro Horibata[1], Hiromi Ando[1], Masahiko Itoh[1], Hiroyuki Sugimoto[1]

[1]Department of Biochemistry, Dokkyo Medical University School of Medicine, Tochigi, Japan

Cytosolic acetyl-CoA is a precursor for biosynthesis of lipids, such as fatty acids and cholesterol. Acyl-CoA thioesterase 12 (ACOT12) is an enzyme that hydrolyzes the thioester bond of acetyl-CoA to produce acetate and CoA. Because ACOT12 is the major enzyme responsible for determining the rate of degradation of cytosolic acetyl-CoA, the regulatory mechanism of ACOT12 is important for the control of lipid biosynthesis. In this study, we analyzed the enzymatic and transcriptional regulation of ACOT12. ACOT12 contains a catalytic thioesterase domain at the N terminus and a steroidogenic acute regulatory protein-related lipid transfer (START) domain at the C terminus. We investigated the effects of lipids (phospholipids, sphingolipids, fatty acids, and sterols) on ACOT12 thioesterase activity. We found that the activity was noncompetitively inhibited by phosphatidic acid (PA). In contrast, the enzymatic activity of a mutant form of ACOT12 lacking the START domain was not inhibited by the lipids. We also found that PA could bind to the thioesterase domain but not to the START domain. ACOT12 is detectable in the liver but not in hepatic cell lines, such as HepG2, Hepa-1, and Fa2N-4. ACOT12 mRNA and protein levels in rat primary hepatocytes decreased following treatment with insulin. These results suggest that cytosolic acetyl-CoA levels in the liver are controlled by lipid metabolites and hormones, which result in allosteric enzymatic and transcriptional regulation of ACOT12.

10

The Heterotrimeric G Protein Subunits $G\alpha_q$ and $G\beta_1$ Have Lysophospholipase D Activity

Chieko Aoyama[1], Hiromi Ando[1], Hiroyuki Sugimoto[1]

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The enzyme lysophospholipase D (lysoPLD) converts lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA), a bioactive phospholipid that activates specific receptors. Previously, we purified a novel lysoPLD from the rat brain. In this study, we identified the purified 42- and 37-kDa proteins as the heterotrimeric G protein subunits $G\alpha_q$ and $G\beta_1$, respectively. When FLAG-tagged $G\alpha_q$ was expressed in Hepa-1, COS-7, and Neuro2A cells and purified, significant lysoPLD activity was observed in microsomal fractions. Levels of the hydrolyzed product, choline, increased over time, and the Mg^{2+} dependency and substrate specificity of $G\alpha_q$ were similar to those of lysoPLD purified from the rat brain. Mutation of $G\alpha_q$ at amino acid Thr-186, predicted to interact with catalytic Mg^{2+}, dramatically reduced lysoPLD activity. Levels of enzymatic activities were different when expressed in different cell types, and the activity from Neuro2A cells was 137.4 nmol/min/mg. The calculated K_m and V_{max} values for 1-*O*-hexadecyl-*sn*-glycero-3-phosphocholine obtained from Neuro2A cells were 21 μM and 0.16 μmol/min/mg, respectively. Because the FLAG-tagged $G\alpha_q$ obtained from the cytoplasmic fraction did not have lysoPLD activity, we surmised that translocation of FLAG-tagged $G\alpha_q$ to the membrane is important for acquiring enzyme activity. Therefore, we prepared mutated constructs at palmitoylation (C^9C^{10} to S^9S^{10}) and interaction with $G\beta_1$ ($I^{25}E^{26}$ to $A^{25}A^{26}$) were prepared. As a result, both mutated proteins lost their enzyme activity. These results reveal a new function for $G\alpha_q$ as an enzyme with lysoPLD activity and that translocation to the membrane and interaction with $G\beta_1$ are important.

11

Targeted Mass Spectrometric Analysis (SWATH-MS) of the Cellular Response following the Perturbation of the LXR and SREBP Pathway

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Cellular cholesterol regulation is a complex biological process underlying different diseases, such as cardiovascular disease and non-alcoholic fatty liver disease (NAFLD). The transcription factors SREBP and LXR regulate the expression of different proteins that play crucial roles in cellular cholesterol regulation. Analysis of the downstream targets of these transcription factors has been typically performed on the transcript level. However, recent advances in mass spectrometry allow the accurate quantitative measurements of several thousand proteins or phosphorylation sites in a whole cell lysate across many different conditions. We employ SWATH-MS, a new mass spectrometric approach developed in our laboratory that results in increased completeness of proteomic measurements, in order to quantitatively characterize the proteomic response downstream of the SREBP and LXR pathway. Different perturbations of the SREBP and LXR pathway have been performed, and the resulting changes in the abundance of >2000 proteins were measured in four different human cell lines (Huh7, HepG2, HeLa, and HEK). Proteins well known to be regulated by SREBP or LXR, such as HMGCS1 and FASN, were measured in different cell lines, and their different regulation was quantitatively described. Interestingly, about 30% of the regulated proteins that we identify have not been previously reported in the literature to be regulated by the LXR or SREBP pathway. Moreover, the proteomic data was correlated to metabolomics measurements (>1000 metabolites) performed in the same cell lines, and validation of our findings was performed by interrogating transcript and metabolite data that has been acquired across >40 different mouse lines in the BXD mouse family. In addition, these high-content data have been used to construct a network model explaining mechanistically the observed proteomic and metabolic response. Differences in this network among the cell lines point to differently regulated processes within cellular cholesterol regulation. Our results show how the LXR and SREBP pathway can affect the proteome and result in perturbed metabolic profiles. A quantitative and mechanistic understanding of cellular cholesterol regulation is crucial to understand pathogenic mechanisms of different complex diseases. We present here an efficient approach using mass spectrometry-based proteomics in order to reach this goal.

12

Transcriptional Suppression of CTP:Phosphoethanolamine Cytidylyltransferase and HMG-CoA Reductase by 25-Hydroxycholesterol Is Mediated by Nuclear Factor-Y and Yin Yang1

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CTP:phosphoethanolamine cytidylyltransferase (Pcyt2) is the rate-limiting enzyme involved in mammalian phosphatidylethanolamine (PE) biosynthesis, and its expression is highly regulated. Previously, we reported that in several types of cells, *Pcyt2* mRNA levels increased after serum starvation, an effect that could be suppressed by supplementation with fetal bovine serum (FBS), low density lipoprotein (LDL), or 25-hydroxycholesterol (25-HC). Transcription of *Hmgcr*, which encodes 3-hydroxy-3-methylglutaryl-CoA reductase, is also suppressed by 25-HC in the same dose-dependent manner. Nevertheless, a sterol regulatory element was not detected in the *Pcyt2* promoter region as might have been expected. The important element for transcriptional control of *Pcyt2* by 25-HC (1.25 μM) was determined to reside between −56 and −36 based on analysis with several deleted *Pcyt2* promoter-luciferase reporters in NIH3T3 cells. Using the yeast one-hybrid system, we found that Yin Yang1 (YY1) binds at $^{-42}$CAT^{-40} and nuclear factor-Y (NF-Y) binds at $^{-37}$CCAAT^{-41} in the *Pcyt2* promoter. Endogenous YY1 and NF-Y clearly and competitively bind to these sites and are important for basal *Pcyt2* transcription. Moreover, by gel-shift analysis, we found that NF-Y binds to the *Hmgcr* promoter at $^{-14}$CCA^{-12}. Transcriptional suppression of both *Pcyt2* and *Hmgcr* by 25-HC was reduced following knockdown targeting of YY1 or NF-YA. Based on these results, we conclude that NF-Y and YY1 are important for basal transcription of *Pcyt2* and are involved in the inhibitory effects of 25-HC on *Pcyt2* and *Hmgcr* transcription.

13

CIDEC/Fsp27 Is Regulated by PPARα and Plays a Critical Role in Fasting- and Diet-induced Hepatosteatosis

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The cell death-inducing DFFA-like effector c (CIDEC; also known in rodents as FSP27 or fat-specific protein 27) is a lipid droplet-associated protein that promotes intracellular triglyceride storage. CIDEC/Fsp27 is highly expressed in adipose tissue but undetectable in normal liver. Its hepatic expression, however, rises during fasting or under genetic or diet-induced hepatosteatosis in both mice and patients. Herein, we demonstrate that CIDEC/Fsp27 is a direct transcriptional target of the nuclear receptor PPARα (peroxisome proliferator-activated receptor α) in both mouse and human hepatocytes and that preventing Fsp27 induction accelerates PPARα-stimulated fatty acid oxidation. We show that adenovirus-mediated silencing of hepatic Fsp27 abolishes fasting-induced liver steatosis in the absence of changes in plasma lipids. Finally, we report that anti-Fsp27 shRNA and PPARα agonists synergize to ameliorate hepatosteatosis in mice fed a high fat diet. Together, our data highlight the physiological importance of CIDEC/Fsp27 in triglyceride homeostasis under both physiological and pathological liver steatosis. Our results also suggest that patients taking fibrates probably have elevated levels of hepatic CIDEC, which may limit the efficient mobilization and catabolism of hepatic triglycerides.

14

Seipin/BSCL2 Organizes Lipid Droplet Formation Sites

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Lipid droplets (LDs) are ubiquitous organelles for cellular lipid storage and metabolism. The biogenesis of LDs from the ER remains poorly understood. Seipin/BSLC2 is among several candidate ER proteins that have been proposed to regulate LD formation. Seipin is a highly conserved membrane protein, and recessive seipin mutations cause congenital generalized lipodystrophy. Depletion of seipin in several cell models leads to the formation of very large LDs. However, the molecular function of seipin in LD biology is unknown. Here, we show that seipin depletion leads to enlarged LDs in *Drosophila* cells. This phenotype is not due to alterations in neutral lipid synthesis. Instead, seipin deficiency alters the packaging of lipids into LDs. By using confocal time-lapse and light-sheet microscopy, we show that initial LD formation from the ER is disorganized in seipin-depleted cells. Seipin that is tagged with GFP at its endogenous genomic locus by CRISPR/Cas9-mediated genome engineering localizes to discrete foci on the ER that are directly adjacent to LDs. In wild-type cells, LD formation occurs in distinct steps, and we showed previously that the lipid synthesis enzyme GPAT4 is excluded from nascent LDs but targets later to a subset of LDs in a distinct, Arf1/COPI-mediated reaction. In seipin-depleted cells, GPAT4 localizes to many more LDs as soon as they form. Based on our results, we suggest that seipin is crucial for organizing LD formation sites, channeling TG made in the ER into LDs, and controlling the access of specific proteins to nascent LDs.

15

Unsaturated Fatty Acids Stimulate Tumor Growth through Stabilization of β-Catenin

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Aberrant accumulation of β-catenin drives development and progression of various cancers. Although Wnt signaling is known to block degradation of β-catenin, the roles of other pathways involved in this process remain elusive. In our study, excess unsaturated fatty acids block degradation of β-catenin through a mechanism different from Wnt signaling. We identified Fas-associated factor 1 (FAF1), a protein known to bind, thereby facilitating degradation of, β-catenin as the fatty acid sensor involved in the regulation. In the absence of unsaturated fatty acids, FAF1 binds to β-catenin, resulting in rapid degradation of the protein. Excess unsaturated fatty acids block the interaction between FAF1 and β-catenin, resulting in stabilization of β-catenin. Interestingly, unlike Wnt, which stabilizes β-catenin by inhibiting ubiquitination of the protein, unsaturated fatty acids stabilize β-catenin at a postubiquitination step. Our discovery is of clinical significance, because excess unsaturated fatty acids promote growth of clear cell renal cell carcinoma through stabilization of β-catenin. Our study suggests that FAF1 may be a novel drug target for cancers whose proliferation is dependent on unsaturated fatty acid-mediated stabilization of β-catenin.

16

Adiponectin Accumulates in Cardiovascular Tissue and Exhibits Protective Effects via T-cadherin

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Adiponectin, a multimeric adipocyte-secreted protein abundant in the circulation, is implicated in cardiovascular protective functions. Recent work has documented that adiponectin locally associates with responsive tissues through interactions with T-cadherin/Cdh13, an atypical, glycosylphosphatidylinositol (GPI)-anchored cadherin cell surface glycoprotein.

Although adiponectin and T-cadherin protein were highly expressed in aorta and heart in wild-type (WT) mice, mice deficient for T-cadherin lack tissue-associated adiponectin, accumulate adiponectin in the circulation, and mimic the adiponectin knockout cardiovascular phenotype. In search of a possible mechanism, we found that enzymatic cleavage of tissue T-cadherin by administration of phosphatidylinositol-specific phospholipase C (PI-PLC) to WT mice increases plasma adiponectin while decreasing tissue-bound adiponectin levels. Similarly, pretreatment of cultured endothelial cells with serum containing adiponectin showed accumulation of adiponectin on cells, but this phenomenon was eliminated by inhibition of T-cadherin expression with siRNA or PI-PLC. In immunohistochemical staining, adiponectin and T-cadherin proteins were colocalized in aortic endothelium of WT mice. On the other hand, in atherosclerotic plaques of ApoE-deficient mice, adiponectin protein accumulated even in the intimal smooth muscle layer and colocalized with T-cadherin. Furthermore, adiponectin protein in the smooth muscle layer colocalized with vimentin, a proliferative smooth muscle cell marker, but not with calponin, a contractile smooth muscle cell marker. We investigated the localization of vascular adiponectin using the immunoelectron microscopic technique. In WT mice, adiponectin was mainly detected on the luminal surface membrane of endothelial cells (ECs). In the atherosclerotic lesions of ApoE-KO mice, adiponectin was detected in ECs, on the cell surface membrane of synthetic smooth muscle cells, and on the surface of monocytes adherent to ECs.

In human aortic smooth muscle cells (HASMC), T-cadherin expression was reduced by inducing cells from the proliferative phenotype to the contractile phenotype with TGF-β1 treatment, and then accumulation of adiponectin protein on cells was inhibited in such conditions. In addition, adiponectin was observed in calponin-negative and vimentin-positive cells. Adiponectin suppressed TNF-α-induced elevation of gene expression levels of MCP-1 and monocyte adhesion molecules, but this effect was cancelled by T-cadherin siRNA. These results suggested that adiponectin accumulates in endothelial cells and proliferative smooth muscle cells of aorta and exhibits protective effects in a T-cadherin-dependent manner.

17

Efficacy and Safety of Sebelipase Alfa in Children and Adults with Lysosomal Acid Lipase Deficiency: Results of a Phase 3 Trial

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Lysosomal acid lipase (LAL) deficiency is a progressive multisystem disease that is an underappreciated cause of cirrhosis, severe dyslipidemia, and early-onset atherosclerosis. The purpose of this work was to assess the safety and efficacy of sebelipase alfa, a recombinant human LAL developed as an enzyme replacement therapy for LAL deficiency. This phase 3, double-blind, placebo-controlled trial randomized children and adults with LAL deficiency ($n = 66$) to placebo or sebelipase alfa, 1 mg/kg every other week for 20 weeks. Primary end point was alanine aminotransferase (ALT) normalization. Secondary end points included changes in lipid levels, additional important efficacy assessments, safety, and immunogenicity. The double-blind period was followed by an open-label period, where all patients received sebelipase alfa. Baseline median LDL-C was 204.0 mg/dl (range 70–378 mg/dl). LDL-C was ≥190 mg/dl in 58%, including 24% ($n = 9/38$) who were on lipid-lowering medications. Liver abnormalities included fibrosis (100%), bridging fibrosis (47%), and cirrhosis (31%) in biopsied patients ($n = 32$; mean age 12 years). After 20 weeks, ALT normalization (upper limit of normal range 34–43 U/L) was achieved in 31% of the sebelipase alfa group and 7% of the placebo group ($p = 0.0271$). Multiple secondary efficacy end points were also met, including relative reduction in LDL-C (–22.2%; $p < 0.0001$), non-HDL-C (–21.0%; $p < 0.0001$), and triglycerides (–14.3%; $p = 0.0375$) and relative increase in HDL-C (+19.9%; $p < 0.0001$) compared with placebo. In patients transitioned to sebelipase alfa during the open label period, treatment effects were consistent with sebelipase alfa-treated patients in the double-blind period. Further improvements were observed in sebelipase alfa-treated patients. During the double-blind period, most AEs were mild and unrelated to sebelipase alfa; 6 patients experienced infusion-associated reactions (4 placebo; 2 sebelipase alfa). Dosing was paused in 1 patient after an atypical infusion-related reaction following sebelipase alfa treatment. The safety profile in the open-label period was consistent with that of the double-blind period. Sebelipase alfa treatment for 20 weeks demonstrated statistically significant improvements in ALT normalization and in a number of other important disease-related abnormalities, including marked reductions in LDL-C and improvement in other lipid parameters. The safety profile appears favorable, and infusions were generally well tolerated; efficacy continued during open-label treatment.

18

Visualizing Sphingomyelin in the Secretory Pathway

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Secretory cargo exits the Golgi apparatus at the trans-Golgi network (TGN) after sorting into distinct transport carriers that ferry the cargo to distinct domains of the plasma membrane (PM) or to other organelles. Lipid-based mechanisms for sorting of secretory cargo have long been postulated to exist, but support for the existence of TGN-derived vesicles with distinct lipid compositions is lacking. The TGN is a major site of sphingomyelin (SM) synthesis, a lipid that is a major component of the plasma membrane outer leaflet. SM synthesis is tightly coordinated with the influx of ceramide, a precursor to SM and a potent signaling molecule, and cholesterol, which partitions with SM to form microdomains that have been implicated in protein sorting and signaling. In order to gain insight into the role of SM in secretory vesicle biogenesis and the regulation of SM synthesis, we have developed a genetically encoded fluorescent probe that recognizes SM in the secretory pathway and traffics in secretory vesicles to the PM. Two-color TIRF microscopy of individual fusion events at the PM demonstrates that SM-containing vesicles are enriched in apical secretory cargo and are largely distinct from vesicles that contain basolateral cargo. These tools also reveal surprisingly distinct behaviors of secretory cargo immediately after fusion that are correlated with lipid content of secretory vesicles.

19

Targeted Next-Generation Sequencing to Diagnose Disorders of HDL Cholesterol

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A low level of high-density lipoprotein cholesterol (HDL-C) is the most common lipid abnormality in patients with premature coronary artery disease. Many patients with very low or very high HDL-C have a rare mutation in one of several genes, but identification of the molecular abnormality in patients with extreme HDL-C is rarely performed in clinical practice. The objective of this study was to investigate the analytic validity and diagnostic yield of a targeted next-generation sequencing (NGS) assay for extreme levels of HDL-C. We developed a targeted NGS panel to capture the exons, intron/exon boundaries, and untranslated regions of 26 genes with highly penetrant effects on plasma lipid levels. We sequenced 90 patients with extreme HDL-C levels, 6 patients with known Mendelian disorders of HDL-C in whom pathogenic mutations had previously been identified, and 1 family with a suspected Mendelian disorder of HDL in which no mutation had previously been identified. We prioritized variants in accordance with medical genetics guidelines. Overall, a molecular diagnosis was established in 40% of patients with low HDL-C and 8% with high HDL-C. One hundred percent of prioritized variants identified by NGS were confirmed by Sanger sequencing, and all previously known variants in patients with established diagnoses were detected by NGS. We validated the functional impact of a subset of these variants using *in vitro* assays and showed that they represent loss-of-function alleles. Our results suggest that a molecular diagnosis can be identified in a substantial proportion of patients with low HDL-C and that our customized NGS assay has positive predictive value and sensitivity approaching 100% for identifying these variants.

20

In vitro Interaction of Drugs with Canalicular Lipid Transporters

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Canalicular bile formation is maintained by an elaborate array of ABC transporters and requires BSEP (bile salt export pump), MDR3 (ABCB4), and ABCG5/G8. Dysfunctions in the biliary cholesterol-bile salt-phospholipid secretion (*e.g.* an increased ratio of cholesterol to bile salts or phospholipids) lead to cholestasis or result in cholesterol crystallization followed by cholelithiasis. The aim of this project is to test the hypothesis that some drugs may induce cholestatic liver disease by specifically interfering with biliary lipid secretion and to identify such inhibitors/stimulators. To this end, we developed a cell-based and polarized model system consisting of stably transfected LLC-PK1 cell lines (overexpressing human sodium-taurocholate cotransporting polypeptide or NTCP, BSEP, MDR3, and ABCG5/G8) cultured on Transwell® plates. In the Transwell® system, cells grown on the filter are polar (*i.e.* the basolateral membrane faces the lower compartment, and the apical membrane faces the upper compartment). Our cells expressing the respective carriers, display a vectorial transport of bile salts ([^{3}H]taurocholate) from the basolateral to the apical compartment and an increased efflux of phosphatidylcholine (endogenous and fluorescent or NBD-PC) and cholesterol ([^{14}C]cholesterol) to the apical compartment, as is the case in human hepatocytes. Currently, we are at the stage of testing the effect of candidate drugs on lipid secretion by applying the generated model. Our experiments show that the antimycotic agent itraconazole inhibits phosphatidylcholine secretion into the apical compartment as previously reported by another group. Taking this result together with the vectorial transport of [^{3}H]taurocholate and lipids, we have successfully established an *in vitro* model for canalicular bile salts and lipid secretion. Consequently, we plan to use this model to characterize the effect of additional drugs suspected to interfere with canalicular bile salts and lipid transporters. This knowledge will help us to better understand the relation between drugs and cholestasis and may help to identify potential therapeutic agents for gallstone disease.

21

Characterization of the Effects of a Novel DGAT2 Inhibitor on Hepatic Lipid Metabolism

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Diacylglycerol acyltransferases (DGAT) catalyze the terminal step in triacyglycerol (TG) synthesis, specifically the esterification of a fatty acid with diacylglycerol resulting in the formation of TG. Here we report the identification of PF-06424439, a potent and selective DGAT2 inhibitor, and characterize its effects on hepatic lipid synthesis and secretion. In sucrose-fed rats, PF-06424439 dose-dependently lowered plasma TG concentrations with a half-maximal effect dose between 0.1 and 0.3 mg/kg. A single dose of PF-06424439 decreased the hepatic very low-density lipoprotein (VLDL)-TG secretion rate by 65%, which, in turn, was associated with reduced circulating low-density lipoprotein (LDL)- and VLDL-associated cholesterol. Treatment of rats with PF-06424439 over 52 h resulted in a 76% reduction in circulating triglyceride concentrations and a 51% decrease in total plasma cholesterol. Liver TG content was reduced following 52 h of PF-06424439 treatment, whereas hepatic levels of diacylglycerol (1.6-fold) and cholesteryl ester (2-fold) were both significantly elevated at this time point. The reduction in liver TG concentrations was paralleled by a dramatic decrease in the expression of the gene encoding the lipogenic transcription factor sterol regulatory element-binding transcription factor 1c (SREBP-1c) and its associated target genes. These data present an integrated view of the direct and adaptive changes that occur in the liver and plasma following inhibition of DGAT2 and support evaluation of this target in the setting of metabolic disease.

22

Apolipoproteins C-I and C-III Inhibit Lipoprotein Lipase Activity by Displacement of the Enzyme from Lipid Droplets

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Apolipoproteins C-I and C-III (apoC-I and apoC-III) are known to inhibit the activity of lipoprotein lipase (LPL), but the molecular mechanisms underlying this inhibition remain obscure. Here, we show that both apoC-I or apoC-III, when bound to triglyceride-rich lipoproteins (TRLs), prevent the association of LPL to the lipid/water interface, resulting in decreased lipolytic activity. TRLs are known to stabilize LPL and protect the enzyme from inactivating factors, such as angiopoietin-like protein 4 (angptl4). The addition of apoC-I or apoC-III to TRLs severely diminished their protective effect on LPL and rendered the enzyme more susceptible to inactivation by angptl4. These observations were seen using chylomicrons as well as the synthetic lipid emulsion Intralipid. The addition of apoC-II, the LPL co-activator, to TRLs partially prevented LPL from being displaced from the lipid/water interface by apoC-I or apoC-III. Using site-directed mutagenesis, we identified the amino acid residues within apoC-III that are critical for its attachment to lipid emulsion particles and its ability to inhibit LPL activity. In conclusion, we show that apoC-I and apoC-III inhibit lipolysis by displacing LPL from lipid emulsion particles and TRLs. We also propose a role for these apolipoproteins in the irreversible inactivation of LPL by angptl4.

23

Hepatocyte-specific ATP Binding Cassette Transporter A1 Deletion Impairs Hepatic Insulin Signaling by Increasing Plasma Membrane Free Cholesterol Accumulation

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ATP binding cassette transporter A1 (ABCA1) effluxes free cholesterol (FC) and phospholipid across the plasma membrane to combine with apolipoprotein A-I, forming nascent high density lipoproteins (HDL). Although nascent HDL formation is the presumed primary function of ABCA1, recent studies report a positive association between ABCA1 sequence variants and type 2 diabetes across multiple ethnic groups. We generated hepatocyte-specific ABCA1 knockout (HSKO) mice to explore the relationship between hepatocyte ABCA1 expression and metabolic regulation. Liver or hepatocytes isolated from chow-fed HSKO mice had decreased insulin-stimulated AKT phosphorylation, compared with their control counterparts, whereas glucagon-induced cAMP concentration and substrates of PKA phosphorylation were increased in HSKO hepatocytes. However, except for plasma lipids, chow-fed HSKO and control mice had a similar metabolic phenotype, including body weight; food intake; energy expenditure; liver lipids; blood/plasma glucose, insulin, glucagon, and ketone body levels; and glucose, insulin, pyruvate, and glucagon tolerance tests. When challenged with a Western-type diet, obese HSKO mice had lower body weight due to increased energy expenditure without changes in food intake or physical activity and had reduced hepatosteatosis due to decreased insulin-stimulated hepatic lipogenesis without changes in fatty acid uptake or export via very low density lipoprotein triglyceride secretion from liver. Notably, liver plasma membrane FC content was increased, resulting in decreased insulin-stimulated insulin receptor phosphorylation in the absence of changes in plasma membrane insulin receptor protein expression in obese HSKO mice. Liver or hepatocytes isolated from obese HSKO *versus* control mice displayed decreased insulin-stimulated AKT phosphorylation, which was normalized when the HSKO hepatocytes were subjected to acute FC depletion. Last, glucagon-induced substrates of PKA phosphorylation were increased in obese HSKO hepatocytes. These findings support a novel role for hepatocyte ABCA1 expression in modulating plasma membrane FC, which in turn, regulates hepatic insulin receptor activation and glucagon signaling.

24

Structural Evidence for Conserved Conformational Plasticity and Ligand Binding of Monoacylglycerol Lipases in Bacteria, Yeast, and Humans

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Monoacylglycerol lipases (MGLs) play an important role in lipid catabolism across all kingdoms of life by catalyzing the release of free fatty acids from monoacylglycerols (MGs). MG-hydrolyzing lipases were first described in the 1960s, and several orthologs of MGL have been characterized over the years. The physiological function of MGL is best understood in mammals, where it has an essential role in lipid metabolism of maintaining energy homeostasis. Additionally, human MGL plays an important part in mediating endocannabinoid-based signaling, rendering it an important pharmacological target. In bacteria, MGLs are thought to have a role in detoxification processes because short chain MGs are highly toxic to these organisms. Despite the longstanding research efforts invested in this enzyme class, structural data were scarce until 2010, when the structures of human MGL (hMGL) were published from three independent groups. Very recently, our group determined the three-dimensional structure of an MGL from a bacterial species and just now solved the structure of MGL from the yeast *Saccharomyces cerevisiae* (YJU3p). These data provide an in-depth structure-function elucidation of these different MGLs. The structures allow identification of conserved and non-conserved structural features of MGLs that will be discussed in this contribution. MGLs adopt an α/β-hydrolase fold core, which is covered by a cap region. The hydrolytic reaction is catalyzed by a catalytic triad that resides within the core domain. The cap regions of MGLs differ in length, amino acid sequence, and secondary structure yet still adopt a strikingly similar overall architecture. Our three-dimensional structures of MGLs in complex with natural substrate and substrate analogs show that substrate binding is facilitated by residues from the cap and the core region. Structure analysis also enables correlation between size and shape of substrate binding cavities and substrate specificities. Using protein crystallography and NMR spectroscopy, we could take snapshots of these lipases undergoing conformational changes between open and closed conformations.

25

apoc2 Knockout Zebrafish as a Genetic Model of Hyperlipidemia

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Apolipoprotein CII is an obligatory activator of lipoprotein lipase. Human patients with APOC2 deficiency display severe hypertriglyceridemia while consuming a normal diet, often manifesting xanthomas, lipemia retinalis, and pancreatitis. Hypertriglyceridemia is also an important risk factor for development of cardiovascular disease. Animal models to study hypertriglyceridemia are limited, with no *Apoc2* knockout mouse reported. To develop a genetic model of hypertriglyceridemia, we generated an *apoc2* knockout zebrafish. *apoc2* mutants show decreased plasma lipase activity and display severe hypertriglyceridemia, which closely resembles the phenotype observed in human patients with APOC2 deficiency. The hypertriglyceridemia in *apoc2* mutants is rescued by injection of plasma from wild type zebrafish or by injection of a human apoC-II mimetic peptide. Consistent with the previous report of a transient *apoc2* knockdown, *apoc2* mutant larvae have a minor delay in yolk consumption and angiogenesis. Furthermore, *apoc2* mutants fed a normal diet develop vascular lipid deposits, resulting in many lipid-laden macrophages, which resemble macrophage foam cells, a hallmark of human atherosclerotic lesions. In addition, *apoc2* mutants show ectopic overgrowth of pancreas. Taken together, our data suggest that the *apoc2* knockout zebrafish is a robust and versatile animal model to study hypertriglyceridemia and the mechanisms involved in pathogenesis of associated human diseases.

26

MAFG Is a Transcriptional Repressor of Bile Acid Synthesis and Metabolism

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Specific bile acids are potent signaling molecules that modulate metabolic pathways affecting lipid, glucose, and bile acid homeostasis and the microbiota. Bile acids are synthesized from cholesterol in the liver, and the key enzymes involved in bile acid synthesis (*Cyp7a1* and *Cyp8b1*) are regulated transcriptionally by the nuclear receptor FXR. We have identified an FXR-regulated pathway upstream of a transcriptional repressor that controls multiple bile acid metabolism genes. We identify *MafG* as an FXR target gene and show that hepatic MAFG overexpression represses genes of the bile acid synthetic pathway and modifies the biliary bile acid composition. In contrast, loss-of-function studies using *MafG*$^{+/-}$ mice cause derepression of the same genes with concordant changes in biliary bile acid levels. Finally, we identify functional MafG response elements in bile acid metabolism genes using ChIP-seq analysis. Our studies identify a molecular mechanism for the complex feedback regulation of bile acid synthesis controlled by FXR.

27

Naringenin Prevents Insulin Resistance and Atherosclerosis Independent of Fgf21

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Dietary supplementation with the citrus flavonoid, naringenin, attenuates the overproduction of apoB100-containing lipoproteins; ameliorates hepatic steatosis; and attenuates dyslipidemia, insulin resistance, and atherosclerosis in high-fat diet (HFD)-fed *Ldlr*$^{-/-}$ mice. The primary metabolic pathways responsible for the reduction in plasma and hepatic lipid were increased hepatic fatty acid oxidation and prevention of sterol regulatory element-binding protein 1-c (SREBP1c)-mediated lipogenesis; however, the molecular mechanisms responsible remain unknown. Because treatment of mice with naringenin increases both *Fgf21* mRNA and plasma FGF21, and FGF21 is a known regulator of energy homeostasis, we hypothesized that FGF21 might be part of the naringenin mechanism, whereby deficiency of FGF21 would potentiate HFD-induced metabolic dysregulation and compromise metabolic protection by naringenin. We therefore performed a series of studies in C57BL6/J wild-type (WT) mice and fibroblast growth factor 21 null (*Fgf21*$^{-/-}$) mice, to determine the requirement of FGF21 in mediating naringenin's beneficial effects. We report here that the absence of FGF21 exacerbated the metabolic response to HFD. Although HFD-induced obesity was 2-fold greater in *Fgf21*$^{-/-}$ mice compared with WT, the addition of naringenin to the HFD completely prevented obesity in both *Fgf21*$^{-/-}$ and WT mice. Analysis of gene expression suggested that naringenin was not primarily targeting fatty acid metabolism in white adipose tissue. Hepatic triglyceride concentrations, which were 2-fold higher in HFD-fed *Fgf21*$^{-/-}$ mice, were corrected by naringenin in both genotypes. Furthermore, naringenin normalized hepatic expression of *Pgc1a*, *Cpt1a*, and *Srebf1c*, in both WT and *Fgf21*$^{-/-}$ mice. HFD-fed *Fgf21*$^{-/-}$ mice displayed greater muscle triglyceride deposition, hyperinsulinemia, and impaired glucose tolerance as compared with WT mice, confirming the role of FGF21 in insulin sensitivity. However, treatment with naringenin improved these metabolic parameters in both genotypes. Thus, we conclude that the absence of FGF21 exacerbates HFD-induced obesity, hepatic steatosis, and insulin resistance. Furthermore, FGF21 is not required for naringenin to protect mice from HFD-induced metabolic dysregulation. Collectively, these studies add to the body of literature demonstrating that naringenin has potent lipid-lowering effects and may act as an insulin sensitizer *in vivo.*

28

SREBP-1, a Central Player in Brain Tumor Metabolism

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Dysregulated lipid metabolism is emerging as a new hallmark in malignancies. Understanding the lipid biology in cancer cells is important to identify the key player in regulating lipid reprogramming and develop an effective therapeutic strategy to treat cancer. Our studies have shown that sterol regulatory element-binding protein (SREBP-1), an endoplasmic reticulum-bound transcription factor with central roles in lipid metabolism, is highly up-regulated in glioblastoma (GBM), a common primary brain tumor with a median survival of only 12–15 months even after advanced therapies. Epidermal growth factor receptor (EGFR), the most amplified and mutated oncogene in GBM, via activating PI3K/Akt signaling promotes SREBP-1 activation and lipogenesis in GBM patients, xenografts, and cell lines. Furthermore, we found that EGFR/PI3K/Akt signaling via SREBP-1 up-regulates low-density lipoprotein receptor (LDLR) for elevated cholesterol uptake. These data demonstrate that SREBP-1 plays a central role in mediating oncogenic signaling-driven lipid metabolism reprogramming in GBM. Moreover, genetic inhibition of SREBP-1 markedly reduces brain tumor growth and significantly prolongs the overall survival of GBM-bearing mice. Interestingly, genetic ablation of SREBP-2 is shown to have no significant inhibitory effects on GBM tumor growth. Taken together, our studies demonstrate that SREBP-1 is a central player in GBM lipid metabolism and suggest that targeting SREBP-1 is a promising therapeutic strategy to treat malignancies.

29

The Terminal Enzymes of Cholesterol Synthesis, DHCR24 and DHCR7, Interact Physically and Functionally

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Cholesterol is essential to human health, and its levels are tightly regulated by a balance of synthesis, uptake and efflux. Cholesterol synthesis requires the actions of more than 20 enzymes to reach the final product, through two alternate pathways. Here we describe a physical and functional interaction between the two terminal enzymes. 24-Dehydrocholesterol reductase (DHCR24) and 7-dehydrocholesterol reductase (DHCR7) co-immunoprecipitate, and when the *DHCR24* gene is knocked down by siRNA, DHCR7 activity is also ablated. Conversely, overexpression of DHCR24 enhances DHCR7 activity but only when a functional form of DHCR24 is used. DHCR7 is important for both cholesterol and vitamin D synthesis, and we have identified a novel layer of regulation, whereby its activity is controlled by DHCR24. This suggests the existence of a cholesterol "metabolon," where enzymes from the same metabolic pathway interact with each other to provide a substrate channeling benefit. We predict that other enzymes in cholesterol synthesis may similarly interact, and this possibility should be explored in future studies.

30

Metabolic Regulation by miR-33 in Macrophages Controls Immune Effector Responses

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Cellular metabolism is increasingly recognized to control immune cell fate and functions. MicroRNA-33 (miR-33) is a central regulator of cellular lipid metabolism that represses genes involved in cholesterol efflux and HDL biogenesis (*Abca1* and *Abcg1*) and fatty acid oxidation (*Cpt1a*, *Crot*, and *Ampk*). Here we show that by altering the balance of aerobic glycolysis and mitochondrial oxidative phosphorylation, miR-33 inhibition directs macrophage polarization to an M2 phenotype and shapes innate and adaptive immune responses. Targeted deletion of miR-33 in macrophages increases oxidative phosphorylation, enhances spare respiratory capacity, and induces the expression of genes that define M2 macrophage polarization (*Arg1*, *Fizz1*, *Cd206*, and *Ym1*). Furthermore, inhibition of miR-33 in *Abca1*$^{-/-}$ macrophages showed that these changes are independent of effects on cholesterol efflux but instead require miR-33 targeting of the energy sensor AMP-activated protein kinase (AMPK). Notably, inhibition of miR-33 markedly increased macrophage expression of the retinoic acid-producing enzyme *Aldh1a2* and retinal dehydrogenase activity both *in vitro* and *in vivo*. Consistent with the ability of retinoic acid to foster inducible regulatory T cells, these macrophages had an enhanced capacity to induce FoxP3 expression in naive CD4^{+} T cells. Finally, treatment of Western diet-fed *Ldlr*$^{-/-}$ mice with miR-33 inhibitors for 8 weeks (conditions that do not alter HDL cholesterol levels) reduced atherosclerosis progression by 40% and promoted the accumulation of M2 macrophages and FoxP3^{+} T regulatory cells in plaques. Collectively, these results identify a novel role for miR-33 in the regulation of macrophage inflammation and show that antagonism of miR-33 is atheroprotective, in part, by reducing plaque inflammation by promoting M2 macrophage polarization and regulatory T cell induction.

31

Inflammasome Activation Stimulates Lipoxin Biosynthesis via Dual Phospholipase Specificities and Enzyme Coupling

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Initiation and resolution of inflammation are considered to be tightly connected processes. Lipoxins (LX) are proresolution lipid mediators that inhibit phlogistic neutrophil recruitment and promote wound-healing macrophage recruitment in humans via potent and specific signaling through the LXA_4 receptor (ALX). One model of lipoxin biosynthesis involves sequential metabolism of arachidonic acid by two cell types expressing a combined transcellular metabolon. It is currently unclear how lipoxins are efficiently formed from precursors or if they are directly generated after receptor-mediated inflammatory commitment. Here, we provide evidence for a pathway by which lipoxins are generated in macrophages as a consequence of sequential activation of Toll-like receptor 4 (TLR4), a receptor for endotoxin, and $P2X_7$, a purinergic receptor for extracellular ATP. Initial activation of TLR4 results in accumulation of the cyclooxygenase-2-derived lipoxin precursor 15-hydroxyeicosatetraenoic acid (15-HETE) in esterified form within membrane phospholipids, which can be enhanced by aspirin (ASA) treatment. Subsequent activation of $P2X_7$ results in efficient hydrolysis of 15-HETE from membrane phospholipids by group IVA cytosolic phospholipase A_2 and its conversion to bioactive lipoxins by 5-lipoxygenase. Our results demonstrate how a single immune cell can store a proresolving lipid precursor and then release it for bioactive maturation and secretion, conceptually similar to the production and inflammasome-dependent maturation of the proinflammatory IL-1 family cytokines. These findings provide evidence for receptor-specific and combinatorial control of pro- and anti-inflammatory eicosanoid biosynthesis and potential avenues to modulate inflammatory indices without inhibiting downstream eicosanoid pathways.

32

Oxidized Low Density Lipoprotein-binding Cationic Peptides and Proteins in Inflammation and Atherosclerosis: Novel Insights

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Apolipoprotein A1 and apolipoprotein E mimetic peptides have attracted attention due to their ability to reduce atherosclerosis and exhibit antioxidant, anti-inflammatory, and hypolipidemic properties. We previously reported that the non-lipoprotein-related Lys-Arg-rich cationic peptides have anti-inflammatory properties both *in vitro and in vivo*. In this study, we have determined whether $ApoB_{100}$ of low density lipoprotein (N-LDL), which is Lys-Arg rich, has similar properties. 5F-mimetic peptide of ApoA1, LL27 derived from the anti-microbial peptide CAMP, and a human glycodelin derived peptide were commercially synthesized. N-LDL was prepared and used. The ability of these peptides and protein to neutralize charges of modified lipoproteins as well as attenuate macrophage uptake and inflammation were analyzed. Oxidized LDL (Ox-LDL) was pretreated with increasing concentrations of peptides and N-LDL to evaluate charge-neutralizing properties of the peptides as well as that of the protein ($ApoB_{100}$). RAW cells were incubated with LPS or Ox-LDL pretreated with peptides and N-LDL. RNA was isolated from treated cells, and real-time PCR was performed using mouse IL-1α and IL-6 primers. Cationic peptides as well as $ApoB_{100}$ protein of N-LDL decomposed the peroxide content of 13-HPODE. Incubation of Ox-LDL and Ac-LDL with the peptides as well as $ApoB_{100}$ resulted in charge neutralization as noted by agarose gel electrophoresis. Preincubation of the peptides and N-LDL with modified lipoproteins reduced the uptake of the latter by macrophages and foam cell formation as detected by Oil-Red O staining. Reduced inflammation was observed in the presence of N-LDL as compared with LPS/Ox-LDL. Based on these studies, we postulate that cationic peptides and protein might have properties that (*a*) would affect events that are unrelated to lipid lowering, (*b*) might play an additional role in immune competent cells, including macrophages, and (*c*) might interact with other biologically important anionic molecules, including lipids and proteins. We also predict that lysine-rich cationic peptides and proteins could have therapeutic potential in reducing CVD/atherosclerosis-associated inflammation.

33

Glucagon-like Peptides Play Opposing Roles in Hepatic Lipid Metabolism

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Glucagon-like peptide-1 (GLP-1) and GLP-2 are gut-derived hormones that are co-secreted in equimolar amounts in response to nutrient ingestion. The paradoxical regulation of these gut peptides on postprandial lipemia have been previously demonstrated by our laboratory, with GLP-2 acutely enhancing postprandial lipemia and GLP-1 decreasing lipemia. Interestingly, GLP-1 has also been shown to decrease hepatic steatosis and very low density lipoprotein (VLDL) production, thereby decreasing fasting dyslipidemia. Alternatively, GLP-2 has been shown to target the liver and modulate hepatic glucose production; however, the role of GLP-2 in hepatic lipid or lipoprotein metabolism has not been investigated. Given that GLP-2 can target the liver and has also been shown to play an opposing role to GLP-1 in lipid metabolism, we postulate that GLP-2 will increase fasting dyslipidemia, VLDL production, and hepatic steatosis. To test this hypothesis, Syrian Golden hamsters received twice daily intraperitoneal injections of GLP-2 (20 pmol/kg) for 14 days. At the end of the study, plasma was collected following intraperitoneal poloxamer administration to prevent lipoprotein uptake, and livers were excised. GLP-2 treatment induced fasting hypertriglyceridemia independent of changes in weight or food consumption. Changes in dyslipidemia were also associated with increased VLDL production as indicated by elevated plasma and VLDL triglyceride, cholesterol, and apolipoprotein B100 (apoB100) accumulation. GLP-2 treatment also increased fasting glucose levels, although no changes in glucose tolerance were observed. Liver excised from GLP-2-treated hamsters had increased hepatic cholesterol levels accompanied by a 2–4-fold increase in Srebp1c, ACC, FAS, and SCD1 mRNA expression. In summary, GLP-2 appears to up-regulate VLDL production and hepatic *de novo* lipogenesis, thereby inducing fasting dyslipidemia and hepatic steatosis. Additional co-infusion studies are in progress to determine which peptide plays a more dominant role *in vivo*. Further experimental evidence to test this role can lead to identification of putative drug targets to fight the metabolic complications of hepatic steatosis and fasting dyslipidemia.

34

Presence of Oxidized Low Density Lipoprotein in the Left Ventricular Blood of Subjects with Cardiovascular Diseases

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Heart failure (HF) patients have lower ejection fraction (EF) as compared with healthy subjects. Because of this, a given molecule is likely to have a longer residence time in the left ventricle of HF patients. It is assumed that long residence of LDL might promote its oxidation. In this study, we tested the hypothesis that Ox-LDL is likely to be formed in the left ventricular blood (LVB) when EF is low. We tested the hypothesis that there is an increased level of Ox-LDL in the LVB as opposed to peripheral blood (PB) and whether the former's presence correlated with EF. Also, we examined whether a higher level of Ox-LDL negatively correlated with the activity of paraoxonase 1 (PON1). Finally, we explored the possibility of cholesterol efflux in the presence of HF subject plasma samples. Following Institutional Review Board approval, 62 HF patients were enrolled in the study. All patients underwent preoperative transthoracic echocardiographic assessment of ventricular function. LVEFs were determined using Simpson's biplane technique. 2 ml of LVB and 5 ml of PB samples were taken before surgery. Blood levels of Ox-LDL were determined by ELISA, and PON1 activity was determined. Foam cells were developed by incubating RAW 264.7 macrophages with labeled cholesterol as well as mixed micelles and used for the efflux studies in the presence of HF plasma samples. A significant increase in the levels of Ox-LDL in LVB was noted as compared with its levels in the PB in HF subjects even when EF was nearly normal. In contrast, Ox-LDL levels increased in the PB of subjects with lower EF and reached those of LVB. PON1 activity and cholesterol efflux studies indicated an increased oxidative stress in LVB and a decreased ability to promote cholesterol efflux from lipid-enriched macrophages. The results suggest that LVB is more oxidatively stressed than PB, and as a result, LV tissue might be affected differently as compared with peripheral tissues. Because we recently reported that BNP, a marker for HF, is induced by Ox-LDL, it is possible that localized factors within LV could profoundly affect markers of HF.

35

Combination Treatment with Eicosapentaenoic Acid and Atorvastatin Active Metabolite Reverses Endothelial Dysfunction in HUVECs Exposed to Oxidized LDL

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Endothelial cell (EC) dysfunction is an early marker of atherosclerosis and associated with increased risk for cardiovascular events. Eicosapentaenoic acid (EPA), an ω-3 fatty acid, has been shown to improve EC function. This effect may be enhanced in combination with select statins, including atorvastatin active metabolite (ATM). In this study, we tested the separate *versus* combined effects of EPA and ATM on changes in EC function following exposure to oxidized LDL (oxLDL). The effects of EPA were compared with docosahexaenoic acid (DHA), fenofibrate, niacin, and gemfibrozil. Human umbilical vein endothelial cells (HUVECs) were incubated with oxLDL for 25 min, followed by treatment with EPA, DHA, fenofibrate, niacin, or gemfibrozil (each at 10.0 μM), alone or in combination with ATM (1.0 μM), *versus* vehicle, for 1 h. Changes in nitric oxide (NO) and peroxynitrite ($ONOO^-$) release were measured with porphyrinic nanosensors following maximal stimulation with calcium ionophore. Exposure to oxLDL decreased HUVEC NO release by 22% (from 376 ± 85 to 292 ± 53 nM) as compared with untreated cells. In HUVECs exposed to oxLDL, treatment with EPA and ATM separately increased NO release by 45 and 64%, respectively, whereas EPA-ATM combination treatment increased NO release by 200% ($p < 0.01$), as compared with vehicle alone. The $NO/ONOO^-$ ratio, an indicator of normal EC function, increased approximately 3-fold with the EPA-ATM combination treatment as compared with vehicle-treated controls ($p < 0.05$). An improvement in NO release over ATM alone was not observed with DHA or any of the other TG-lowering agents in combination with the statin. EPA and ATM increased NO bioavailability in oxLDL-treated ECs in a manner that was significantly enhanced by their co-administration. This effect was not observed for DHA or any of the other TG-lowering agents in combination with ATM. Although the exact mechanism for this combinatorial effect remains unclear, these data suggest that EPA-ATM interactions may provide atheroprotective benefits beyond therapeutic changes in lipid levels alone.

36

Adipocyte Hypertrophy Induces Insulin Resistance Independent of Inflammation

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In obesity, adipocyte hypertrophy and proinflammatory responses are closely associated with the development of insulin resistance in adipose tissue. However, it is largely unknown whether adipocyte hypertrophy in the absence of inflammation is sufficient to induce insulin resistance. Here, we developed an *in vitro* model of adipocyte hypertrophy by treating adipocytes with long-chain fatty acids. Treatment with saturated or monounsaturated fatty acids resulted in adipocyte hypertrophy, but a proinflammatory response was only observed in adipocytes treated with saturated fatty acids. Regardless of inflammation, hypertrophic adipocytes with unilocular lipid droplets exhibited impaired insulin-dependent glucose uptake with a defect in GLUT4 trafficking to the plasma membrane. Moreover, Toll-like receptor 4 mutant mice (C3H/HeJ) with diet-induced obesity were not protected against insulin resistance, although they were resistant to adipose tissue inflammation. Together, our *in vitro* and *in vivo* data suggest that adipocyte hypertrophy alone would be sufficient to cause insulin resistance in early obesity.

37

Blocking Lipid Droplet Formation by Inhibition of ACAT1 Suppresses SREBP-1-regulated Lipogenesis and Inhibits Glioblastoma Growth

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Altered lipid metabolism is one of the major characteristics of malignancies. Recent studies have shown that lipid droplets (LDs) exist in cancer cells. However, the link between LD formation and tumorigenesis is unclear. Here, by analyzing a large cohort of patient tissues, including low grade to high grade glioma and glioblastoma (GBM) xenografts, we show that LDs are a unique signature of GBM. Importantly, GBM patients with a greater number of LDs had significantly shorter survival time. We found that inhibition of cholesteryl ester (CE) synthesis by targeting ACAT1 significantly blocked LD formation, suppressed GBM tumor growth, and markedly prolonged the survival of GBM-bearing mice through inhibition of SREBP-1-regulated lipogenesis. Taken together, our data strongly suggest that LD could be a novel diagnostic marker in GBM and reveal that LDs and ACAT1 could be novel therapeutic targets in malignant tumors.

39

FBXL10/KDM2B Regulates 3T3-L1 Adipogenesis via PRC1-dependent Transcriptional Repression

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Polycomb-repressive complex PRC1 plays an essential role in cellular differentiation. However, its function during adipogenesis is totally unknown. Here we show that FBXL10/KDM2B is an antiadipogenic factor of 3T3-L1 preadipocyte differentiation. Overexpression of FBXL10 in 3T3-L1 inhibited the second round of mitotic clonal expansion. This change was associated with repression of the cell cycle gene and percentage of S-phase. FBXL10 inhibits adipogenesis in an F-box- and LRR-dependent but JmjC motif- and C*XX*C domain-independent manner. F-box and LRR motifs of Fbxl10 are required for the interaction with RING1B, SKP1, PCGF1, and BCOR, forming a PRC1. Knockdown of either RING1B or SKP1 restored FBXL10-mediated repression of 3T3-L1 preadipocyte differentiation, indicating that PRC1 formation is sufficient for FBXL10 function. ChIP-seq analysis showed that FBXL10 recruits RING1B, a mediator of chromatin compaction and gene repression, to the specific genomic loci, including *Cdk1*, *Uhrf1*, *Pparg1*, and *Pparg2*, in an F-box-dependent manner. These results provide evidence that FBXL10 recruits non-canonical PRC1 complex to the genes of the cell cycle and terminal differentiation and plays a role as a negative regulator in the orchestrated events during adipogenesis.

40

The Nuclear Receptor FXR Uncouples the Actions of miR-33 from SREBP-2

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The objective of this work was to determine whether activation of farnesoid X receptor (FXR) alters cellular and plasma cholesterol homeostasis as a result of regulation of *Srebp-2* and miR-33. Chromatin immunoprecipitation sequencing (ChIP-seq) data identified an FXR-response element within intron 10 of the sterol regulatory element-binding protein-2 (*Srebp-2*) gene. Consistent with this observation, treatment of mice with FXR-specific agonists (GSK2324 or GW4064) rapidly increased hepatic levels of *Srebp-2* mRNA, precursor SREBP-2 (pSREBP-2) protein, and miR-33. Further, miR-33 targets, which include ATP binding cassette transporter A1 (ABCA1), *N*-ethylmaleimide-sensitive factor (NSF), and carnitine palmitoyltransferase 1 (CPT1), were all reduced in GSK2324-treated mice. In contrast, neither nuclear SREBP-2 (nSREBP-2) protein, nor SREBP-2 target genes were induced following FXR activation. The inability to process pSREBP-2 to nSREBP-2 is probably a consequence of the induction of insulin-induced gene-2a (INSIG-2a) by FXR agonists. Finally, we show that the FXR-dependent induction of both *Srebp-2* and miR-33 is ablated in *Scap*$^{-/-}$ mice that lack nSREBP-2. We demonstrate that activation of FXR uncouples the expression of nSREBP-2 and miR-33 and the regulation of their respective target genes. Further, we conclude that the FXR agonist-dependent increase in miR-33 requires transcription of the *Srebp-2* gene.

41

Lineage-specific Gene Body DNA Methylation Recruits SETDB1 to Form Non-canonical Bivalent Chromatin Domains That Inhibit Cebpα and Pparγ Expression and Adipogenesis

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Bivalent chromatin domains in embryonic stem cells contain activating H3K4me3 and repressive H3K27me3 histone modifications and keep developmental regulatory genes expressed at very low levels and poised for activation. Here, we show an alternative bivalent modification configuration in lineage-committed preadipocytes and mesenchymal stem cells. The novel modification configuration harbors H3K4me3 and, in tandem, small intragenic H3K9me3 modifications, but not H3K27me3, and keeps adipogenic master regulatory genes (*Cebpa* and *Pparγ*) poised for activation. Via lineage-specific gene body DNA methylation, H3K9 methyltransferase SETDB1 is recruited to gene bodies proximal to transcription start sites marked with H3K4me3 to form the novel chromatin domains. This novel domain prevents transcription factor C/EBPβ binding and H3K4me3 deposition to the *Cebpa* gene body, keeps RNA polymerase II pausing, and inhibits adipogenesis. These results demonstrate that H3K4me3/H3K9me3 chromatin domains function as non-canonical bivalent domains that keep gene expression low and poised for activation in lineage-committed cells during differentiation.

42

PKA-dependent Phospho-switch on JMJD1A Regulates Higher Order Chromatin Dynamics via SWI/SNF Association for Thermogenesis

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JMJD1A, a histone H3 lysine 9 (H3K9) demethylase, contributes to β-adrenergic-induced systemic metabolism and body weight control. We show that JMJD1A is phosphorylated at Ser-265 by protein kinase A (PKA), and this is pivotal to activate expression of the β1-adrenergic receptor gene (*Adrb1*) and downstream targets, including the *Ucp1* gene, in brown adipocytes. Phosphorylation of JMJD1A by PKA increases its interaction with the switch/sucrose nonfermentable (SWI/SNF) nucleosome remodeling complex and DNA-bound PPARγ. This complex confers β-adrenergic-induced JMJD1A recruitment to target sites, which is a prerequisite for subsequent genome-wide JMJD1A demethylation. Interestingly, mutation of Ser-265 to alanine abolished interactions with SWI/SNF and prevented long-range chromatin interactions without affecting JMJD1A demethylase activity. Thus, our results show that JMJD1A has two important roles in regulating hormone-stimulated higher order chromatin dynamics that modulates thermogenesis in brown adipocytes. In one role, JMJD1A is recruited to target sites and functions as a cAMP-responsive scaffold that facilitates long-range chromatin interactions, and in the second role, JMJD1A demethylates H3K9 dimethylation.

43

Pharmacological and Toxicological Characterization of Novel LXR Modulators

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Liver X receptors (LXR) are important regulators of cholesterol metabolism and potential targets for the development of anti-atherosclerotic drugs. However, most synthetic LXR agonists to date also cause hepatosteatosis and plasma triglyceridemia. We identified two chemical series of LXR modulators using high throughput screening, and they were further developed using a combination of *in vitro* and *in vivo* assays. To identify compounds with desirable profiles (*i.e.* effect on genes involved in reverse cholesterol transport but without liver steatosis and plasma hypertriglyceridemia), we focused on an unbiased *in vivo* screen in hamsters. The link between receptor modulation and effects on atherosclerosis was established in both progression and regression studies in mouse atherosclerosis disease models. Interestingly, the LXR-treated mice had less atherosclerosis compared with controls and also a striking survival benefit over control animals. In addition, we could show that these compounds stimulated reverse cholesterol transport in both CETP and non-CETP species (mice and hamsters). AZD1437 was chosen as a candidate drug and was further evaluated in preclinical toxicological models. The initial safety assessment in rat and dog looked promising with no adverse effects. However, unexpectedly, studies with longer duration and high exposure of AZD1437 led to adverse effects in the hearts of both species. Several studies to shed light on these unexpected findings were initiated, and based on those data, a number of follow-up compounds from different chemical series were identified. AZD3971 was nominated as a back-up compound and went through a similar *in vivo* safety assessment as AZD1437, and although the adverse events were much less severe compared with AZD1437, the safety margins were still not sufficient to continue the development of the compound. Why did two unrelated LXR agonists from different chemical series cause adverse cardiac events? Data supporting likely explanations will be shown and possible ways to avoid these types of effects for future LXR drug development will be discussed.

44

Brown Adipose Tissue Plays a Role in Regulating Fetal Growth by Undergoing a Gestation-dependent Phenotypic Change

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Pregnancy is associated with increased maternal plasma lipid levels, an important physiological response to support the nutritional demand of the developing fetus and fulfill the maternal requirement for the production of pregnancy hormones. The homeostatic controls of metabolically active organs are known to change in response to pregnancy signals. We hypothesized that pregnancy affects brown adipose tissue (BAT) phenotype and function and, as a consequence, plays a role in the regulation of fetal development and growth.

Using mice at gestational day 14 of pregnancy (GD14) or non-pregnant controls, we showed that the interscapular BAT (iBAT) was hypertrophied and contained enlarged lipid droplets. This was accompanied by a decrease in the expression of uncoupling protein 1 and other markers of brown adipose tissue. In parallel, the expression of white adipose markers was increased at GD14, indicating that iBAT at GD14 loses a BAT phenotype but gains a white adipose phenotype. At a functional level, the norepinephrine-mediated energy expenditure and increase in respiratory quotient observed in terminally anaesthetized non-pregnant mice were both abrogated in GD14 mice. This suggests that inducible thermogenesis and fuel utilization in thermogenic tissue are modified by pregnancy. To study the impact of the pregnancy-dependent phenotypic change on maternal and fetal parameters during the growth phase of the fetus (GD14–GD19), the iBAT was surgically ablated in female mice prior to mating, and the mice were sacrificed at GD18. Ablation of iBAT resulted in a significant increase in normalized maternal body weight at GD18. Additionally, fetal weight at GD18 was significantly higher, with a wider distribution than the sham-operated group, indicating that the removal of the main BAT depot results in the dysregulation of fetal growth. These data shed new light on a pregnancy-brown adipose tissue axis that appears to play a role in the regulation of fetal growth driven by a gestational reduction in classical BAT phenotype.

45

T-cadherin-mediated Endocytosis of Adiponectin Produces Exosome Containing T-cadherin and Adiponectin

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Adiponectin, an adipocyte-secreted abundant protein, accumulates in the heart, vascular endothelium, and skeletal muscles through interactions with T-cadherin, a glycosylphosphatidylinositol (GPI)-anchored protein. Although our studies have strongly suggested that this accumulation is essential for adiponectin-mediated cardiovascular protection, the molecular mechanism of it has not been fully elucidated. Several GPI-anchored proteins have been revealed to be internalized into cells and secreted as an exosome cargo. Exosomes, extracellular vesicles secreted from the endosomal membrane compartment, have recently been recognized as playing a critical role in intercellular communication and also are suggested to be involved in cardiovascular protection. We hypothesized that adiponectin enters the endosomal route by binding to T-cadherin, is retroendocytosed, and exerts cardiovascular protective effects as an exosome cargo. The adiponectin accumulation in endothelial cells depended on T-cadherin expression and was indeed increased by overexpression, and decreased by knockdown, of T-cadherin in endothelial cells. Confocal microscopy analysis revealed that adiponectin and T-cadherin were localized not only on the cell surface but also inside of cells. They co-localized with markers of multivesicular bodies and exosomes, such as *N*-rhodamine-phosphatidylethanolamine and CD63. Electron microscopy analysis confirmed adiponectin localization in exosomes (intraluminal vesicles) included in multivesicular bodies, where exosome is generated by budding off of limiting membrane. An Optiprep™ density gradient following standard differential centrifugation-based purification from the conditioned medium revealed the existence of a substantial amount of adiponectin and T-cadherin in the exosome fraction. Adiponectin associating with exosome was also increased by overexpression, and decreased by knockdown, of cellular T-cadherin, as was seen in cellular accumulation. Exosomes containing both adiponectin and T-cadherin were also detected in mouse and human serum. Altogether, our current data have clearly demonstrated a unique molecular model, where adipose-derived adiponectin enters into the endosomal route to multivesicular bodies by binding to cell surface T-cadherin and is resecreted as an exosome cargo with T-cadherin. This is the first demonstration that a secretion factor can be retroendocytosed as an exosome cargo. Finally, the possibility that adiponectin, a so-called pleiotropic adipose-secreted factor, might exert its cardioprotective functions by modulating intercellular communication and activities of exosomes warrants future investigation.

46

Thermogenic Activity of UCP1 in Human White Fat-derived Beige Adipocytes

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Heat-producing beige/brite (brown-in-white) adipocytes in white adipose tissue (WAT) have the potential to suppress metabolic disease in mice and hold great promise for the treatment of obesity and type 2 diabetes in humans. Here, we demonstrate that human adipose-derived stromal/progenitor cells (hASCs) from subcutaneous WAT can be efficiently converted into beige adipocytes. Upon pharmacological activation of Pparg, hASC-derived adipocytes activated beige fat-selective genes and a unique brown/beige fat-specific electron transport chain (ETC) gene program. Importantly, hASC-derived beige fat cells displayed the bioenergetic characteristics of genuine brown fat cells, including a capacity for increased respiratory uncoupling in response to β-adrenergic agonists. Furthermore, knockdown experiments underscore that the thermogenic capacity of human beige fat cells was entirely dependent on the presence of UCP1. In summary, this study reveals that hASCs can be readily differentiated into beige adipocytes that, upon activation, undergo UCP1-dependent thermogenesis.

47

Adaptor Protein PID1 Is a Molecular Switch for LRP1 Function in Liver and Adipose Tissue

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The LDL receptor-related protein 1 (LRP1) is important for the rapid clearance of proatherogenic lipoprotein remnants into the liver. Furthermore, in adipose tissue, LRP1 is located within endosomal vesicles containing the insulin-dependent glucose transporter Glut4. The phosphotyrosine interaction domain-containing protein 1 (PID1) has been identified as an adaptor protein for LRP1. In the current study, we investigated the functional consequences of PID1 loss with regard to LRP1 localization and its role in systemic lipoprotein and glucose metabolism. Silencing of PID1 was mediated by siRNA and LRP1, and Glut4 localization was visualized by immunhistochemistry. PID1 knockout mice were generated and fed with a high fat diet. Subsequently, oral glucose as well as insulin tolerance tests were performed. The organ-specific glucose uptake was investigated using radioactively labelled tracers. Gene expression analysis was performed by TaqMan® analysis. In the absence of PID1, LRP1 in liver and LRP1 as well as Glut4 in adipose tissue were sorted to the plasma membrane independent of insulin signaling under fasting conditions. Notably, loss of PID1 enhances triglyceride and glucose clearance *in vitro* and *in vivo*, which was associated with facilitated glucose-dependent expression of *de novo lipogenesis* genes. Our data support the model that phosphorylation of LRP1 induced by exogenous stimuli interrupts the LRP1-PID1 interaction, resulting in LRP1 translocation to the cell surface. Next to an altered lipoprotein uptake into the liver, PID1 might also be involved in the sorting of Glut4 storage vesicles in insulin-responsive tissues, thereby influencing systemic plasma glucose metabolism.

48

Insulin-dependent Triglyceride-rich Lipoprotein Uptake into Brown Adipose Tissue

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Catabolic, cold-activated brown adipose tissue (BAT) burns triglycerides stored in lipid droplets for heat production. Consequently, endogenous lipid stores need to be replenished by anabolic processes. The canonical lipid uptake pathway involves the hydrolysis of triglyceride-rich lipoproteins (TRL) by active lipoprotein lipase (LPL) and the subsequent fatty acid uptake by active adipocytes. In addition to fatty acid uptake, there is evidence for a whole TRL particle internalization into active BAT. The aim of this study was to investigate the catabolic and anabolic processes in lipid handling of activated BAT. BAT activity was stimulated by cold exposure or CL316,243 (CL) treatment in wild-type and transgenic mice. The uptake processes were investigated by metabolic turnover studies, intravital microscopy, and electron microscopy after the injection of nanoparticle-labeled TRL. Increased BAT activity is accompanied by enhanced insulin secretion. CL treatment stimulated anabolic processes in BAT via insulin-dependent phosphorylation of Akt kinase. This process is dependent on CD36 expression and associated with facilitated whole particle uptake, which was visualized by electron microscopy. Inhibition of insulin secretion using the potassium channel agonist diazoxide during activation abolished TRL uptake into BAT. Our data show that cold promotes catabolic as well as anabolic processes in BAT, whereas insulin orchestrates metabolic pathways that control lipoprotein handling for the replenishment of endogenous energy stores. Impaired lipoprotein processing mediated by BAT in insulin-resistant states could lead to dyslipidemia observed in patients suffering from type 2 diabetes mellitus.

49

Lpcat3-dependent Production of Arachidonyl Phospholipids Is a Key Determinant of Triglyceride-rich Lipoprotein Production

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The phospholipid composition determines the biophysical character of membranes and impacts the membrane-associated biological processes. However, the physiological role of specific phospholipids *in vivo* has been difficult to assess due to an inability to selectively manipulate membrane composition in animals. Here, we define a nuclear receptor pathway for the dynamic modulation of membrane composition in response to changes in cellular lipid metabolism. Ligand activation of liver X receptors (LXRs) preferentially drives the incorporation of polyunsaturated fatty acids into phospholipids through induction of the remodeling enzyme lysophosphatidylcholine acyltransferase 3 (Lpcat3). Using Lpcat3-deficient mice, we further demonstrate that Lpcat3 is uniquely required for the incorporation of arachidonic acid into hepatic and intestinal membranes i*n vivo* and that an absence of arachidonyl phospholipids profoundly affects lipid transport and lipoprotein production. Biophysical studies indicate that Lpcat3-dependent production of arachidonyl phospholipids is important for lipid movement within membranes. These studies identify Lpcat3-dependent phospholipid remodeling as a critical, LXR-regulated step in lipid transportation and highlight a previously unrecognized requirement for a specific membrane lipid class in lipoprotein metabolism.

50

Perilipin 5 Overexpression in Skeletal Muscle Increases Fibroblast Growth Factor 21 Expression and Benefits Systemic Metabolism

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Perilipin 5 (PLIN5) coats lipid droplets and regulates lipid metabolism by recruiting enzymes that function in lipolysis or lipid synthesis to lipid droplets. PLIN5 increases triacylglycerol storage. In fact, its overexpression in the heart leads to cardiac steatosis and dysfunction. This is consistent with the notion that ectopic lipid is metabolically disruptive. In contrast, expression of PLIN5 in skeletal muscle (SM) positively correlates with insulin sensitivity despite increasing triacylglycerol storage. Lipid accumulation in the SM of obese individuals is linked to the development of insulin resistance, a precursor to developing diabetes. SM lipid accumulation is also observed in insulin-sensitive athletes. These contradictory findings have been termed the "athlete's paradox." Interestingly, obese individuals have lower PLIN5 expression in adipose tissue and SM compared with athletes. This suggests that PLIN5-regulated lipid metabolism is beneficial, especially in the context of SM lipid storage. To examine whether increased PLIN5 expression enhances metabolic flexibility, we developed a mouse line with muscle creatine kinase (MCK) promoter-driven PLIN5 overexpression in skeletal muscle (MCK-Plin5 mice). We show that MCK-Plin5 mice accumulate significantly more triacylglycerol in their muscle, and their insulin sensitivity is unaffected compared with non-transgenic mice. MCK-Plin5 mice also have increased metabolism and are resistant to some of the adverse effects of high fat feeding. For instance, high-fat-fed MCK-Plin5 mice have lower liver cholesterol and expression of inflammatory markers. SM secretes hormones (myokines) that regulate glucose and lipid metabolism in muscle and target peripheral tissues, such as liver and adipose tissue. We found that PLIN5 overexpression in SM caused an 80-fold increase in the gene expression of the myokine fibroblast growth factor 21 (FGF21) and a concomitant rise in serum FGF21 protein. Thus, PLIN5 expression leads to enhanced metabolic flexibility, which occurs, at least in part, through the FGF21 signaling pathway.

51

ANGPTL3 Antibody Reduces Circulating Lipids in Dyslipidemic Mice and Monkeys

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Angiopoietin-like protein 3 (ANGPTL3) is an important post-translational regulator of lipoprotein lipase (LPL) and endothelial lipase, key enzymes involved in the hydrolysis of triglycerides (TG) and phospholipids in plasma lipoproteins. Because humans with loss-of-function variants in *ANGPTL3* have low levels of circulating triglycerides and cholesterol, we assumed a similar phenotype following antibody blockade of ANGPTL3. Thus, we generated a fully human monoclonal antibody (REGN1500) that binds ANGPTL3 from humans, monkeys, and mice with high affinity and reverses ANGPTL3-induced inhibition of LPL activity *in vitro*. Administration of REGN1500 increased post-heparin plasma LPL activity and caused a profound decrease in plasma TG levels in normolipidemic and dyslipidemic mice. Studies in *Lipg* (endothelial lipase) knockout mice revealed that REGN1500 affects serum HDL-C through an endothelial lipase-dependent mechanism. Finally, single administration of REGN1500 to dyslipidemic cynomolgus monkeys caused a rapid and pronounced decrease in plasma TG, non-HDL-C, and HDL-C. REGN1500 normalized circulating TG levels even in monkeys with baseline plasma TG ≥400 mg/dl. Collectively, these data demonstrate that the ANGPTL3-neutralizing antibody REGN1500 consistently reduces plasma lipids in dyslipidemic mice and monkeys and thus provides a potential therapeutic avenue for the treatment of patients with hyperlipidemia.

52

GPIHBP1 and ANGPTL4: Regulators of Lipoprotein Lipase Function

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The absorption, packaging, and delivery of fat to appropriate peripheral tissues is essential for maintaining metabolic homeostasis, and defects or dysregulation of these processes can contribute to metabolic disorders such as diabetes, obesity, and hyperlipidemia. In the intestine, dietary fat is packaged into triglyceride-rich lipoprotein particles and delivered to peripheral tissues through the circulatory system. Lipolysis of these triglycerides requires the enzyme lipoprotein lipase (LPL) and takes place on the luminal surface of capillary endothelial cells. Lipolysis by LPL is regulated in part by two proteins, GPIHBP1 and ANGPTL4. GPIHBP1, a GPI-anchored protein of capillary endothelial cells, is responsible for transporting LPL across endothelial cells to the capillary lumen. Without this transport, LPL becomes mislocalized to the interstitial space and cannot access triglyceride-rich lipoproteins, resulting in severe hypertriglyceridemia. Conversely, ANGPTL4 inhibits LPL, and ANGPTL4 deficiency results in increased LPL activity and lower plasma triglyceride levels. Our goal is to understand how the interactions between LPL, GPIHBP1, and ANGPTL4 influence the delivery of triglyceride-derived fatty acids to tissues. Here, we focus on two peculiar findings. 1) Although GPIHBP1 and ANGPTL4 appear to have opposing functions with regard to LPL, expression of both genes is induced by fasting. 2) Unlike GPIHBP1-deficient mice, which are severely hypertriglyceridemic, *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice have relatively normal triglyceride levels. We show evidence that whereas *Angptl4* and *Gpihbp1* expression both increase in multiple tissues upon fasting and *Angptl4* expression increases relatively quickly after the onset of fasting, *Gpihbp1* expression only increases after a prolonged fast. We also demonstrate that the peculiar phenotype in *Gpihbp1*$^{-/-}$*Angptl4*$^{-/-}$ mice is not due to overexpression of *Lpl* or increased lipase activity.

53

Knocking Down Hsc70 Curtails VLDL Secretion from the Liver by Disrupting the ER-to-Golgi VLDL Transport

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Aberrant secretion of very low-density lipoproteins (VLDL) by the liver leads to the pathogenesis of dyslipidemia, which is associated with various metabolic disorders, such as type-2 diabetes and cardiovascular diseases. The rate-determining step in the secretion of VLDLs from the liver is their transport from the endoplasmic reticulum (ER) to the Golgi, and this step represents a potential therapeutic target in controlling VLDL secretion. We have discovered a distinct ER-derived vesicle, VLDL transport vesicle (VTV), which mediates the targeted delivery of VLDLs from the ER to the Golgi. We reported earlier that a specific fusion complex, composed of a unique set of soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, mediates VTV-Golgi fusion and that the assembly of fusion complex requires hepatic cytosol. However, cytosolic proteins regulating the fusion complex formation remain unidentified. To identify cytosolic proteins, we performed a series of chromatographic steps, including FPLC, on liver cytosol and isolated a complex of three cytosolic proteins, which we found is required for *in vitro* VTV-Golgi fusion. We carried out a detailed proteomic analysis utilizing two-dimensional gels and nano-MS/MS techniques and identified one of the three proteins as hsc70. Immunodepletion of hsc70 from the hepatic cytosol resulted in a significant reduction in fusion complex formation and subsequent VTV-Golgi fusion, whereas the addition of recombinant hsc70 to the depleted cytosol completely restored the activity. Consistent with the role of hsc70 in fusion complex formation, knockdown of hsc70 significantly inhibited *in vitro* VTV-Golgi fusion and VLDL secretion from hepatocytes. We conclude that hsc70 regulates the ER-to-Golgi transport of VLDL by facilitating the formation of fusion complex and plays an important role in VLDL secretion from the liver.

54

High-performance Gel Permeation Chromatography Analysis Characterizes Lipoprotein Profiles in Patients with Cholesteryl Ester Transfer Protein Deficiency

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Recent studies have suggested that HDL efflux capacity could be a potentially beneficial assay to predict the risk of cardiovascular diseases, compared with HDL cholesterol (HDL-C) levels. Here, we developed a novel methodology using high-performance gel permeation chromatography (HPLC) to evaluate lipoprotein subclasses. Five CETP-deficient (CETP-D) patients, whose serum CETP mass was <0.1 μg/ml, and five normolipidemic controls were investigated. We examined particle numbers of each 20 lipoprotein subclasses by HPLC, which is a newly developed LipoSEARCH® system (Skylight Biotech Inc., Akita, Japan). As we reported previously, serum HDL-C levels were markedly elevated in CETP-D patients compared with controls. In addition, the number of very small LDLs, which is known to be atherogenic, was significantly higher in CETP-D patients than that in controls (254.0 ± 53.5 *versus* 167.8 ± 24.3 nM, $p = 0.011$). On the other hand, the number of small and very small HDLs, which have anti-atherogenic function, was significantly lower in CETP-D patients (4.36 ± 0.55 *versus* 5.52 ± 0.41 μM; $p = 0.005$, 1.91 ± 0.34 *versus* 3.14 ± 0.18 μM; $p = 0.0001$, respectively). We have characterized lipoprotein subclasses in CETP-D patients, suggesting that the particle number of very small LDL was increased and that the particle number of small and very small HDL was decreased. Our results may indicate a proatherogenic lipoprotein profile by CETP deficiency.

55

Lamin B Receptor Null Mice Show Cutaneous Lipid Abnormalities Consistent with a Critical Role for LBR in Epidermal Lipid Homeostasis and the Late Steps of Cholesterol Biosynthesis

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We identified in the Lamin B receptor (*LBR*) murine gene a spontaneous mutation consisting of a 49-bp deletion spanning the end of exon 6 and its splice donor site. *LBR* mRNA was not detected by qPCR in tissues from these mice, showing that it produces a null mutation. On an inbred C57BL/6J genetic background, *LBR* null mice had embryonic and early lethality. However, approximately 10% of null mice survived to about 2–3 weeks of age and developed neonatal ichthyosis, alopecia, a compromised skin barrier, and peripheral lipoatrophy. In addition to these phenotypes, *LBR* null mice bred on the mixed 129-C57BL/6J genetic backgrounds, developed kyphosis, and survived to approximately 5 months of age. Skin histology showed extensive keratinization of the surface and hair follicle epithelia. In the dermis, a marked decrease in or absence of adipocytes was replenished by a proliferative epidermis. Electron microscopy of the epidermis uncovered the presence of abnormal lamellar bodies, which normally package cholesterol, fatty acids, and ceramides. Analyses of epidermal and dermal lipids from *LBR* null mice with ichthyosis of both genetic background strains revealed substantial accumulation only in the epidermis of a lipid that we unveiled by mass spectrometry to consist of 5α-cholesta-8,14-dien-3β-ol, an intermediary sterol synthesized during the late steps of cholesterol biosynthesis. Furthermore, saturated very long chain fatty acids (22:0, 24:0, 26:0) and their respective conjugated ceramides were 4–10-fold higher in null than in control mice. The role of *LBR* in the distant cholesterol biosynthesis pathway is consistent with its predicted cytoplasmic domain as a sterol 14α-reductase. Taken together, our studies demonstrate that perturbed epidermal lipid biosynthesis associated with packaging of abnormal lipids into lamellar bodies results in the secretion of detrimental lipids to the stratum corneum, resulting in ichthyosis and its sequelae. Thus, the susceptibility of the epidermis to the absence of *LBR* protein supports a critical role for *LBR* in epidermal murine lipid homeostasis. Because the other protein domain of *LBR* is localized in the nucleus and anchors chromatin, further characterization of the mice is under way to uncover potentially detrimental effects of *LBR* nuclear function.

56

The Multitissue cis-eQTL Landscape in Coronary Artery Bypass Grafting Patients: The Stockholm Atherogenesis and Gene Expression (STAGE) Study

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Genome-wide association studies (GWAS) have identified well over 100 loci showing genome-wide significance for association with cardiovascular and coronary artery disease (CAD). However, with the exception of a few examples, such as the SORT1 locus, the mechanisms underlying these associations are not well understood. With the aim to better comprehend the relevance of genetic variation for CAD, we here dissect the consequences of single nucleotide polymorphisms (SNPs) on gene expression in carotid plaques and other CAD-relevant tissues.

Blood and biological specimens from 100 patients who underwent coronary artery bypass grafting (CABG) surgeries were collected for genome-wide genotyping and expression profiling. We performed expression quantitative trait loci (eQTL) analysis using Matrix_EQTL of 7M 1000G-imputed variants and 40K protein-coding probe sets in individual tissues and across seven tissue types: atherosclerotic arterial wall (AAW, $n = 63$), internal mammary artery (IMA, $n = 74$), liver ($n = 72$), skeletal muscle ($n = 74$), whole blood ($n = 94$), subcutaneous ($n = 60$), and visceral ($n = 82$) fat. We identified 370,871 unique eQTLs in 7,194 genes (FDR ≤ 0.05), present in at least one tissue. Initial screening against the GWAS catalog among Caucasians yielded 489 GWAS SNP-anchoring eQTLs; in 60% of these ($n = 302$), reported genes do not contain the corresponding eQTL gene. This shows that the biological functions of association loci are often obscure and that eQTL studies are informative resources for unveiling additional insights into the association of complex phenotypes. To understand the roles of the identified eQTLs in coronary plaques in the 100 CABG patients in STAGE, we first restricted our attention to the top ~5% (FDR ≤ 5×10^{-10}, $n = 19,333$) that are putatively functional (contain SNPs in coding/regulatory or ncRNAs regions, $n = 7,157$) and are not present in population negative controls (GTEx version 1) (335 genes in 7,076 eQTLs). Gene ontology enrichment analysis of the top cis-genes confirms the importance of metabolic/biosynthetic processes of lipids, lipoprotein, and fatty acids (in liver, muscle, and whole blood) and antigen processing and peptidase activities in the context of inflammatory response (in liver and visceral fat). This work provides an improved understanding of the underlying genetic mechanisms and multitissue eQTL landscape in CAD patients, paving the path forward to informed molecular validations for disease biology.

57

Glucagon-like Peptide-1 Reduces Chylomicron Secretion Despite Adequate Intestinal Lipid Availability

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Chylomicron overproduction and associated postprandial dyslipidemia are common features of insulin resistance and the metabolic syndrome. This results in heightened levels of atherogenic chylomicron remnants that can increase the risk of cardiovascular disease. Recently, the hormone glucagon-like peptide-1 (GLP-1) has been shown to impair chylomicron production and prevent the development of diabetic dyslipidemia. In healthy humans who received lipid intraduodenally, these effects on chylomicron particle number took place despite normal postprandial lipemia. Thus, our study aimed to assess whether GLP-1 could lower chylomicron production despite adequate lipid availability by impairing intracellular particle trafficking within intestinal enterocytes. To test this, hamsters were administered olive oil intraduodenally to bypass the stomach, preceded by an intraperitoneal injection of the GLP-1 receptor agonist exendin-4 or vehicle. They then received an intravenous infusion of Triton to prevent lipoprotein catabolism and clearance. Plasma was collected over a 4-h period, and the triglyceride (TG)-rich lipoprotein (TRL) fraction of the plasma was isolated for TG and apolipoprotein B48 (apoB48) measurements, the latter reflecting the number of chylomicron particles present. Exendin-4 treatment had no effect on plasma or TRL TG levels but significantly lowered TRL-apoB48 levels at 4 h. This resulted from an increase in lipoprotein particle size (TG loading), as determined by FPLC, despite the presence of fewer particles. Whereas exendin-4 had no effect on 4 h jejunal activity of microsomal TG transfer protein, responsible for apoB48 lipidation, there was an increasing trend in jejunal tissue TG levels ($p = 0.084$). Furthermore, a reduction in jejunal CD36 levels was observed at 4 h with exendin-4 treatment, and CD36 is needed for proper formation of prechylomicron transport vesicles for intracellular chylomicron trafficking within the enterocyte. Overall, we demonstrate a role for GLP-1 in reducing the number of chylomicron particles secreted by the intestine, even during adequate lipid availability and elevated particle lipidation. This impairment in apoB48 secretion was associated with lower jejunal levels of CD36, involved in intracellular chylomicron trafficking. Future studies will aim to assess the effects of GLP-1 on intracellular signaling pathways linked to chylomicron trafficking, providing mechanistic insight into the actions of this antidiabetic hormone.

58

The Antioxidant Enzyme PRDX1 Alters Glucocorticoid Receptor Interaction with Chromatin in Human Lung Cancer

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Cancer cells maintain their transcriptional machinery and survive under extreme metabolic stress. To do so, many types of cancers and especially lung cancer up-regulate the levels of antioxidant peroxiredoxin (PRDX) family enzymes in the nucleus. Another highly expressed protein in lung cancer is the glucocorticoid receptor (GR). We used metastatic and primary lung cancer cell lines with different glucocorticoid sensitivities as well as normal human bronchial epithelial cells (HBECs) to study the role of PRDX1 and GR in lung cancer. Whereas PRDX1 and GR interacted in both normal and cancer lung cells, PRDX1 knockdown (KD) resulted in the death of cancer cells but not HBECs. Utilizing protein binding arrays (PBMs), we found that GR and PRDX1 were co-localized at numerous AT-rich promoter sequences in normal and lung cancer cells, but >100 sequences from promoters of disease-related genes (including 15 well known cancer driver genes) were differentially bound by GR-PRDX1 in metastatic and primary tumor cells *versus* normal cells. To investigate the cause of differential DNA binding, we employed rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME) to analyze the protein complexes on DNA. GR formed complexes with proteins known to be implicated in lung cancer, metastasis, and drug resistance in the cancer but not the normal cells. We also found that PRDX1 KD disrupted the interaction of GR with numerous transcription and chromatin-remodeling factors while promoting the interaction with DNA methyltransferases and other factors in lung cancer cells. Co-IP experiments showed that the PRDX1 KD increased tyrosine nitration of HBEC GR complexes but decreased nitration of lung cancer GR complexes. RIME also revealed nitration sites on GR, co-repressors, chromatin remodelers, and histone acetyltransferases, some of which were affected by PRDX1 KD. Because in plants and bacteria, PRDX1 has been shown to reduce protein nitration, we propose that PRDX1 removes nitration from certain chromatin remodelers, which in turn affects PRDX1 binding to AT-rich motifs in the promoter region of GR target genes as well as the interactions between GR and co-activators. The net result could be altered sensitivity to glucocorticoids.

59

Receptor-medicated ER Exit: Two Tails Are Better than One?

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The cellular secretory pathway packages numerous cargoes into the transport vesicles coated by the COPII complex for exiting the endoplasmic reticulum (ER) and delivery to the Golgi. This process is often aided by transmembrane cargo receptors that efficiently recruit specific secretory cargoes. Moreover, cargo receptors contain specific sorting motifs that are directly recognized by the SEC24 subunit of COPII. Nevertheless, the mechanism to achieve high capacity while maintaining specificity in the interaction between cargo receptors and the COPII complex remains to be fully illustrated. We recently adapted a proximity-dependent biotinylation approach to track the dynamics of COPII-medicated ER exiting in cells. With this approach, we found that the cargo receptor LMAN1 is highly enriched in COPII-coated vesicles. The recruitment of LMAN1 into COPII vesicles is dependent on the FF sorting motif located at the cytosolic tail of LMAN1. Surprisingly, we found that LMAN1 recruitment into COPII vesicles also requires its dimerization within the ER lumen, constituting a dimeric sorting signal for SEC24 recognition. Indeed, an LMAN1 mutant missing the FF sorting signal displays a "posing" effect in the recruitment of wild type LMAN1 into COPII vesicles. We propose that dimeric sorting signals may represent a mechanism for the COPII complex to expand its capacity while maintaining specificity during cargo recognition. Current efforts are aimed at illustrating the process with structure-function studies.

60

Visualization of Tissue-specific, Subcellular ApoA-I Dynamics and Peripheral Cholesterol Clearance in Live Zebrafish

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The biological significance of why apolipoprotein A-I (APOA-I), the main structural component of high-density lipoproteins, is expressed by both the human liver and intestine is not currently understood. Additionally, subcellular processing and function of APOA-I remains poorly characterized. The optically clear larval zebrafish presents an excellent opportunity to visualize global and cellular APOA-I dynamics in a live animal. Zebrafish have two ApoA-I proteins; our *in situ* hybridization analysis shows that both expressed in the yolk syncytial layer during development. In larvae, ApoA-Ia is expressed strongly in the intestine and weakly in the liver, whereas ApoA-Ib is expressed only in the liver. To study APOA-I of hepatic *versus* intestinal origin *in vivo*, we created transgenic zebrafish expressing fluorescently labeled human or zebrafish APOA-I driven by liver- or intestine-specific promoters. Confocal microscopy of live larvae reveals secretion of the fusion proteins into the circulation and localization of APOA-I-mCherry to specific tissues and subcellular domains. Fluorescent puncta were observed in the apical lysosomal/late endosomal compartment of intestinal enterocytes of larvae expressing hepatic APOA-I-mCherry. APOA-I-mCherry of intestinal origin colocalizes with LysoTracker-positive and -negative hepatocyte organelles. These observations suggest previously unappreciated roles for the intestine and liver in the recycling and/or degradation of human and zebrafish ApoA-I of hepatic origin and intestinal origin, respectively. Ongoing work is investigating intracellular transport dynamics with fish expressing APOA-I fused to the photoconvertible Eos protein. APOA-I transports peripheral cholesterol to the liver for excretion in the bile (reverse cholesterol transport; RCT). We developed an assay of RCT: fluorescently labeled cholesteryl ester (F-CE) is injected into the somites of live larvae, and fluorescence clearance is quantified. Forty-eighty hours following injection, a 70% decrease in fluorescence and macrophage recruitment was observed; extraction of total lipids and subsequent fluorescence quantification suggests that the F-CE is excreted, as occurs in RCT. We hypothesize that F-CE clearance will be accelerated in larvae overexpressing zebrafish ApoA-I fusion proteins. In conclusion, we harnessed the larval zebrafish to visualize lipoprotein dynamics at both the multiorgan and subcellular levels and peripheral cholesterol clearance. To our knowledge, this is the first time tissue-specific apolipoprotein transport has been visualized *in vivo*.

61

Reduced PPARγ-driven Lipogenesis Protects ATGL-deficient Mice from Diet-induced Obesity

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Adipose triglyceride lipase (ATGL) initiates triglyceride (TG) hydrolysis in adipose and non-adipose tissues. In humans, ATGL deficiency causes neutral lipid storage disease with myopathy (NLSDM) characterized by a systemic neutral lipid accumulation, yet NLSDM patients are not obese. In mice, a genetic deletion of ATGL (AKO) also causes TG accumulation in many non-adipose tissues, but again, mutant mice are only moderately obese. The absence of massive obesity in humans and in mice is unexpected considering the importance of the enzyme for TG catabolism. In this study, we identify the counteracting mechanism that prevents excessive adipose lipid accumulation in the absence of ATGL. We used a "healthy" AKO mouse model expressing ATGL exclusively in cardiac muscle (AKO/cTg) to circumvent the cardiomyopathy and premature lethality observed in AKO mice. Counterintuitively, AKO/cTg mice were protected from high-fat diet (HFD)-induced obesity despite complete ATGL deficiency in adipose tissues. As expected from leaner animals, hyperinsulinemic-euglycemic clamp experiments revealed that AKO/cTg mice were highly insulin-sensitive. Instead, ATGL deficiency caused aberrant peroxisome proliferator-activated receptor-γ (PPARγ) signaling, leading to delayed adipogenesis and impaired lipid synthesis in white adipose tissue of AKO/cTg mice. Luciferase reporter assays provided direct evidence that ATGL-mediated lipolysis activates PPARγ. These results argue for the existence of an interdependence between lipolysis and lipid synthesis via the regulation of PPARγ by ATGL. Pharmacological inhibition of ATGL may prove useful to prevent obesity.

62

Knockdown of Hepatic Apoc3 Reduces Hypertriglyceridemia and Atherosclerosis

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A common characteristic of diabetic dyslipidemia is increased plasma concentrations of triglyceride (TG)-rich very low-density lipoprotein (VLDL), an independent risk factor for the development of coronary heart disease. Apolipoprotein C-III (apoC-III) is a known negative regulator of lipoprotein lipase activity, and loss-of-function mutations in apoC-III have been associated with significantly reduced plasma TG and reduced cardiovascular disease risk. Hence, antagonism of apoC-III is a potential therapeutic strategy for the treatment of hypertriglyceridemia and atherosclerosis. However, the effect of inhibiting *Apoc3* on atherogenesis, in a preclinical model of atherosclerosis, has not been reported. We used siRNA to silence *Apoc3* in male CETP-transgenic, *Ldlr*$^{-/-}$ mice fed a high-fat diet for 12 weeks. At the end of the study, liver *Apoc3* mRNA was markedly reduced (−95%), whereas intestinal *Apoc3* mRNA was unchanged. The hepatic knockdown of *Apoc3* was accompanied with decreased plasma apoC-III and decreased plasma TG (−90 and −54%, respectively); total plasma cholesterol was unaffected. Liver TGs were reduced (−40%) in animals treated with *Apoc3* siRNA. Aortic root Oil Red-O staining was attenuated by *Apoc3* siRNA treatment (−15%), which was associated with a 30% reduction in aortic root TG content. Plaque cholesteryl esters and lysophosphatidylcholines were unaffected by hepatic *Apoc3* knockdown. These studies suggest that liver-specific silencing of *Apoc3* reduces hypertriglyceridemia and attenuates atherogenesis.

63

Adaptors Dab2 and Arh in LDL Receptor Endocytosis and Lipoprotein Metabolism

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Disabled-2 (Dab2) is a widely expressed clathrin-binding endocytic adaptor protein with a phosphotyrosine-binding (PTB) domain and has some features similar to those of another adaptor, Arh (autosomal recessive hypercholesterolemia protein). Dab2 knockout mice die at an early embryonic stage around E5.5 because of disorganization of the extraembryonic endoderm. We constructed a floxed Dab2 conditional mutant line and generated a Dab2 mosaic deletion using SOX2-Cre to restrict gene deletion within the embryo proper. By 3 weeks of age, Dab2 gene deletion was found in the majority (99%) of the cells of the conditional mutant mice. The mutant mice appear normal and fertile and have a normal life span. Further investigation uncovered several defects in the Dab2 conditional knockout mice, including LDL and cholesterol metabolism. Furthermore, we were able to generate mutant mice null for both Dab2 and ARH. The double knockout mice are viable and grossly normal but had serum cholesterol level comparable with those of LDL receptor knockouts. The essential role of Dab2 and Arh in LDL uptake was observed in mouse embryonic fibroblasts prepared from the mutant mice. These data suggest Dab2 and Arh play complementary but not redundant roles as endocytic adapters for LDL receptor endocytosis and lipoprotein metabolism.

64

Structure of the WD40 Domain of SCAP from Fission Yeast Reveals the Molecular Basis for SREBP Recognition

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The sterol regulatory element-binding protein (SREBP) and SREBP cleavage-activating protein (SCAP) are central players in the SREBP pathway, which controls cellular lipid homeostasis. SCAP binds to SREBP through their respective carboxyl (C) domains and escorts SREBP from the endoplasmic reticulum to the Golgi upon sterol depletion. A conserved pathway, with the homologues of SREBP and SCAP being Sre1 and Scp1, was identified in the fission yeast *Schizosaccharomyces pombe*. Here we report the *in vitro* reconstitution of the complex between the C domains of Sre1 and Scp1 as well as the crystal structure of the WD40 domain of Scp1 at 2.1 Å resolution. The structure reveals an eight-bladed β-propeller that exhibits several features distinct from those of a canonical WD40 repeat domain. Structural and biochemical characterization led to the identification of two Scp1 elements that are involved in Sre1 recognition: an Arg/Lys-enriched surface patch on the top face of the WD40 propeller and a 30-residue C-terminal tail. The structural and biochemical findings were corroborated by *in vivo* examinations. These studies serve as a framework for the mechanistic understanding and further functional characterization of the SREBP and SCAP proteins in fission yeast and higher organisms.

65

Synthesis of GABA in Endothelial Cells: Its Role in Fatty Acid Oxidation

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γ-Aminobutyric acid (GABA) synthesized by some neuronal cells is a principal non-peptidal inhibitory neurotransmitter with a well-characterized role as an inhibitor of neuronal firing in the CNS. We now demonstrate its synthesis by both human umbilical vein endothelial cells (HUVEC) and human aortic endothelial cells (HAEC) and demonstrate some of its physiological functions. Both HUVEC and HAEC exhibited the capacity to synthesize ^{14}C-labeled GABA from ^{14}C-labeled glutamate and also expressed both of the isoforms of glutamate decarboxylase (GAD65 and GAD67). Exogenous GABA (4 μM) added to endothelial cells increased the mRNA of the GABA receptors (*i.e.* GABAR1, GABAR2, and GABAR3) as well as that of its synthesizing enzymes (GAD65 and GAD67). The release of GABA from endothelial cells in cultures was significantly (~60 ± 2%) decreased following treatment with L-allylglycine (LAG) (100–300 μM), a specific pharmacological inhibitor of GABA synthesis. Inhibition of GABA synthesis by LAG in HUVEC was associated with a significant decrease in the steady state levels of free fatty acid oxidation (FFAO) as assessed by the rate of oxidation of ^{14}C-labeled palmitate. The decrease in FFAO was associated with a significant decrease (~55 ± 5%) in the steady state levels of intracellular ATP. We concluded that GABA (*a*) is synthesized in HUVEC and HAEC cells, (*b*) plays an important role in intracellular free fatty acid oxidation in endothelial cells, and (*c*) plays an important role in the maintenance of intracellular ATP levels in endothelial cells. Decreased intracellular synthesis of GABA in endothelial cells may be associated with pathophysiological conditions of the vascular system.

66

The Polyhedral Three-dimensional Structure of Human Very Low-density Lipoprotein by Electron Cryotomography

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Human plasma very low-density lipoprotein (VLDL) is the origin of other apoB100-containing lipoproteins, including its lipolytic remnants, intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL). VLDL particles consist of various types and copies of lipids as well as numerous proteins in addition to apoB100. Due to the compositional heterogeneity, structural characterization of VLDL particles has been very challenging. We employed electron cryotomography and a local refinement algorithm, IPET, to build the three-dimensional structures of individual VLDL particles. The refined structures showed polyhedral morphology instead of the smooth globular shape that many would assume. On each particle, several large flat faces that extend over 30 nm can be identified. Some neighboring faces are at or near right angles to each other. The dihedral angles become bigger as the particle diameter increases. The faces are high density meshwork filled with low density in the holes. They span a few nanometers radially, which coincides with the thickness of a phospholipid single layer. We further decorated VLDL particles with a monoclonal antibody that targets the N terminus of apoB100. The antibody is located at a vertex on VLDL where three large faces converge. The geometry of the three-face cone with the apoB100 N terminus at the vertex provides direct evidence for the lipid pocket model for assembly of apoB-containing lipoprotein particles.

67

An Open-label, Phase I Trial of Transplantation Therapy with Allogeneic Adipose Tissue-derived Multilineage Progenitor Cells in Homozygous Familial Hypercholesterolemia Patients

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Familial hypercholesterolemia (FH) is an inherited disorder, mainly caused by defects in the low-density lipoprotein (LDL) receptor gene. The patients are characterized by high LDL cholesterol levels in the blood and premature cardiovascular disease. Although most of heterozygous FH patients are usually treated with statin, ezetimibe, and bile acid sequestrants, homozygous FH patients are resistant to drug therapy. Therefore, in Japan, many homozygous FH patients are treated by LDL-apheresis. LDL-apheresis is an effective procedure to remove LDL cholesterol from the blood and contribute to improving the prognosis of homozygous FH patients. However, the effect of removing LDL cholesterol is temporary and still not sufficient. As a definitive therapy, liver transplantation is one option to recover LDL receptor, but the donor pool is always limited in Japan. With the increase in evidence of the safety of mesenchymal stem cells and the percutaneous transhepatic portal approach in islet transplantation, we developed a cell transplantation therapy with allogeneic adipose tissue-derived multilineage progenitor cells (ADMPCs) as an alternative treatment instead of liver transplantation. We have already demonstrated that xenogenic transplantation of human ADMPCs into Watanabe heritable hyperlipidemic (WHHL) rabbits via the portal vein resulted in significant reductions in total cholesterol, and the reductions were observed within 4 weeks and maintained for 12 weeks. These results suggested that hADMPC transplantation could correct the metabolic defects and be a novel therapy for inherited liver diseases. Therefore, we generated a protocol for the first-in-human clinical trial, and it has been approved by the institutional review board and Ministry of Health, Labor, and Welfare of Japan.

68

Cholesterol Efflux Pathways in Endothelial Cells Suppress Atherosclerosis

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High density lipoprotein (HDL) has several putative antiatherogenic effects, including preserving endothelial function. The cholesterol transporters ATP binding cassette A1 and G1 (ABCA1 and ABCG1) are highly expressed in endothelial cells (ECs) and mediate cholesterol efflux to apoA-1 and HDL. We have shown previously that whole body *Abca1/g1* deficiency decreases endothelium-dependent vasorelaxation due to decreased endothelial nitric-oxide synthase (eNOS) activity in mice fed cholesterol-rich diets. These observations suggested that endothelial ABCA1/G1 are antiatherogenic. Studies in zebrafish have suggested that endothelial ABCA1/G1 suppress angiogenesis, which could be antiatherogenic in advanced lesions. However, the role of endothelial ABCA1/G1 in atherosclerosis and angiogenesis in mammals has not been directly investigated. We generated $Ldlr^{-/-}$ mice with endothelial *Abca1/g1* or *Abcg1* deficiency. After 22 weeks of a Western type diet, both endothelial *Abca1/g1* and *Abcg1* deficiency accelerated atherosclerosis in the aortic root and whole aorta, with a more pronounced effect of endothelial *Abca1/g1* than *Abcg1* deficiency (2-fold; EC-*Abca1/g1* knockouts compared with controls; $p < 0.001$). Cholesterol levels (~1000 mg/dl) and blood leukocyte levels were similar. In aortic ECs, *Abca1/g1* deficiency suppressed eNOS activity (~50%; $p < 0.05$) and increased inflammatory mRNA expression (VCAM, ICAM, E-selectin, MCP-1, TNFα, and IL-6) and inflammasome priming (NLRP3 and IL-1β) following a lipopolysaccharide (LPS) stimulus (2-fold; $p < 0.01$). These findings were recapitulated in human aortic ECs. In aortic rings stimulated with vascular endothelial growth factor, endothelial *Abcg1* deficiency and *Abca1/g1* deficiency increased the formation of new sprouts, suggesting increased angiogenesis (4-fold; EC-*Abca1/g1* knockouts compared with controls; $p < 0.001$). However, very few new blood vessels were observed in advanced atherosclerotic lesions of the aortic root with no difference between the groups.
These observations suggest that endothelial *Abca1/g1* and, to a lesser extent, endothelial *Abcg1* deficiency accelerates atherosclerosis due to decreased eNOS activity and increased endothelial inflammation. These are the first studies to show directly that cholesterol efflux pathways in ECs are athero-protective.

69

Fatty Acid Oxidation Defects in Pre-eclampsia: Role of GABA

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Pre-eclampsia is a disorder of pregnancy (characterized by high blood pressure and large amounts of protein in the urine) that affects between 2 and 8% of all pregnancies worldwide. The mechanism(s) leading to the pathogenesis of this disease is not yet completely elucidated. We have already shown, that human umbilical cord endothelial cells (HUVEC) are capable of synthesizing and releasing γ-aminobutyric acid (GABA). Moreover, specific inhibition of GABA synthesis leads to compromised free fatty acid oxidation (FFAO) and subsequently lowered steady state levels of intracellular ATP. Utilizing the different metabolic profile of HUVEC from normal and pre-eclamptic patients, we now demonstrate that GABA may play a significant role in the manifestation of pre-eclampsia. Metabolomic studies performed to assess the differences in metabolism between HUVEC from normal and pre-eclamptic patients showed significantly decreased levels of intracellular GABA (~4-fold) in pre-eclamptic HUVEC. ELISA-based assays, utilizing specific antibodies to detect GABA, revealed that pre-eclamptic HUVEC released significantly less (~70%) GABA compared with normal cells. Moreover a dipeptide, Met-GLU, which was found in significantly increased levels in pre-eclamptic HUVEC (compared with normal ones), significantly inhibited glutamate dehydrogenase activity (which synthesizes GABA), in *in vitro* assays. Upon monitoring free fatty acid oxidation (FFAO), it was observed that pre-eclamptic HUVEC (when compared with normal HUVEC) exhibited significantly less FFAO, as evidenced by extracellular flux analysis experiments measuring released carbon dioxide following exposure to palmitate. Measurement of oxygen consumption rates indicated that pre-eclamptic HUVEC exhibited significantly less respiration compared with normal HUVEC and, unlike the latter, did not exhibit a further lowering of oxygen consumption when exposed to etomoxir, a specific inhibitor of FFAO. This effect was reversed upon exogenous addition of GABA. Interestingly, the mitochondria (the major organelle for FFAO) from pre-eclamptic HUVEC exhibited significantly distorted morphology when compared with normal ones. We conclude that compromised GABA levels in pre-eclamptic HUVEC may be associated with compromised FFAO in pre-eclampsia. This may compromise energy reserves in endothelial cells lining the umbilical cord, further contributing to the disease manifestation.

70

Molecular Mechanisms of Lupus Dyslipidemia in Mice

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Lupus dyslipidemia is characterized by increased total cholesterol, triglycerides, LDL, and VLDL and decreased HDL. The molecular mechanisms of abnormal lipid profiles in lupus patients currently are not properly understood. In this study, we generated mice that have lupus dyslipidemia on a chow diet and studied the pathways that are responsible for increased cholesterol and triglycerides in plasma. LDLr$^{-/-}$ and Fas$^{-/-}$ (*lpr/lpr*) mice on the B6 background were purchased from the Jackson Laboratories (Bar Harbor, ME). These mice were interbred to produce mice homozygous for both LDLr$^{-/-}$ and *lpr* confirmed by PCR genotyping. LDLr$^{-/-}$(LDLr) and LDLr$^{-/-}$lpr$^{-/-}$ (LDLrLpr) mice were fed on a chow diet. Thirteen female mice from each group were sacrificed at the age of 24–28 weeks. LDLrLpr mice developed a lupus phenotype, demonstrated by autoantibody production (ANA, anti-dsDNA, anti-sm, and anti-cardiolipin antibodies), increased body weight, splenomegaly, hepatomegaly, and generalized lymphadenopathy compared with LDLr mice. LDLrLpr mice had a significantly increased proteinuria score (1.5 ± 0.2 *versus* 1.1 ± 0.06; $p < 0.02$) and renal histology score (1.8 ± 0.4 *versus* 0.4 ± 0.1; $p < 0.004$) compared with LDLr$^{-/-}$ mice. The lymphocyte population in the spleen measured by flow cytometry resulted in significantly increased CD4^{+} T cells (21 *versus* 16%; $p < 0.003$), double negative T cells (59 *versus* 26%; $p < 0.001$), and CD138^{+} plasma cells (12 *versus* 0.4%; $p < 0.001$) and decreased CD8^{+} T-cells (6 *versus* 14%; $p < 0.001$) in LDLrLpr mice compared with LDLr mice similar to human lupus. Lipid analyses in LDLrLpr mice compared with LDLr mice on a chow diet demonstrated significantly elevated total cholesterol (mg/dl) (347.0 ± 31.65 *versus* 220.9 ± 8.235; $p < 0.0008$), triglycerides (118.8 ± 18.51 *versus* 42.85 ± 3.665; $p < 0.0005$), VLDL (66.85 ± 17.54 *versus* 3.385 ± 0.4875; $p < 0.0014$), and LDL cholesterol (214.9 ± 22.22 *versus* 148.1 ± 8.049; $p < 0.0093$) without a significant difference in HDL level. We conclude that increased cholesterol synthesis, decreased fatty acid oxidation, increased triglyceride secretion, and decreased lipase activity are the molecular mechanisms of dyslipidemia in lupus mice.

71

Identification of Putative Fat Regulatory Elements of the Leptin Gene

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Leptin is a fat specific hormone that is expressed in direct proportion to the lipid content of adipocytes. This suggests that there may be a lipid sensing mechanism in adipocytes that is read out with the expression of the leptin gene. This basic question has remained elusive since the discovery of leptin 20 years ago. It is analogous to the question of how cells sense membrane cholesterol content and the rationale for these studies is similar to that which led to the identification of SREBP as a transcriptional regulator of the LDL receptor.
To study the transcriptional mechanisms responsible for leptin gene expression, we created deletion series of bacterial artificial chromosome (BAC) transgenic mouse lines with a luciferase reporter gene, which allows noninvasive imaging of the leptin expression of mice in vivo. We have used this approach to define the enhancer regions that are necessary for leptin gene regulation.
We also hypothesize that there is a single switch-like fat-sensing transcription factor that could turn on leptin expression in adipose tissue from obese, but not fasted animals. To further identify putative fat regulatory elements for the leptin gene, we are utilizing a recently developed unbiased ATAC-seq approach to interrogate the open chromatin. This method could independently predict the ARE7 sequence of the aP2(Fabp4) enhancer, which was crucial for the elucidation of the role of PPARg in adipogenesis. This method has accurately reproduced and verified the BAC transgenic data and indicated several putative transcription factor binding sites.

72

Identification of Putative Fat Regulatory Elements of the Leptin Gene

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Leptin is a fat-specific hormone that is expressed in direct proportion to the lipid content of adipocytes. This suggests that there may be a lipid-sensing mechanism in adipocytes that is read out with the expression of the leptin gene. An answer to this basic question has remained elusive since the discovery of leptin 20 years ago. It is analogous to the question of how cells sense membrane cholesterol content, and the rationale for these studies is similar to that which led to the identification of SREBP as a transcriptional regulator of the LDL receptor.

To study the transcriptional mechanisms responsible for leptin gene expression, we created deletion series of bacterial artificial chromosome (BAC) transgenic mouse lines with a luciferase reporter gene, which allows noninvasive imaging of the leptin expression of mice *in vivo*. We have used this approach to define the enhancer regions that are necessary for leptin gene regulation. We also hypothesize that there is a single switchlike fat-sensing transcription factor that could turn on leptin expression in adipose tissue from obese but not fasted animals. To further identify putative fat regulatory elements for the leptin gene, we are utilizing a recently developed unbiased ATAC-seq approach to interrogate the open chromatin. This method could independently predict the ARE7 sequence of the aP2(Fabp4) enhancer, which was crucial for the elucidation of the role of PPARγ in adipogenesis. This method has accurately reproduced and verified the BAC transgenic data and indicated several putative transcription factor binding sites.

73

Smooth Muscle Cells, Origin of Foam, and Macrophage-like Cells in Human Atherosclerotic Lesions

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Cholesterol accumulation in atherosclerotic plaque has previously been thought to occur primarily in monocyte-derived macrophages. Using coronary artery sections from hearts explanted at the time of heart transplantation, we have determined the relative contribution of smooth muscle cells (SMCs) to foam cell formation. Lipids in formalin-fixed coronary artery tissues were preserved for staining in paraffin sections, followed by immunohistochemical staining with SM α-actin and Oil Red-O. Studies of coronary artery lesions with a high content of foam cells show that, at a minimum, SMCs comprise 50 ± 7% (average ± S.E., $n = 14$ subjects) of foam cells in human coronary atherosclerosis. Further estimation of plaque foam cell content using fluorescence-activated cell sorting suggests that the contribution of SMCs to foam cells in human atherosclerosis may be much higher. We also found that SMCs in advanced lesion intima have a specific reduction in ABCA1 expression not seen in early or advanced lesion myeloid lineage cells or in early lesion SMCs. These results suggest that an inability of SMCs to release cholesterol via the ABCA1-apoAI-HDL axis contributes to the large contribution of SMCs to the foam cell population. Previous studies have suggested that cultured SMCs can express macrophage markers upon lipid loading. We found that 40 ± 6% ($n = 15$) of cells expressing the macrophage marker CD68 also expressed the SMC marker SMα-actin. In addition, 34 ± 8% ($n = 11$) of CD68-positive cells lacked expression of the myeloid lineage marker CD45. These results indicate that up to one-third or more of cells considered to be myeloid lineage macrophages in human atherosclerosis are in fact SMCs exhibiting a macrophage phenotype. Further studies are examining the relationship of lipid loading to expression of macrophage markers by SMCs. We are also characterizing SMCs that express macrophage markers in human coronary atherosclerotic plaques.

74

Hepatic Expression of SREBP-1c Requires SREBP-2-mediated Generation of Sterol Ligand for LXR

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Transgenic overexpression of SREBP-2 in liver results in the activation of all genes involved in cholesterol synthesis, whereas SREBP-1c overexpression preferentially activates genes involved in fatty acid synthesis. SREBP-2 also regulates the transcription of the LDL receptor and PCSK9. To further define the *in vivo* function of SREBP-2, we generated mice that lack SREBP-2 only in hepatocytes (L-BP2 KO). L-BP2 KO mice exhibited lower plasma and liver cholesterol levels compared with wild-type mice as a result of reduced mRNA levels of all genes in the cholesterol biosynthetic pathway. Unexpectedly, the mRNA and protein levels of SREBP-1c were also markedly decreased, resulting in reduced expression of all genes involved in fatty acid synthesis and reduced rates of *de novo* fatty acid synthesis. Inasmuch as SREBP-1c gene expression is largely dependent on the nuclear receptor, LXR, we determined whether the loss of SREBP-1c expression was due to the loss of LXR activity. Thus, L-BP-2 KO mice were fed a diet supplemented with the LXR agonist *T*0901317. Supplementation with the LXR agonist restored hepatic expression of SREBP-1c and its target genes. SREBP-1c activity in L-BP2 KO mice was also rescued when L-BP2 KO mice were fed a diet supplemented with cholesterol. Combined, our data suggest that SREBP-2 is required for the basal expression of all cholesterol biosynthetic genes and that flux through the sterol synthesis pathway provides an endogenous LXR ligand that is required for LXR activity and SREBP-1c expression.

75

Mutations in ABCA8 underlie reduced plasma high density cholesterol levels in humans

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The relationship between cardiovascular disease risk and high density lipoprotein cholesterol (HDLc) levels is unclear, raising the need for further studies. We identified and characterized the impact of a novel HDLc gene, ATP binding cassette transporter A8 (*ABCA8*). We sequenced *ABCA8* in 80 low (HDLc %ile<10th) and 120 high HDLc (HDLc %ile≥90th) individuals, and identified three *ABCA8* variants exclusively in low-HDLc subjects: Proline609Arginine (in the ATP-binding domain), E17-2 A>G (disruption of essential splice site) and Threonine741Stop. Genotyping of expanded families identified additional mutation carriers and first-degree relative controls. Compared with controls, heterozygous mutation carriers showed a significant 26.5% decrease in plasma HDLc levels and 55.5% decrease HDLc percentiles (age and sex adjusted). Overexpression of human *ABCA8* in mouse livers via adenoviral injection led to a 23.1% increase in HDLc levels. Wild-type ABCA8 localized at the plasma membrane and the ER. However, P609R- and T741X-ABCA8 are only present at the ER. A significant 181% increase in cholesterol efflux to lipid free APOA-I was observed with wild type ABCA8, but not with the mutants P609R or T741X. It has been described that ABCA8 regulates levels of sphingomyelin, an essential lipid in the formation and maturation of HDL. Compared to controls, HDL sphingomyelin content of *ABCA8* mutation carriers was decreased, and HDL sphingomyelin levels of mice overexpressing ABCA8 in the liver was significantly increased. We show here that ABCA8 is a cholesterol transporter that modulates HDLc and sphingomyelin levels in humans and mice.

76

Lymphatic HDL Can Act as a Donor for TICE and This Pathway Is Impaired under Conditions of Insulin Resistance and/or High Fat Diet

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Emerging evidence shows that proximal small intestine secretes cholesterol into the intestinal lumen through the trans-intestinal cholesterol excretion (TICE) pathway. This contributes up to 30–40% for fecal neutral sterols. Plasma apoB-containing lipoproteins can donate cholesterol to this pathway, whereas conflicting evidence exists on the role for plasma HDL as donor. Due to anatomical proximity of lymphatic HDL, it may serve as a candidate donor to TICE, but this has not been tested. The objective of this work was to determine whether lymphatic HDL derived from mesenteric lymph can act as cholesterol donor for TICE in the JCR:LA cp rat model of insulin resistance (IR). Mesenteric lymph was collected following intralipid infusion via lymphatic cannulation from control rats. Lymphatic HDL was isolated using ultradensity centrifugation and labeled with ^{3}H cholesterol. Jejunal explants were obtained from control and IR rats (as a model of reduced TICE) fed chow or high fat/cholesterol diet. TICE was measured with Ussing chambers as the appearance of [^{3}H]cholesterol-labeled lymphatic HDL using micelles as acceptors. Relative to free cholesterol (FC; used as a marker of nonspecific lipid permeability), lymph-derived HDL TICE was 77% higher in control tissue ($n = 4–5$, $p < 0.05$) under chow-fed conditions, suggestive of an effective donor for TICE. Lymph HDL TICE was reduced (89%, $p < 0.05$) in insulin-resistant (IR) rats compared with control. Furthermore, SR-B1 mRNA was reduced (–65%) in enterocytes of IR rats compared with control, which may explain reduced TICE by lymph HDL in IR. Under conditions of high fat/cholesterol-fed diet, TICE from FC (and mannitol as a marker of paracellular transport) was increased in both control and IR rats, suggesting an elevated nonspecific permeability of lipids by the basolateral membrane. Consistent with recent reports on lymphatics and reverse cholesterol transport, these data suggest that lymphatic derived HDL may be an effective donor for TICE, possibly by the SR-B1 pathway. Although we have shown that the lymph HDL TICE pathway may be impaired during insulin resistance, further insult to the intestinal basolateral membrane with a high fat/cholesterol diet may increase lipid permeability via a nonspecific efflux pathway.

77

Reducing Macrophage Proteoglycan Sulfation Increases Atherosclerosis and Obesity through Enhanced Type I Interferon Signaling

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Heparan sulfate proteoglycans (HSPGs) are an important constituent of the macrophage glycocalyx and extracellular microenvironment. To examine their role in atherogenesis, we inactivated the biosynthetic gene *N*-acetylglucosamine *N*-deacetylase-*N*-sulfotransferase 1 (Ndst1) in macrophages and cross-bred the strain to Ldlr(–/–) mice. When placed on an atherogenic diet, Ldlr(–/–)Ndst1(f/f)LysMCre(+) mice had increased atherosclerotic plaque area and volume compared with Ldlr(–/–) mice. Diminished sulfation of heparan sulfate resulted in enhanced chemokine expression; increased macrophages in plaques; increased expression of ACAT2, a key enzyme in cholesterol ester storage; and increased foam cell conversion. Motif analysis of promoters of up-regulated genes suggested increased type I interferon signaling, which was confirmed by elevation of STAT1 phosphorylation induced by IFN-β. The proinflammatory macrophages derived from Ndst1(f/f)LysMCre(+) mice also sensitized the animals to diet-induced obesity. We propose that macrophage HSPGs control basal activation of macrophages by maintaining type I interferon reception in a quiescent state through sequestration of IFN-β.

78

Hypoxia-inducible Lipid Droplet-associated (HILPDA) Is a Novel PPAR Target Involved in Hepatic Triglyceride Secretion

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Peroxisome proliferator-activated receptors (PPARs) play major roles in the regulation of hepatic lipid metabolism through the control of numerous genes involved in processes such as lipid uptake and fatty acid oxidation. Here we identify hypoxia-inducible lipid droplet-associated (*Hilpda/Hig2*) as a novel PPAR target gene and demonstrate its involvement in hepatic lipid metabolism. Microarray analysis revealed that *Hilpda* is one of the most highly induced genes by the PPARα agonist Wy14643 in mouse precision cut liver slices. Induction of *Hilpda* mRNA by Wy14643 was confirmed in mouse and human hepatocytes. Oral dosing with Wy14643 similarly induced *Hilpda* mRNA levels in livers of wild-type mice but not *Ppara*$^{-/-}$ mice. Transactivation studies and chromatin immunoprecipitation showed that *Hilpda* is a direct PPARα target gene via a conserved PPAR response element (PPRE) located 1200 base pairs upstream of the transcription start site. Hepatic overexpression of *Hilpda* in mice via adeno-associated virus led to a 4-fold increase in liver triglyceride storage, without any changes in key genes involved in *de novo* lipogenesis, β-oxidation, or lipolysis. Moreover, intracellular lipase activity was not affected by *Hilpda* overexpression. Strikingly, *Hilpda* overexpression significantly impaired hepatic triglyceride secretion. Taken together, our data uncover *Hilpda* as a novel PPAR target that raises hepatic triglyceride storage via regulation of triglyceride secretion.

79

ApoA-I-binding Protein (AIBP) Deficiency Promotes Retinal Angiogenic Sprouting

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Angiogenesis, a process of blood vessel formation, is essential for embryonic development, tissue homeostasis and repair, and disease progression, such as fueling tumor growth. Enormous progress has been made toward understanding the molecular mechanisms underlying angiogenesis, and some of the findings have been translated into therapeutic applications. We have recently connected apoA-I-binding protein (AIBP)-regulated cholesterol metabolism with angiogenesis. We showed that AIBP accelerates cholesterol efflux from endothelial cells (ECs) to HDL and thereby regulates angiogenesis. AIBP- and HDL-mediated cholesterol depletion reduces lipid rafts, disrupts VEGFR2 dimerization and signaling, and inhibits VEGF-induced angiogenesis *in vitro* and mouse aortic neovascularization *ex vivo*. In agreement, Aibp limits angiogenesis in zebrafish in a cholesterol transporter Abca1- and Abcg1-dependent manner. Our findings demonstrate that secreted AIBP positively regulates cholesterol efflux from ECs and that effective cholesterol efflux is critical for proper angiogenesis.

We extended our studies by generating $Aibp^{fl/fl}$ and $Aibp^{-/-}$ mice, which are viable and fertile. Here we explored the role of AIBP in VEGF-mediated vascularization using the postnatal murine retina model. Retinal vessels characteristically begin to sprout from the optic disc on postnatal day 1 (P1) and reach the retinal margin on P7. We examine retinal angiogenesis in P5 embryos. Compared with wild type controls, which showed an evenly distributed migration front along the vascular circumference, AIBP deficiency resulted in the loss of a synchronized sprouting phenotype, with some vascular sprouts migrated substantially beyond the advancing plexus. Increased HDL levels were shown to correct cell functions with dysregulated cholesterol content. Thus, we generated $Aibp^{-/-} \times Apoa1^{Tg}$ mice. We postulate that overexpression of *Apoa1* in AIBP-deficient mice will rescue the angiogenic defects. We intend to show a conserved role of cholesterol-enriched lipid rafts in controlling angiogenesis in $Abcg1^{-/-}$ mice. We expect that the inhibitory effect of AIBP on angiogenesis will be abolished with ABCG1 deficiency. Our studies will uncover a conserved mechanism for AIBP-mediated cholesterol regulation of angiogenesis and suggest the application of AIBP as a possible therapy targeting angiogenesis.

80

Fine-tuning Roles of the MIG12/S14 Complex in *de Novo* Lipogenesis

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MIG12 is a small protein whose expression is controlled by the transcription factors SREBP-1c, CHREBP, and LXR. It is known to induce polymerization of acetyl-CoA carboxylase (ACC) and up-regulate its activity. This, in turn, promotes total fatty acid synthesis by increasing the availability of cellular malonyl-CoA. MIG12 shows significant sequence homology with another small protein, Spot 14 (S14). S14 is regulated by SREBP-1c and CHREBP but is also responsive to the thyroid hormone T_3. Both MIG12 and S14 exist as homodimers in solution but can also heterodimerize with each other. When it forms a heterodimer with S14, the ability of MIG12 to polymerize and activate ACC is significantly attenuated *in vitro*. Curiously, MIG12 knockout mice show little change in hepatic fatty acid synthesis rates. We show high expression of liver S14, leading to complete MIG12 heterodimerization, as the likely cause behind this discrepancy. Furthermore, we investigate the *in vitro* mechanism behind S14 regulation of MIG12 activity by producing and purifying recombinant MIG12:S14 heterodimer. Finally, we indicate a possible role for MIG12 protein in mouse pulmonary surfactant production.

81

Induction of Lysosomal Biogenesis in Macrophages Reduces Atherosclerosis in an Autophagy-dependent Manner

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Recent reports of the proatherogenic phenotype of mice with a macrophage-specific autophagy deficiency have renewed interest in the role of the autophagy-lysosomal system in atherosclerosis. Lysosomes have the unique role of processing both exogenous material, such as excess atherogenic lipids, and endogenous cargo that includes dysfunctional proteins and organelles via autophagy. Previously, we demonstrated that oxidized LDL and cholesterol crystals, two of the commonly encountered lipid species in the atherosclerotic plaque, create a profound lysosomal and autophagy dysfunction in cultured macrophages. Overexpression of TFEB, a transcription factor that is the only known master regulator of lysosomal and autophagy biogenesis, in macrophages initiates a robust prodegradative response, including induction of lysosomal and autophagy genes. This in turn ameliorates several deleterious effects of the lipid-mediated dysfunction, namely the blunting of inflammasome activation, enhancement of cholesterol efflux, and acceleration of the degradation of protein aggregates. Our *in vitro* data suggest that the induction of a lysosomal biogenesis program in macrophages can have atheroprotective effects. Indeed, myeloid-specific TFEB overexpression in mice significantly reduces atherosclerotic plaque burden as well as plaque complexity as gauged by reduced necrotic core and markers of apoptosis. Interestingly, this protection is autophagy-dependent because these TFEB-overexpressing mice on a background of myeloid-specific autophagy (ATG5) deficiency no longer demonstrate plaque reduction. Mechanistically, this indicates that suppression of the inflammasome and enhancement of cholesterol efflux and protein aggregate removal are dependent on the TFEB-autophagy axis. Taken together, our data support the notion that harnessing the prodegradative response in macrophages via TFEB can be atheroprotective and provide the impetus to evaluate mechanisms by which macrophage lysosomal and autophagy biogenesis can be modulated therapeutically.

82

Macrophage p62/SQSTM1 Ameliorates Atherosclerosis by Sequestering Inclusion Bodies and Mediating Mitophagy

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Protein and organelle turnover is critical for cellular homeostasis and is prominently mediated by autophagy. Disruptions in autophagy lead to accumulation of protein aggregates and dysfunctional organelles, such as mitochondria. Recent evidence suggests that the chaperone protein p62 is a critical link for targeting polyubiquitinated protein aggregates/damaged mitochondria to autophagosomes for degradation. Herein, we describe a p62-centric mechanism of handling protein aggregates and dysfunctional mitochondria in atherosclerosis. Macrophages deficient in autophagy ($ATG5^{-/-}$) or rendered deficient by incubation with atherogenic lipids have significantly increased levels of p62. This coincides with 1) the accumulation of polyubiquitinated proteins co-localizing with p62 and present as cytoplasmic inclusion bodies, and 2) p62 co-localization with mitochondrial markers. Aortas from atherosclerotic ($ApoE^{-/-}$) mice also have progressive and marked elevations in p62, polyubiquitinated proteins, and mitochondrial reactive oxygen species that predominantly co-localize with plaque macrophages, a process further exacerbated in the autophagy-deficient setting. The formation of cytoplasmic inclusions and maintenance of adequate mitochondrial function appear to be dependent on p62. Lipid-loaded p62-null macrophages show polyubiquitinated protein accumulation present in a diffuse/disrupted cytoplasmic pattern. These macrophages also develop larger dysmorphic mitochondria with increased polarization and decreased oxidative phosphorylation capacity. As a result, p62-null macrophages display apoptotic susceptibility to atherogenic lipids and increased IL-1β secretion, probably through mitochondria-dependent inflammasome activation. Consistent with our *in vitro* observations, mice with either whole-body p62 deficiency or transplanted with p62-deficient bone marrow show significantly increased atherosclerotic plaque burden and lesion complexity with increased apoptosis and necrotic cores. Taken together, these data demonstrate a previously unrecognized atheroprotective role for macrophage p62 by facilitating the formation of inclusion bodies and maintaining healthy mitochondria.

83

Flavin Monooxygenase 3 Is Required for FoxO1 Expression and Development of the Diabetic Phenotype

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Hepatic flavin monooxygenase 3 (*Fmo3*) has recently been identified as a novel regulator of cholesterol metabolism that contributes to development of cardiovascular diseases (CVD). We find that FMO3 and its product trimethylamine *N*-oxide (TMAO) are markedly increased in the livers of male liver insulin receptor knockout (LIRKO) mice, streptozotocin-induced diabetic mice, and *ob/ob* mice. Knockdown of insulin receptor in *ob/ob* livers also further increases FMO3 levels. In addition, hepatic *FMO3* mRNA is found increased in obese humans. Therefore, FMO3 appears to be increased in states of defective insulin signaling *in vivo*. Consistent with this, we find that *Fmo3* mRNA is acutely suppressed by insulin in primary hepatocytes. Knockdown of hepatic FMO3 using antisense oligonucleotides in both LIRKO and *ob/ob* mice markedly suppresses the transcription factor forkhead box O1 (FoxO1), a crucial node of metabolic control. Thus, ablation of FMO3 in LIRKO mice completely normalizes glucose tolerance and plasma cholesterol levels. It also completely prevents the development of atherosclerosis. Taken together, these data indicate that FMO3 is a novel target of insulin and that FMO3 is required for FoxO1 expression and development of multiple diabetes-associated phenotypes. The results of this work suggest that therapies to reduce FMO3 and/or TMAO to normal levels may be particularly helpful in the prevention of diabetes-associated CVD.

84

Lack of Phosphatidylethanolamine *N*-Methyltransferase Alters the Phospholipid Composition and Causes Stress in Hepatic Endoplasmic Reticulum

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Endoplasmic reticulum (ER) stress is associated with the development and progression of steatohepatitis. Phosphatidylethanolamine *N*-methyltransferase (PEMT), catalyzing phosphatidylcholine (PC) synthesis via methylation of phosphatidylethanolamine (PE), is essentially a liver-specific enzyme located on the ER and mitochondria-associated membranes. We propose that PEMT deficiency alters the PC and PE content of the ER, thereby inducing ER stress and sensitizing *Pemt*$^{-/-}$ mice to diet-induced steatohepatitis. The mass of PC and PE in hepatic ER fractions was determined by phosphorus assay. Levels of proteins involved in ER stress and the unfolded protein response (UPR) were measured by immunoblotting in livers and McArdle-RH7777 rat hepatoma cells. The chemical chaperone, 4-phenylbutyric acid (PBA), was administered to McArdle cells and high fat (HF)-fed *Pemt*$^{-/-}$ mice to alleviate ER stress. The PC/PE ratio in ER fractions from livers of chow-fed *Pemt*$^{-/-}$ mice was lower than in *Pemt*$^{+/+}$ mice, whereas levels of CHOP and BIP were higher without activating the UPR. In HF-fed *Pemt*$^{-/-}$ mice, the UPR was more activated than in *Pemt*$^{+/+}$ mice because all three arms of the UPR (PERK-eIF2α, IRE1α-XBP1s, and ATF6a) were more activated in HF-fed *Pemt*$^{-/-}$ compared with *Pemt*$^{+/+}$ mice. Similarly, the UPR was activated, and CHOP and BIP were higher in McArdle cells lacking PEMT than in McArdle cells expressing PEMT. PBA reduced activation of the UPR and levels of CHOP and BIP in McArdle cells lacking PEMT but only minimally reduced hepatic ER stress in HF-fed *Pemt*$^{-/-}$ mice. Lack of PEMT alters ER phospholipid composition and leads to ER stress, thereby sensitizing the mice to development of HF-induced steatohepatitis. PBA reduces ER stress in McArdle cells lacking PEMT but not in livers of HF-fed *Pemt*$^{-/-}$ mice.

85

Initial results from deep sequencing of 9 candidate genes regulating HDL cholesterol (HDL-C) metabolism in rhesus macaque half-sibs with discordant HDL-C levels

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Sex-biased genetic effects on HDL cholesterol (HDL-C) have been reported in human cohorts, but have not been investigated in the rhesus macaque (*M. mulatta*), an important preclinical animal model. Previously, we demonstrated substantial heritability for HDL-C levels in female macaques, which appeared to be absent altogether in males (h^2=0.84 in females vs. h^2=0.00 in males; N=193). To explore the possibility of sex-biased genetic effects on HDL-C levels in this species, we conducted deep sequencing of exons and associated regulatory regions across the genome in 19 macaques that were maximally discordant for HDL-C levels, selected from the original 193 animals. Equal numbers of male and female half-sib pairs were selected based on percentile differences in HDL-C levels and matched for age-class (percentile differences within pairs ranged from 50-91%, representing unadjusted differences of 28-58 mg/dL; among all animals, unadjusted HDL-C levels ranged from 24-87 mg/dL). Exome enrichment was performed using the Illumina Nextera Exome Enrichment System, and animals were sequenced to 100X coverage using paired-end reads on the Illumina HiSeq 2500 sequencer. Reads were aligned to the latest version of the macaque genome as recommended by GATK Best Practices, and single nucleotide variants (SNVs) were called using GATK (v3.2) with HaplotypeCaller. SNVs were filtered for quality, and we explored predicted functionality of these variants using SnpEff (v3.6c). Here, we describe variants found in *ABCA1*, *ABCG1*, *APOA1*, *CETP*, *LCAT*, *LDLR*, *NR1H2*, *NR1H3*, and *SREBF2* as key genes regulating HDL-C metabolism in humans. We found a total 341 functional SNVs across all 9 candidate genes, including 57 synonymous and 19 non-synonymous (missense) coding variants, 2 variants resulting in new start sites, and 1 splice site variant. Of these, 9 variants are also found in humans. Of note, one variant (rs2738466) in the 3' UTR of human *LDLR* and now described in macaques, has been associated previously with variation in lipid levels and incident coronary heart and cardiovascular disease in a sex-dependent manner. Although further analyses are ongoing, these initial findings support the value of the rhesus macaque as an important animal model for genetic effects on inter-individual variation in lipoprotein cholesterol levels.

86

Developing a Novel Platform for Quantifying Non-Steady State Fatty Acid and Cholesterol Net Synthetic Fluxes In Vitro

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Altered lipid metabolism is a key feature of many pathologies, including obesity, diabetes, and cancer. One of the largest barriers to progress in this field is the difficulty in measuring lipid metabolic fluxes in an intact cell. Here, we develop a novel platform for simultaneously quantifying fatty acid and cholesterol synthetic rates for cells in vitro. Cells can be labeled with any ^{13}C source (e.g. glucose) or combination of sources, and a simple but efficient in situ derivatization procedure produces fatty acid methyl esters and trimethylsilyl ethers of cholesterol from cell pellets. Nearly quantitative yields were obtained from esterified and free fatty acids and cholesterol. After analysis via GC/MS, the isotopologue distributions are fit using a novel mathematical model. Importantly, we demonstrate how the model parameters can be used to calculate net synthetic flux in a cellular system for an analyte pool that is neither at isotopic nor metabolic steady state. This novel platform allows researchers to quantify a larger subset of lipid metabolic phenotypes in a broader range of normal and pathologic states.

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