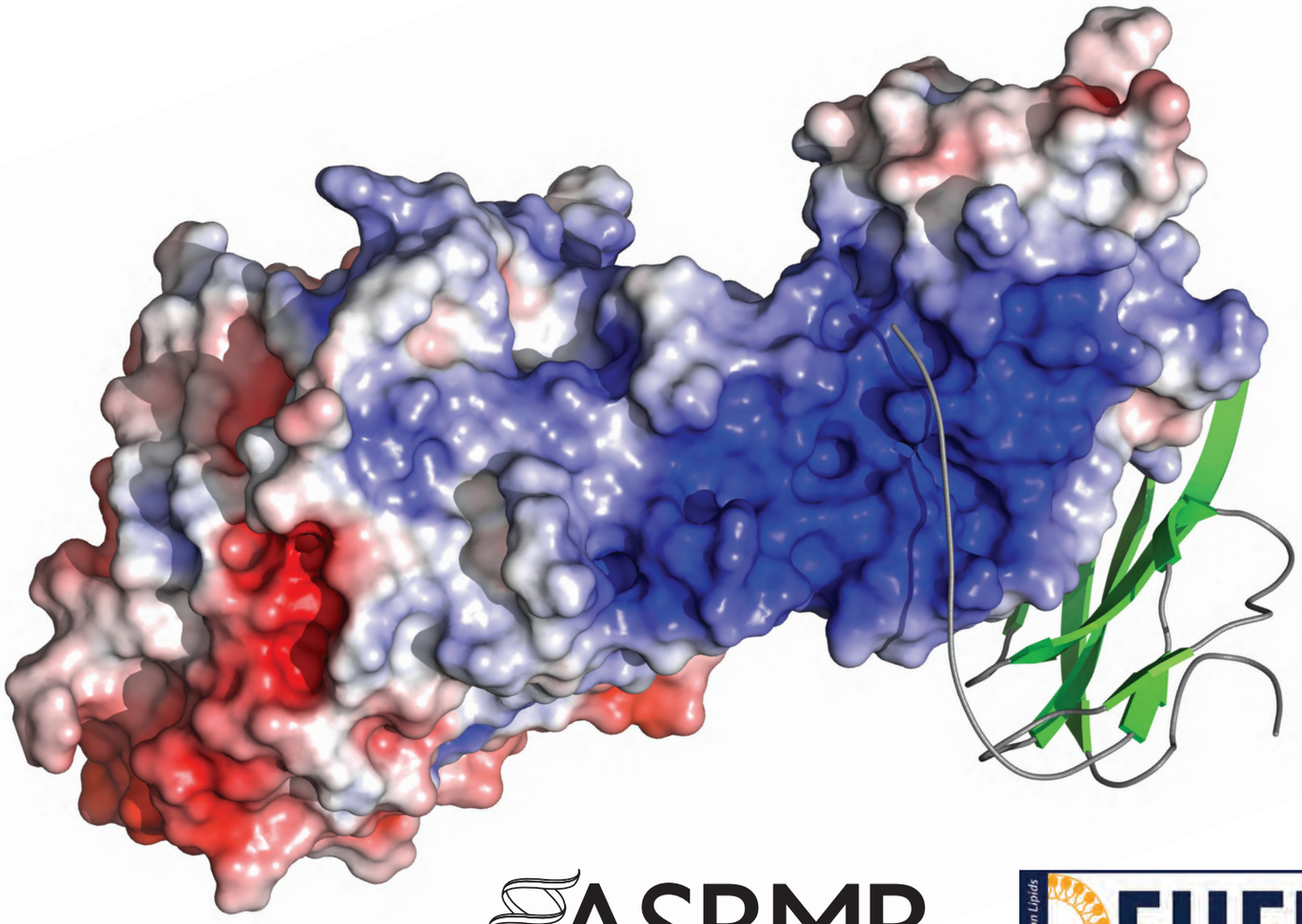
ASBMB DEUEL CONFERENCE ON LIPIDS

March 5 – 8, 2019
Dana Point, Calif.

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



ASBMB
American Society for Biochemistry and Molecular Biology

Conference on Lipids
DEUEL

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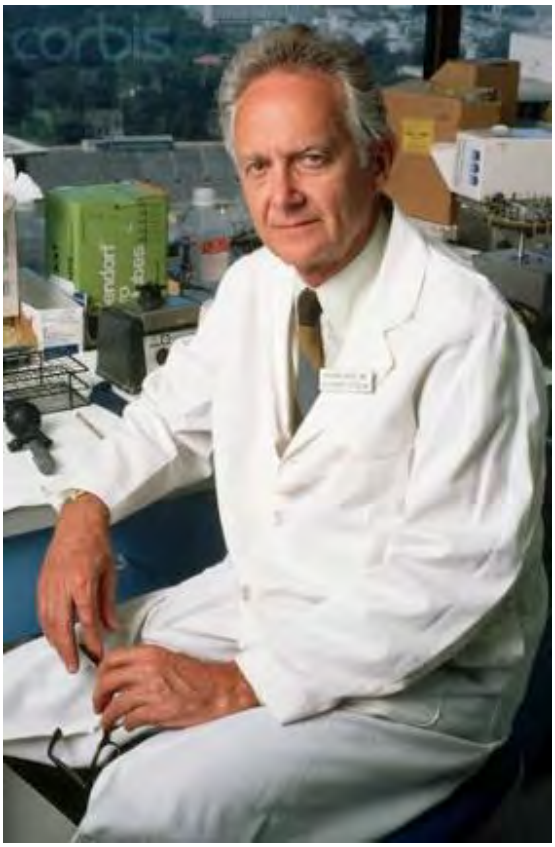
Table of Contents

The ASBMB-Deuel Conference on Lipids, March 5-8, 2019

Laguna Cliffs Marriott, Dana Point, Calif.

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

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



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

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

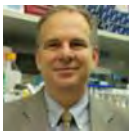
Dr. Havel has published over 300 manuscripts. The quality of his publications is reflected in his election to the National Academy of Sciences in 1983; the Institute of Medicine in 1989; and the American Academy of Arts and Sciences in 1992. He has received many other honors including the Bristol-Myers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

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

2019 HAVEL AWARD LECTURE

Jake Lusis,
University of California, Los Angeles
"The power of natural variation: Sex differences and mitochondrial functions"

PAST HAVEL AWARDEES

2018		**Michael Czech, University of Massachusetts Medical School** *"Crosstalk between fat metabolism and neuronal signaling"*	2011		**Christopher K. Glass, University of California, San Diego** *"Oxysterol regulation of macrophage gene expression"*
2017		**Peter Tontonoz, University of California, Los Angeles** *"Transcriptional control of lipid metabolism in physiology and disease"*	2010		**David J. Mangelsdorf, University of Texas Southwestern Medical Center** *"Nuclear receptor control of lipid metabolism"*
2016		**Sir Stephen O'Rahilly, University of Cambridge** *"Obesity and insulin resistance; lessons from human genetics"*	2009		**Stephen G. Young, University of California, Los Angeles** *"Adventures in lipid metabolism"*
2015		**Thomas Sudhof, Stanford University** *"Brown & Goldstein-inspired science off field: lipid membrane fusion at the synapse"*	2008		**Helen H. Hobbs, University of Texas Southwestern Medical Center** *"Going to extremes to identify genetic variations contributing to cardiovascular risk*
2014		**Rudolf Zechner, University of Graz** *"Lipolysis - more than just the breakdown of fat"*	2007		**" Ronald Evans, The Salk Institute** *"PPARdelta and the marathon mouse: running around physiology*
2013		**Rick Lifton, Yale University** *"From human genetics to validated therapeutic targets"*	2006		**David Russell, University of Texas Southwestern Medical Center** *"The enzymes of cholesterol breakdown"*
2012		**Gokhan Hotamisligil, Harvard University** *"Inflammation, endoplasmic reticulum stress and lipids: emerging networks regulating metabolism"*	2005		**Johann Deisenhofer, University of Texas Southwestern Medical Center, HHMI** *"Structure of the LDL receptor"*

2004		**Jeffrey M. Friedman, Rockefeller University** *"Oxysterol regulation of macrophage gene expression"*	2003		**Bruce Spiegelman, Harvard Medical School** *"Transcriptional control of energy and glucose metabolism"*
2002	 	**Michael S. Brown and Joseph L. Goldstein, University of Texas Southwestern Medical Center** *"SREBPs: Master regulators of lipid metabolism"*			

Schedule At-a-Glance

	Tuesday, 3/5	Wednesday, 3/6	Thursday 3/7		Friday, 3/8
7AM		**Breakfast** 7:00 - 8:15	**Breakfast** 7:00 - 8:15	**Board Meeting** 7:00 - 8:15	**Breakfast** 7:00 - 8:15
8AM					
9AM		**Session 1** 8:30 - 9:30	**Session 3** 8:30 - 11:05		**Session 5** 8:30 - 9:30
		Coffee Break 9:30-10:00			**Coffee Break** 9:30-10:00
10AM		**Session 1 cont.** 10:00 - 11:15	**Coffee Break** 10:00 - 10:30		**Session 5 cont.** 10:00 - 11:15
			Session 3 cont.		
11AM		**Free Time** 11:15 - 5:00	**Free Time** 11:10 - 5:00		
12PM					
1PM					
2PM					
3PM	**Registration** 3:00 - 5:00				
4PM					
5PM	**Opening Reception, Dinner** 6:00 -7:00	**Poster Session 1** 5:00 - 6:00	**Poster Session 2** 5:00 - 6:00		
6PM		***'Meet the Leader'*** **Dinner** 6:00 - 7:15	**Dinner** 6:00 - 7:15		
7PM		**Session 2** 7:15 - 9:15	**Session 4** 7:15 - 9:15		
	The Havel Lecture				
8PM					
9PM					

Program Co-Chairs:

Cynthia Hong, Novartis

Peter Tontonoz, University of California, Los Angeles

Tuesday, March 5

3:00 – 5:00 **Meeting Registration, Dana IV Foyer**

5:00 – 6:00 **Opening Reception - Vue Patio**

6:00 - 7:30 **Dinner, Vue**

7:30 – 7:35 **Welcome:** **Peter Tontonoz**, University of California, Los Angeles
Cynthia Hong, Novartis

7:35p - 7:40p **The Havel Lecture Introduction - Dana IV**
Peter Edwards, University of California, Los Angeles

7:40p – 8:30p The Havel Lecture
The power of natural variation: Sex differences and mitochondrial functions
Jake Lusis, University of California, Los Angeles

Wednesday, March 6

7:00 - 8:15 **Breakfast, Laguna Brick**

Session 1: **8:30 am– 11:15 am, Dana VI - VIII**

Session Chair: **Shaun Coughlin, Novartis**

8:30 – 9:00 **Using bacterial and fungal toxins to understand cholesterol sensing and transport in animal cells**
Arun Radhakrishnan, University of Texas, Southwestern

9:00 – 9:30 **Gene editing to prevent and cure metabolic disorders**
Kiran Musunuru, University of Pennsylvania

9:30 – 10:00 **Coffee break, Dana VI Foyer**

10:00 - 10:45 **Molecular insights into intravascular lipolysis**
Gabriel Birrane, Harvard University
Stephen Young, University of California, Los Angeles

10:45 – 11:00 **Statins stimulate hepatic glucose production via the miR-183/96/182 cluster**
Angel Baldan, Saint Louis University

11:00 – 11:15 **The Role of the Mevalonate Pathway in Intestinal Lipid Absorption**
Alexandria Doerfler, Baylor College of Medicine

11:15 – 5:00 **Free time**

5:00 – 6:00 **Poster Session 1**, **Dana IV**

Wednesday, March 6, continued…

6:00 – 7:15 ******Meet the Leaders****** **Dinner, Laguna Brick**
Each table will be hosted by leaders in our field. Open seating for attendees. Please join for lively discussions over dinner

Session 2: 7:15p.m. – 9:15 pm, Dana VI - VIII

Session Chair: **J. Mark Brown,** Cleveland Clinic

7:15 – 7:45 **Novel mechanisms of metabolic reprogramming**
Jean Schaffer, Washington University, St. Louis

7:45 – 8:15 **Unexpected signaling roles of mitochondrial lipids**
Jared Rutter, University of Utah

8:15 - 8:20 Journal of Clinical Investigation Lectureship Award Introduction
Sarah Jackson, Executive Editor, Journal of Clinical Investigation

8:20 – 8:45 Journal of Clinical Investigation Award Lecture
The intersection of bileacid composition and metabolic regulation
Rebecca Haeusler, Columbia University

8:45– 9:00 **Immune cell-Adipocyte Crosstalk Regulates Thermogenesis**
Prashant Rajbhandari, University of California, Los Angeles

9:00 - 9:15 **Mutation in Sortilin identified in an Amish population results in hyperinsulinemia and hypercholesterolemia in humans and mice**
Kelly Mitok, University of Wisconsin, Madison

Thursday, March 7

7:00 am - 8:15 am Breakfast, Laguna Brick

Session 3: 8:30 a.m. – 11:05 am, Dana VI - VIII

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

8:25 - 8:30 Journal of Lipid Research Lectureship Award Introduction
Stephen Young, University of California, Los Angeles

8:30 – 9:00 Journal of Lipid Research Award Lecture
Diabetes, triglyceride-rich lipoproteins, and atherosclerosis
Karin Bornfeldt, University of Washington

9:00 – 9:30 **Targeting nuclear receptors to treat inflammatory bowel disease**
David Moore, Baylor College of Medicine

9:30 – 10:00 **Insights into lipid metabolism by the endothelium**
William Sessa, Yale University

10:00 – 10:20 **Coffee break, Dana VI Foyer**

Thursday, March 7 cont.,

10:20 – 10:50 **The role oftetratricopeptide repeat domain protein 39B (TTC39B) in regulation of lipogenesis and lipoprotein metabolism**
Allan Tall, Columbia University

10:50 - 11:05 **Exploiting altered enhancer landscapes to decode pathogenic changes in gene expression of diverse hepatic macrophages**
Jason Seidman, University of California, San Diego

11:05 – 5:00 **Free time**

5:00 – 6:00 **Poster Session 2, Dana IV**

6:00 – 7:15 **Dinner, Laguna Brick**

Session 4: **7:15 – 9:15 pm, Dana VI - VIII**
Session Chair: **Claudia Villaneuva, University of Utah**

7:15 – 7:45 **Sex differences in obesity associated with a chromatin remodeling protein**
Karen Reue, University of California, Los Angeles

7:45 – 8:15 **Metabolic adaptation and heterogeneity in fat cells**
Shingo Kajimura, University of California, Los Angeles

8:15 – 8:45 **Role of lncRNAs in cholesterol homeostasis and atherosclerosis**
Kathryn Moore, New York University

8:45 – 9:00 **Damaging variation in ACVR1C encoding the activin-receptor like kinase 7 protects against abdominal obesity as well as type 2 diabetes**
Connor Emdin, Broad Institute of Harvard and MIT

9:00 – 9:15 **Physiologic and Genetic Role for C/EBPα in Plasma Lipid Metabolism**
Robert Bauer, Columbia University

Friday, March 8

7:00 - 8:15 **Breakfast, Laguna Brick**

Session 5: **8:30 – 10:45 am, Dana VI - VIII**
Session Chair: **Kerry-Ann Rye, University of New South Wales**

8:30 – 9:00 **The novel designer cytokine IC7Fc protects against obesity-induced metabolic disease**
Mark Febbraio, Garvan Institute of Medical Research, Sydney, Australia

Friday, March 8, cont.,

9:00 – 9:30	**Role of inflammation in control of hepatic lipid metabolism — New insights to the pathogenesis of NASH** Michael Karin, University of California, San Diego, Baylor College of Medicine
9:30 – 10:00	**Coffee break, Dana VI Foyer**
10:00 – 10:30	**Endothelial lipid metabolism and degenerative disease** Clay Semenkovich, Washington University, St. Louis
10:30 – 10:45	**Dissociation of the triglyceride and phospholipid transfer activities of a vertebrate microsomal triglyceride transfer protein** Meredith Wilson, Carnegie Institution for Science, Baltimore
10:45 - 11:00	**AIBP-mediated Cholesterol Efflux Instructs Hematopoietic Stem and Progenitor Cell Fate** Longhou Fang, Houston Methodist Research Institute
11:10 - 11:15	**Closing Remarks**

Abstract #	Last Name	First Name	Title	Institution	Board #
1	**Baldan**	Angel	Statins stimulate hepatic glucose production via the miR-183/96/182 cluster	Saint Louis University	120
2	**Bauer,**	Robert	Physiologic and Genetic Role for C/EBPα in Plasma Lipid Metabolism	Columbia University	117
3	**Beigneux**	Anne	Lipoprotein lipase is active as a monomer	University of California, Los Angeles	113
4	**Cochran**	Blake	Hypercholesterolaemia modifies density and function of circulating human neutrophils	UNSW Sydney	108
5	**dai**	wen	Role of Adipocyte CaMKII in Metabolic Homeostasis	Department of Medicine, Columbia University	119
6	**Davie**	Kelli	Inhibition and Stabilization of Endothelial Lipase by ANGPTL3	University of Iowa	110
7	**Doerfler**	Alexandria	The Role of the Mevalonate Pathway in Intestinal Lipid Absorption	Baylor College of Medicine	121
8	**Emdin**	Connor	Damaging variation in ACVR1C encoding the activin-receptor like kinase 7 protects against abdominal obesity as well as type 2 diabetes	Broad Institute of Harvard and MIT	103
9	**Erbay**	Ebru	Intercepting the Lipid Induced Integrated Stress Response Reduces Atherosclerosis	Bilkent University	111
10	**Fang**	Longhou	AIBP-mediated Cholesterol Efflux Instructs Hematopoietic Stem and Progenitor Cell Fate	Houston Methodist Research Institute	102
11	**Farber**	Steven	LipoGlo: A new strategy to study the cell biology and physiology of lipoproteins in larval zebrafish	Carnegie Institution for Science	112
12	**Feltes**	McKenna	Diazirine alkyne probes as tools for the study of intracellular cholesterol trafficking	Washington University School of Medicine	104
13	**Fonteh**	Alfred	A plasma lipid biomarker of major depressive disorder is increased in chronic migraine	Huntington Medical Research Institutes	100
14	**Fonteh**	Alfred	Enhanced lipolysis and lipogenesis contribute to chronic migraine pathology	Huntington Medical Research Institutes	105
15	**Fonteh**	Alfred	NSAIDs and opioids attenuate lipolysis in chronic migraine	Huntington Medical Research Institutes	115
16	**Fowler**	Joseph	Inflammatory stress increases SREBP-dependent gene expression and exogenous uptake of cholesterol in endothelial cells.	Yale University	109
17	**Gonzalez**	Lila	Agpat2-/- adipocytes lack caveolae and have increased levels of cholesterol and cholesterol-regulatory genes.	Pontificia Universidad Católica de Chile	101
18	**Grevengoed**	Trisha	N-acyl taurines are fish oil-induced biliary lipids that lower lipid absorption and plasma TAG	University of Copenhagen, NNF CBMR	114
19	**Hu**	Xuchen	GPIHBP1, the lipoprotein lipase transporter, is expressed in capillaries of gliomas	University of California, Los Angeles	106
20	**Hu**	Xuchen	Release of cholesterol-rich particles from the plasma membrane of macrophages	University of California, Los Angeles	118

Abstract #	Last Name	First Name	Title	Institution	Board #
21	**Ingram**	Lishann	Phospholipids as Indicators of Docetaxel-Resistant Castration Resistant Prostate Cancer	University of Georgia	116
22	**Izquierdo**	Maria Concepcion	Hepatic FoxO transcription factors are required for HDL-associated Sphingosine-1-phosphate by regulating Apolipoprotein M.	Columbia University	107
23	**Lee**	Sung-Joon	Ectopic olfactory receptor regulates energy metabolism and adiposity	Korea University	203
24	**Lee**	Stephen	Neuronal IDOL regulates systemic energy balance through the VLDL Receptor	University of California, Los Angeles	216
25	**Lee**	Elinor	The Role of Statins in Pulmonary Alveolar Proteinosis	University of California, Los Angeles	220
26	**Loregger**	Anke	A Haploid Mammalian Genetic Screening Approach to Identify Key Determinants of Lp(a) uptake into cells	University of Amsterdam	200
27	**Mitok**	Kelly	Mutation in Sortilin identified in an Amish population results in hyperinsulinemia and hypercholesterolemia in humans and mice	University of Wisconsin	215
28	**Nohara**	Atsushi	Lysosomal Acid Lipase Activities Negatively Correlated with Cholesterol-esterification Rates in Statin Users, and were Lower with Common Functional Variant LIPA T16P Carriers in Heterozygous FH	Kanazawa University Health Service Center	213
29	**Nowinski**	Sara	Mitochondrial fatty acid synthesis is a master regulator of oxidative phosphorylation	University of Utah School of Medicine	214
30	**Pessentheiner**	Ariane	Human milk oligosaccharides and their anti-inflammatory properties can attenuate cardiometabolic disease	University of California, San Diego	209
31	**Qin**	Yuanyuan	Transmembrane protein 55B regulates LDLR lysosomal decay through PI(4,5)P2	UCSF-Children's Hospital Research Institute	222
32	**Rajbhandari**	Prashant	Immune cell-Adipocyte Crosstalk Regulates Thermogenesis	University of California-Los Angeles	201
33	**Seidman**	Jason	Exploiting altered enhancer landscapes to decode pathogenic changes in gene expression of diverse hepatic macrophages	University of California, San Diego	206
34	**Sen**	Progga	Identification of vesicle trafficking factor p115 as a novel ACSL4 interacting protein and its function in proteasome-mediated degradation of ACSL4	Department of Veterans Affairs, Palo Alto Health Care System	210
35	**Singaraja**	Roshni	FURIN inhibition reduces vascular remodeling and atherosclerotic lesion progression in mice	National University of Singapore	207
36	**Sletten**	Arthur	snoRNA U17 rewires cellular metabolism and regulates lipotoxicity.	Washington University, St. Louis, MO	219

Abstract #	Last Name	First Name	Title	Institution	Board #
37	**Smeir**	Elya	Regulation of mitochondrial respiration and lipidome in cardiomyocytes following persistent administration of the β-agonist isoproterenol	Institute of Pharmacology, Berlin, Germany	218
38	**Tanyanyiwa**	Donald	Evaluating the Role of Genetic Variation on the Risk of Dyslipidaemia in the South African Population	University of the Witwatersrand and NHLS	205
39	**Tzoneva**	Gannie	Increased fat oxidation and metabolic rewiring in a murine model of Marfanoid Progeroid Lipodystrophy Syndrome	Regeneron Pharmaceuticals, Tarrytown, NY	211
40	**Wang**	Huan	Lipin1 is required for hepatic adaptation to fasting/feeding and maintenance of mRNA splicing patterns	University of California, Los Angeles	212
41	**Wilson**	Meredith	Dissociation of the triglyceride and phospholipid transfer activities of a vertebrate microsomal triglyceride transfer protein	Carnegie Institution for Science, Baltimore, MD	202
42	**Xu**	Mike	Endocytosis Adaptor Dab2 in Lipid Metabolism	University of Miami	204
43	**Ye**	Cunqi	Transcriptional regulation of sulfur metabolism by phospholipid methylation	University of Texas Southwestern Medical Center	221
44	**Zelcer**	Noam	Haploid mammalian genetic screens empower novel gene identification in lipid metabolism and atherosclerosis	University of Amsterdam	208
45	**Zhang**	Zhengyi	Regulation of lipid metabolism by the heterogenous nuclear ribonucleoprotein RALY	University of California, Los Angeles	217

1

Statins stimulate hepatic glucose production via the miR-183/96/182 cluster

Tyler J. Marquart[1], Ryan M. Allen[1], Mary Chen[1], Gerald W. Dorn[2], Scot J. Matkovich[2], Angel Baldan[1]

[1]Saint Louis University, St. Louis, MO; [2]Washington University in St. Louis, St. Louis, MO

Statins are the most common pharmacologic intervention in hypercholesterolemic patients, and their use is recognized as a key medical advance leading to a 50% decrease in deaths from heart attack or stroke over the past 30 years. The atheroprotective outcomes of statins are largely attributable to the accelerated hepatic clearance of low-density lipoprotein (LDL) cholesterol from circulation following the induction of the LDL receptor. However, multiple studies suggest that these drugs exert additional LDL-independent effects. The molecular mechanisms behind these so-called pleiotropic effects of statins, either beneficial or undesired, remain largely unknown. Here, we determined the coding transcriptome, miRNome, and RISCome of livers from mice dosed with saline or atorvastatin to define a novel *in vivo* epitranscriptional regulatory pathway that links statins to hepatic gluconeogenesis via the SREBP2–miR-183/96/182–TCF7L2 axis. Notably, multiple genome-wide association studies identified TCF7L2 (transcription factor 7–like 2) as a candidate gene for type 2 diabetes, independent of ethnicity. Our data reveal an unexpected link between cholesterol and glucose metabolism, provide mechanistic insight into the elevated risk of diabetes recently observed in patients taking statins, and identify the miR-183/96/182 cluster as an attractive pharmacological candidate to modulate non-canonical effects of statins.

2

Physiologic and genetic role for CCAAT/enhancer-binding protein α in plasma lipid metabolism

Kavita S. Jadhav[1], Noel Walsh[1], Gabriella I. Quartuccia[1], Robert C. Bauer[1]

[1]Columbia University, New York, NY

CCAAT/enhancer-binding protein α (C/EBPα) is a basic leucine zipper transcription factor and a mediator of hepatic lipid and glucose metabolism. In the liver, the pseudokinase Tribbles-1 (TRIB1) promotes the proteasomal degradation of C/EBPα. TRIB1 is a GWAS locus for plasma cholesterol and triglycerides, and hepatic-specific overexpression (OE) or knockout (KO) of Trib1 reduces or increases, respectively, plasma lipid levels in mice. Given the function of TRIB1 OE in down-regulating C/EBPα protein, we sought to determine how loss of C/EBPα reduces plasma lipids. We injected Cebpa_CKO mice with either AAV-null or AAV-Cre to generate liver-specific knockout (LSKO) of *Cebpa* (>95% KO, $n = 5$, $p < 0.01$). Cebpa_LSKO mice fed chow diet (CD) and Western diet (WD) have drastically decreased TC (>50%, $p < 0.01$), mostly in the HDL fraction. Plasma TG was also reduced (50%, $p < 0.05$) in the CD-fed group. Despite its role in *de novo* lipogenesis, there was no difference in VLDL–TG secretion in Cebpa_LSKO mice. Cebpa_LSKO mice have increased hepatic expression of the HDL uptake gene SR-B1, but no changes were observed in other lipoprotein uptake or cholesterol synthesis genes. Given these data and the relationship of C/EBPα to TRIB1, we hypothesized that *CEBPA* should itself be a GWAS hit for plasma lipids if it mediates the effect of Trib1 OE on plasma lipids. Interestingly, an intronic SNP (rs731839) in the gene *PEPD* significantly associates with plasma HDL and TG in multiple GWAS, but *PEPD* has no known function in lipid metabolism. This GWAS locus is annotated to *PEPD*; however, the *CEBPA* gene is 80 kb upstream of the lead SNP. To test whether this SNP lies in a DNA element that regulates *CEBPA* expression, we used CRISPR/Cas9 genome editing to delete ~1100 bp around rs731839 in Huh7 cells. Cells with both heterozygous and homozygous deletions had significant reductions in the gene expression of *CEBPA* (50%, $p < 0.05$ and $p < 0.01$, respectively), whereas the expression of neighboring genes was unchanged. In summary, we show that LSKO of C/EBPα drastically reduces plasma lipids, potentially via altered HDL clearance, and that *CEBPA* may be the causal gene at the Chr19q13.11 plasma lipid GWAS locus.

3

Lipoprotein lipase is active as a monomer

Anne P. Beigneux[1], Christopher M. Allan[1], Norma P. Sandoval[1], Gabriel Birrane[2], Muthuraman Meiyappan[3], Loren G. Fong[1], Stephen G. Young[1]

[1]UCLA, Los Angeles, CA; [2]Beth Israel Deaconess Medical Center, Boston, MA; [3]Shire Pharmaceuticals, Cambridge, MA

Lipoprotein lipase (LPL), the enzyme that hydrolyzes triglycerides in plasma lipoproteins, is assumed to be active only as a homodimer. In support of this idea, several groups have reported that the size of LPL, as judged by density gradient ultracentrifugation, is ~110 kDa, twice the size of LPL monomers (~55 kDa). Of note, however, the LPL in those studies had been incubated with heparin (a polyanionic substance that binds and stabilizes LPL). Here, we revisited the assumption that LPL is active only as a homodimer. When freshly secreted human LPL (or purified preparations of LPL) was subjected to density gradient ultracentrifugation (in the absence of heparin), LPL mass and activity peaks exhibited the size expected of monomers (near the 66-kDa albumin standard). GPIHBP1-bound LPL also exhibited the size expected for a monomer. In the presence of heparin, LPL size increased, overlapping with a 97.2-kDa standard. We also used density gradient ultracentrifugation to characterize the LPL within the high- and low-salt peaks from a heparin–Sepharose column. The catalytically active LPL within the high-salt peak exhibited the size of monomers, whereas most of the inactive LPL in the low-salt peak was at the bottom of the tube (in aggregates). Consistent with those findings, the LPL in the low-salt peak, but not the LPL in the high-salt peak, was easily detectable with “single monoclonal antibody” sandwich ELISAs (in which LPL is captured and detected with the same antibody). We conclude that catalytically active LPL can exist in a monomeric state.

4

Hypercholesterolemia modifies density and function of circulating human neutrophils

Blake J. Cochran[1,2], Yong Chang Lai[1], Damilola Pinheiro[2], Maria Prendecki[2], Ben Jones[2], Victoria Lee[1], Kerry-Anne Rye[1], Jaimini Cegla[2], Kevin J. Woollard[2]

[1]University of New South Wales, Sydney, Australia; [2]Imperial College London, London, UK

Elevated cholesterol levels and increased accumulation of myeloid cells are key risk factors in the development of atherosclerosis. Although there is significant understanding of the impact of hypercholesterolemia on monocyte function, less is known about the impact of cholesterol on neutrophils. The aim of this study was to determine whether cholesterol loading of neutrophils occurs in hypercholesterolemia and characterize the associated phenotypic and functional consequences. Patients were a mixture of familial hypercholesterolemia and mixed dyslipidemia, all with total cholesterol >7.0 mmol/liter. Age- and sex-matched healthy donors were used as controls. Normal and low-density neutrophils were isolated using a Percoll density protocol and further purified by FACS, selecting for $CD66b^+CD15^+$ cells. Low-density neutrophils (LDNs) were only present in hypercholesterolemic patients, accounting for 12.2 ± 9.6% of the cells in the lower-density Percoll fraction ($p < 0.01$ *versus* control). LDNs were morphologically similar to mature high-density neutrophils and stained positive for neutral lipid. Functionally, LDNs secreted significantly more extracellular traps (NETs) in response to PMA treatment relative to normal-density neutrophils isolated from the same patients or healthy controls ($p < 0.005$). Cholesterol-loaded neutrophils were more adhesive to endothelial cells compared with controls ($p < 0.005$). Increased NETosis and neutrophil adhesion have been demonstrated to be atherogenic. Phagocytosis was increased in LDNs *versus* controls ($p < 0.05$). Mitochondria in LDNs contained significantly elevated levels of superoxide compared with normal-density neutrophils isolated from the same patients or healthy controls ($p < 0.005$). These functional changes could all be replicated *in vitro* via treatment of neutrophils from a healthy subject with cholesterol or oxidized LDL. In conclusion, hypercholesterolemia results in cholesterol loading and a pro-atherosclerotic phenotype in neutrophils. Our data provide evidence that this cholesterol loading occurs in the circulation.

5

Role of adipocyte CaMKII in metabolic homeostasis

Wen Dai[1], Lale Ozcan[1]

[1]Department of Medicine, Columbia University, New York, NY

Adipose tissue is an important endocrine organ that regulates whole-body energy homeostasis. During periods of energy excess, adipocytes store energy in the form of triglycerides through *de novo* lipogenesis and release it as free fatty acids (FFAs) during times of energy deprivation through activation of cAMP-mediated lipolysis. Dysfunction of these processes have been implicated in the development of many diseases, including insulin resistance and fatty liver; however, major gaps remain in our understanding of the upstream signaling pathways involved in the regulation of adipocyte function and metabolic homeostasis. Recent work has shown that aberrant activation of hepatic glucagon–cAMP signaling results in activation of a calcium-sensitive enzyme, CaMKII. CaMKII activity is increased in obese mice and human liver, and its inhibition markedly improves hyperglycemia and insulin resistance. Although the role of CaMKII in hepatic insulin resistance has been well characterized, its function in adipose tissue is not known. Previous studies have suggested a role for CaMKII in regulating adipocyte lipolysis in cell culture models; however, in-depth mechanistic and *in vivo* molecular-genetic causation information is lacking. To elucidate the functional role of CaMKII in adipocytes, we generated mice lacking CaMKII specifically in adipocytes (CaMK2g-AKO) and found that CaMK2g-AKO mice had decreased fasting blood glucose levels, improved glucose tolerance, and improved epididymal white adipose tissue insulin signaling compared with control mice on a high-fat diet. Remarkably, there were no significant differences in insulin tolerance between CaMK2g-AKO and control littermates. Besides, we noted reduced plasma FFA levels both under basal condition and upon isoproterenol stimulation in CaMK2g-AKO mice *versus* controls on chow diet. Similarly, our *in vitro* data demonstrated that silencing of CaMKII in OP9 differentiated adipocytes led to lower secreted glycerol levels than in the control group under both basal and isoproterenol-stimulated conditions. Further mechanistic studies revealed that CaMKII silencing resulted in reduced levels of phospho-HSL and total ATGL, two key proteins involved in lipolysis. Conversely, overexpression of constitutively active CaMKII increased HSL activity in adipocytes. Taken together, these data suggest that adipocyte CaMKII is a critical regulator of lipid handling and contributes to systemic metabolic dysfunction upon an imbalanced equilibrium between lipid storage and mobilization.

6

Inhibition and stabilization of endothelial lipase by angiopoietin-like 3

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High plasma triglyceride levels and low high-density lipoprotein (HDL) cholesterol levels are risk factors for atherosclerosis and cardiovascular disease. Both plasma triglyceride and HDL levers are regulated in part by the circulating inhibitor angiopoietin-like 3 (ANGPTL3). ANGPTL3 acts on HDL by inhibiting the phospholipase endothelial lipase (EL), which hydrolyzes the phospholipids of HDL, thus decreasing plasma HDL levels. ANGPTL3 regulates plasma triglycerides by inhibiting lipoprotein lipase (LPL), the lipase primarily responsible for the clearance of triglycerides from the circulation. In this study, we characterized ANGPTL3 inhibition of EL and investigated the role of angiopoietin-like 8 (ANGPTL8) in EL inhibition by ANGPTL3. We found that inhibition of EL by ANGPTL3 was dose- and temperature-dependent and was completely reversible. Surprisingly, ANGPTL3 seemed to have a stabilizing effect on EL. Although ANGPTL3 inhibited EL, it also appeared to prevent the spontaneous inactivation of EL. Our lab has previously found that ANGPTL3 requires ANGPTL8 to efficiently inhibit LPL. However, here we found that ANGPTL8 did not significantly alter the binding or the inhibition of EL by ANGPTL3, indicating that ANGPTL8 is not necessary for EL inhibition.

7

The role of the mevalonate pathway in intestinal lipid absorption

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Statins, which inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), are cholesterol-lowering drugs that are widely prescribed to lower the risk of death from cardiovascular disease (CVD). HMGCR is the rate-limiting enzyme in the cholesterol biosynthetic pathway (mevalonate pathway), which is also required for the synthesis of multiple non-sterol isoprenoids (*i.e.* dolichol, ubiquinone, vitamin K, isopentenyl tRNA, heme A, and farnesyl and geranylgeranyl lipid anchors). The intestine is believed to be one of the primary sites of *de novo* cholesterol synthesis in humans and an important organ that mediates the absorption of dietary lipids. We hypothesized that Hmgcr is required for intestinal lipid absorption and enterocyte viability. Mice harboring floxed alleles for Hmgcr were bred with the Villin-Cre transgene to specifically knock out this enzyme in the villus and crypt epithelial cells of the small intestine and colon (Hmgcr i-KO). Successful deletion of Hmgcr in intestinal epithelial scrapings was confirmed at the mRNA level by Q-PCR and RNA-Seq. Total plasma cholesterol levels were unaffected in Hmgcr i-KO mice of both sexes. However, female i-KO mice had reduced plasma triglycerides compared with controls. The Hmgcr i-KO mice of both sexes had significantly lower body weights at time of weaning but gradually recovered to normal by 12 weeks of age on a chow diet. Prior to this recovery at 5 weeks of age, the Hmgcr i-KO mice had longer intestines, increased intestine to body weight ratios, deeper crypts, and dysmorphic villi. These features are highly suggestive of a lipid malabsorption followed by compensatory regeneration. Preliminary data in female mice suggest increased feed efficiency between 4 and 5 weeks of age, possibly explaining the recovery to normal body weight by 12 weeks of age. Studying the effects of Hmgcr deletion in the intestine will help clarify the importance for the mevalonate pathway in intestinal lipid absorption, leading to improved personalized medicine for hypercholesterolemia and CVD.

8

Damaging variation in ACVR1C encoding the activin receptor–like kinase 7 protects against abdominal obesity as well as type 2 diabetes

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A genetic predisposition to higher waist-to-hip ratio adjusted for body mass index (WHRadjBMI), a measure of abdominal obesity, is associated with increased risk for type 2 diabetes. We conducted an exome-wide association study of coding variation in UK Biobank (405,569 individuals) to identify variants that lower WHRadjBMI and protect against type 2 diabetes. We identified four variants in the gene ACVR1C, encoding the activin receptor–like kinase 7 (ALK7) receptor expressed on adipocytes and pancreatic beta cells, which independently associated with reduced WHRadjBMI: Asn150His (–0.09 standard deviations, $p = 3.4 \times 10^{-17}$), Ile195Thr (–0.15 SD, $p = 1.0 \times 10^{-9}$), Ile482Val (–0.019 SD, $p = 1.6 \times 10^{-5}$), and rs72927479 (–0.035 SD, $p = 2.6 \times 10^{-12}$). Carriers of these variants exhibited reduced percent abdominal fat in dual-energy X-ray imaging. Pooling across all four variants, a 0.2 SD decrease in WHRadjBMI through ACVR1C was associated with a 30% lower risk of type 2 diabetes (OR 0.70, CI 0.63, 0.77; $p = 5.6 \times 10^{-13}$). In an analysis of exome sequences from 55,516 individuals, carriers of predicted damaging variants in ACVR1C were at 54% lower risk of type 2 diabetes (OR 0.46, CI 0.27, 0.81; $p = 0.006$). These findings indicate that variants predicted to lead to loss of ACVR1C gene function reduce abdominal obesity and protect from type 2 diabetes. These findings identify the ALK7 receptor as a potential therapeutic target for type 2 diabetes.

9

Intercepting the lipid-induced integrated stress response reduces atherosclerosis

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Eukaryotic cells can respond to diverse stimuli by converging at serine 51 phosphorylation on eIF2α and activate an adaptive stress response or the integrated stress response (ISR). eIF2α phosphorylation is a key step in translational control and must be tightly regulated; however, persistent phosphorylation is observed in mouse and human atheroma. Potent inhibitors of ISR to modulate neurodegenerative disorders have been identified. In this study we evaluated the potential benefits of intercepting ISR in atherosclerosis, which is a chronic metabolic and inflammatory disease. We investigated ISR's role in lipid-induced inflammasome activation and atherogenesis by taking advantage of three different small molecules and the ATP analog–sensitive kinase allele technology (ASKA) to intercept ISR signaling at multiple molecular nodes. Our results show that lipid-activated eIF2α signaling induces a mitochondrial quality control protease, LONP1, that degrades PINK1 and blocks Parkin-mediated mitophagy, resulting in greater mitochondrial oxidative stress, inflammasome activation, and IL-1β secretion in macrophages. Furthermore, ISR inhibitors are shown to suppress hyperlipidemia-induced inflammasome activation and reduce atherosclerosis. In conclusion, these results reveal that ER controls mitochondrial clearance by activating eif2α–LONP1 signaling, contributing to an amplified oxidative stress response that triggers robust inflammasome activation and IL-1β secretion in the face of a metabolic insult. These findings underscore that the intricate exchange of information and coordination of both organelles' responses to lipids are important for metabolic health and that the miscommunication between ER and mitochondria induced by dietary fats can promote inflammasome activation and atherogenesis. Modulation of ISR may be a novel strategy to reduce atherosclerosis.

10

AIBP-mediated cholesterol efflux instructs hematopoietic stem and progenitor cell fate

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Hypercholesterolemia, the driving force of atherosclerosis, accelerates the expansion and mobilization of hematopoietic stem and progenitor cells (HSPCs). The molecular determinants connecting hypercholesterolemia with hematopoiesis are unclear. Here, we report that a somite-derived pro-hematopoietic cue, AIBP, orchestrates HSPC emergence from the hemogenic endothelium, a type of specialized endothelium manifesting hematopoietic potential. Mechanistically, AIBP-mediated cholesterol efflux activates endothelial Srebp2, the master transcription factor for cholesterol biosynthesis, which in turn transactivates Notch and promotes HSPC emergence. Srebp2 inhibition impairs hypercholesterolemia-induced HSPC expansion. Srebp2 activation and Notch up-regulation are associated with HSPC expansion in hypercholesterolemic human subjects. Genome-wide ChIP-Seq, RNA-Seq, and ATAC-Seq indicate that Srebp2 trans-regulates Notch pathway genes required for hematopoiesis. Our studies outline an AIBP-regulated Srebp2-dependent paradigm for HSPC emergence in development and HPSC expansion in atherosclerotic cardiovascular disease.

11

LipoGlo: A new strategy to study the cell biology and physiology of lipoproteins in larval zebrafish

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Cardiovascular disease is currently the leading cause of death worldwide. Serum levels of atherogenic lipoproteins are one of the strongest determinants of cardiovascular disease risk, but much remains unknown about how these particles are regulated at the level of whole-organism physiology. Using genome editing we fused a chemiluminescent reporter (NanoLuc) to apolipoprotein B, the obligate protein component of atherogenic lipoproteins, in the endogenous locus of the zebrafish genome. Using this reporter (LipoGlo) we characterized numerous aspects of lipoprotein biology with unprecedented speed and sensitivity, including dynamics in the abundance, size distribution, and localization of lipoproteins during zebrafish development. Genetic, dietary, and pharmacological manipulations revealed striking similarity to mammalian lipoprotein physiology in the small, rapidly developing zebrafish embryo, which has unparalleled tractability for genetic and small-molecule screening. Small-molecule screening efforts are underway to identify pharmacological modulators of atherogenic lipoprotein homeostasis, and forward genetic screening has identified phospholipase A_2 group 12B (Pla2g12b) as a potent mediator of lipoprotein biogenesis. Surprisingly, despite being a member of the phospholipase gene family, previous studies have shown that Pla2g12b has neither phospholipid binding or cleavage activity *in vitro*. Although decreased VLDL was reported in a mutant mouse allele, this highly conserved vertebrate gene has no known mechanism of action. Using LipoGlo, we show that *pla2g12b*$^{-/-}$ mutant zebrafish have reduced rates of lipoprotein secretion, and the lipoproteins they are able to secrete are significantly smaller (LDL-like particles rather than VLDL). We also used electron microscopy to demonstrate that significant lipid deposits are left behind in lipoprotein-secreting tissues, potentially in the form of ER-lumenal lipid droplets. These parallel lines of evidence suggest that Pla2g12b may mediate fusion between nascent lipoproteins and ER-lumenal lipid droplets. These data illustrate the power of zebrafish forward genetic approaches to identify a central regulator of vertebrate lipoprotein biogenesis.

12

Diazirine alkyne probes as tools for the study of intracellular cholesterol trafficking

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Cholesterol is an important metabolic precursor, regulator of metabolic processes, and component of cellular membranes. Exogenous cholesterol is acquired principally through receptor-mediated endocytosis of cholesteryl ester–laden low-density lipoproteins (LDLs) and trafficked to the lysosome where cholesteryl esters are hydrolyzed and from which free, unesterified cholesterol is distributed to other cellular organelles. Export of lysosomal cholesterol can occur through both NPC1-dependent and less efficient NPC1-independent mechanisms. However, other components of the post-lysosomal cholesterol trafficking pathways remain poorly understood. This is, in part, due to a lack of suitable cholesterol-trafficking probes. Diazirine alkyne–functionalized probes are minimally modified and enable tagging and enrichment of protein targets for identification by mass spectrometry. Characterization of cholesterol probes demonstrates that diazirine alkyne probes move to authentic cholesterol compartments and interact with established cholesterol-binding proteins. Studies are underway to use these probes to obtain a time-resolved map of post-lysosomal cholesterol-trafficking networks. Diazirine alkyne probes provide a powerful new tool for the discovery and study of previously elusive post-lysosomal cholesterol-trafficking pathways.

13

A plasma lipid biomarker of major depressive disorder is increased in chronic migraine

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Nervonic acid is a major component of myelin sphingolipids and a biomarker of major depressive disorders. Given that depression is a comorbid condition of migraine, we determined whether nervonic acid levels were altered in blood plasma from chronic migraine (CM) patients. Blood plasma was obtained from healthy control volunteers (CT; $n = 10$) and CM patients ($n = 15$). The latter was diagnosed using the International Classification of Headache Disorders-3β criteria, and blood plasma was collected without any change in their medications. Plasma unesterified and esterified nervonic acid and its precursor (lignoceric acid, C24:0) were quantified using gas chromatography–stable-isotope mass spectrometry. Age, sex, and BMI were similar for CT and CM. Although most CM participants were on some combination of migraine medications (NSAIDs, opioids, triptans, antidepressants, and anti-epileptic drugs (AEDs)), no CT subjects were using these medications. Unesterified levels of nervonic acid were significantly higher in CM than in CT ($p = 0.02$). Similarly, esterified levels of nervonic acid were higher in CM than CT ($p < 0.0001$). Desaturase and elongase indices were similar for unesterified nervonic acid, but the elongation of esterified C22:1 to nervonic acid was significantly higher for CM ($p = 0.0004$). PHQ-9 depression scores were lower ($p = 0.0041$) in CT (3.6 ± 2.2) compared with CM (10.4 ± 5.9). Depression scores directly correlated with esterified nervonic acid and lignoceric acid levels. CM duration was inversely correlated with unesterified nervonic acid. Medication usage did not affect nervonic acid levels in CM, although we noticed a slight attenuation in levels of CM participants on NSAIDs and opioid compared with participants who were not using these medications. In conclusion, our data show that levels of myelin precursor known to be a major marker of depression (nervonic acid) are increased in CM by elongation of its monounsaturated fatty acid precursors. Nervonic acid increase in CM plasma is not influenced by migraine medications. We propose that plasma measures of nervonic acid are a potential biomarker of CM. Finally, targeting monounsaturated fatty acid elongase activity to restore normal plasma levels of nervonic acid may lead to a treatment for chronic migraine.

14

Enhanced lipolysis and lipogenesis contribute to chronic migraine pathology

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Fatty acids are a major storage form of energy and the source of inflammatory and pain signaling molecules; however, their contribution to chronic migraine (CM) is not known. Our aim is to determine whether plasma fatty acid metabolism contributes to CM pathology. We obtained plasma from healthy control volunteers (CT; n = 10) and CM patients (n = 15), the latter diagnosed using the International Classification of Headache Disorders-3β criteria. We measured even chain (ec) and odd chain (oc) unesterified (UFAs) and esterified fatty acids (EFAs) using gas chromatography coupled with stable isotope single ion–monitoring mass spectrometry. Protein levels and PLA_2 activities were determined using fluorescent reagents and substrates, respectively. Unesterified levels of three even chain saturated fatty acids (ecSAFAs), two ocSAFAs, the sum of ecSAFAs, the sum of SAFAs, six even chain monounsaturated fatty acids (ecMUFAs), two ocMUFAs, five *n*-3 polyunsaturated fatty acids (PUFAs), and five *n*-6 PUFAs are higher in plasma from CM patients. The sum of all UFAs is significantly elevated in CM. Esterified levels of two ecSAFAs, the sum of ecSAFAs, one ocSAFA, the sum of all SAFAs, five ecMUFAs, three ocMUFAs, five *n*-3 PUFAs, the sum of *n*-3 PUFAs, and three *n*-6 PUFAs are higher in CM plasma. For both UFA and EFA, only the C16:1 composition was significantly higher in CM plasma. The ratios C16:1/C16:0 and C20:4*n*-6/homo-γ-C20:3*n*-6 representative of Δ9 and Δ5 desaturases, respectively, are higher in CM. Although PLA_2 activity was similar in CM and CT, the UFA to EFA ratio of even chain MUFAs and the sum of MUFAs is higher in CM. Elongase indices <C18:0 were lower and >C20:0 were higher in CM. In conclusion, because plasma UFAs are known to correspond to adipose tissue levels, higher plasma fatty acids and UFA/EFA ratios suggest enhanced lipolysis and lipogenesis from adipose storage sites in CM. This is accompanied by increased Δ9 desaturase activity in CM. Together, these studies show that enhanced lipolysis and lipogenesis highlight extensive alteration of lipid metabolism in CM. We propose that controlling adipocyte lipid metabolism may relieve CM symptoms, and monitoring plasma fatty acids has potential to help diagnosis of CM.

15

NSAIDs and opioids attenuate lipolysis in chronic migraine

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Although cyclooxygenase inhibition resulting in a decrease in prostaglandin formation is a known mechanism of action of NSAIDs, it is not known whether NSAIDs and other pain medications affect other lipid metabolic pathways. Our objective is to determine whether higher lipolysis in chronic migraineurs (CM) is influenced by different treatments. Healthy control volunteers (CT; $n = 10$) and CM patients ($n = 15$) were enrolled in an IRB-approved study. CM patients were diagnosed using the International Classification of Headache Disorders-3β criteria. Plasma unesterified (UFAs) and esterified fatty acids (EFAs) were quantified using gas chromatography–mass spectrometry. The groups of fatty acids detected in plasma included even chain
saturated fatty acids (ecSAFAs), odd chain SAFAs (ocSAFAs), even chain monounsaturated fatty acids (ecMUFA), odd chain MUFAs (ocMUFAs), and ω-3 (*n*-3) and ω-6 (*n*-6) polyunsaturated fatty acids (PUFAs). Although age, sex, and BMI were similar, many CM patients were on NSAIDs, opioids, triptans, antidepressants, and anti-epileptic drugs (AEPs), whereas no CT subjects were on these medications. Higher plasma UFA levels of two ecSAFAs, three ocSAFAs, four ecMUFAs, two ocMUFAs, and three *n*-3 and three *n*-6 PUFAs were reduced in plasma from CM patients on NSAIDs. The sum of all UFAs that was significantly elevated in CM plasma was reduced in subjects on NSAIDs. Similarly, CM patients on opioids had lower levels of two ecSAFAs, four ecMUFA, one ocMUFA, and one *n*-3 and four *n*-6 PUFAs. In contrast, elevated blood plasma UFA and EFA levels in CM patients were not reduced by other medications. In conclusion, these data suggest that a novel common mode of action of NSAIDs and opioids is the attenuation of lipolysis that accompanies CM. We propose that identifying lipolytic enzymes that increase plasma UFAs may offer new targets for preventing CM or monitoring therapeutic efficacy.

16

Inflammatory stress increases SREBP-dependent gene expression and exogenous uptake of cholesterol in endothelial cells

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Atherosclerosis is the leading cause of death worldwide and is distinguished by the deposition of lipids into the vessel wall. A growing body of research suggests a relationship between inflammation and sterol metabolism; however, a majority of these studies characterize this relationship solely within macrophages. Little is known about how the cells directly in contact with circulating lipoproteins, endothelial cells (ECs), deal with hypercholesterolemia and inflammatory stress. Here, we investigate how inflammatory stress in EC influences mechanisms promoting endogenous and exogenous accumulation of cholesterol. Treatment of primary human umbilical vein endothelial cells with inflammatory cytokines, TNFα and IL-1β, up-regulated LDL receptor (LDLR) levels 5-fold, amplified LDL uptake, and increased total cholesterol levels within cells. Furthermore, cytokines induced cleavage and activation of SREBP, the main transcription factor of LDLR, leading to a similar increase in transcript levels of several other sterol-regulated genes. Chemical inhibition of NF-κB activation attenuated cytokine-mediated up-regulation of sterol-responsive genes and indicated that SREBP cleavage is downstream of NF-κB DNA binding. We hypothesize that inflammatory stress perturbs EC sterol responsiveness and that when combined with hypercholesterolemia or metabolic disturbances may lead to EC dysfunction and disease. Detailed analysis of the relationship between inflammatory stress and sterol homeostasis in ECs will provide useful information about the pathobiology surrounding the initiation of atherosclerosis.

17

Agpat2$^{-/-}$ adipocytes lack caveolae and have increased levels of cholesterol and cholesterol-regulatory genes

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Caveolae are plasma membrane microdomains important for adipose tissue differentiation. Cholesterol and sphingolipids, as well as proteins caveolin-1 and cavin-1, are necessary for caveolae formation. Mutations in the genes encoding for these proteins determine congenital generalized lipodystrophy. Agpat2$^{-/-}$ mice are born with normal adipose tissue but develop generalized lipodystrophy during the first week of life. Agpat2$^{-/-}$ adipocytes have severely decreased caveolae and impaired adipogenesis i*n vitro*. Herein, we assessed caveolin-1 and cavin-1 levels and subcellular distribution and evaluated cellular cholesterol and the mRNA levels of transcriptions factors and enzymes regulating cholesterol in differentiated Agpat2$^{-/-}$ adipocytes. Pre-adipocytes were harvested from Agpat2$^{-/-}$ and Agpat2$^{+/+}$ mice and adipogenically differentiated *in vitro*. Abundance and subcellular localization of cholesterol were assessed with PFO-recombinant probe staining. mRNA levels were determined by qPCR. Caveolin-1 and cavin-1 levels and subcellular localization were estimated by Western blotting and confocal immunofluorescence, respectively. Cholesterol levels in the adipose tissue of newborn mice were assessed by PFO-recombinant probe. Cholesterol was increased in differentiated Agpat2$^{-/-}$ adipocytes (day 10 of differentiation). Also, subcutaneous white adipose tissue (P0.5 and P2.5) and brown adipose tissue (P4.5 and P6.5) of Agpat2$^{-/-}$ mice have higher cholesterol levels. The mRNA abundance of genes implicated in cholesterol (Abca1, Ldlr, Lrp, Srbi, Srebp2, and Hmg-CoAR) was elevated in differentiated Agpat2$^{-/-}$ adipocytes (day 10 of differentiation). *In vitro* differentiated Agpat2$^{-/-}$ adipocytes have abnormal subcellular distribution of caveolin-1 and cavin-1 without differences in their total abundance. Alterations in cholesterol levels and caveolin-1 and cavin-1 distribution, but not changes in these proteins' levels, are associated with the low abundance of caveolae in Agpat2$^{-/-}$ adipocytes. We hypothesize that abnormal cholesterol metabolism results in impaired caveolae formation and adipogenic failure in Agpat2$^{-/-}$ adipocytes. The specific subcellular structures where cholesterol is accumulated remain to be determined.

18

N-Acyl taurines are fish oil–induced biliary lipids that lower lipid absorption and plasma TAG

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Supplementation with fish oil–type polyunsaturated fatty acids is an established way to lower plasma TAG, but the mechanisms for this beneficial effect are unclear. We hypothesized that bioactive small-molecule PUFA derivatives might contribute to this effect. We performed untargeted metabolomics of bile and plasma from mice fed fish oil. *N*-Acyl taurines (NATs), an amphipathic class of water-soluble lipids, increased 25-fold in bile (up to 1 mM) and 6-fold in plasma after fish oil feeding for 3 days. Interestingly, NATs with polyunsaturated acyl chains predominated in both groups, with C22:6 NAT accounting for 28% of total NATs in bile from chow-fed animals. In humans, C22:6 NAT was 3-fold higher in plasma of subjects receiving DHA/EPA supplements for 1 year compared with placebo controls. To test whether C22:6 NAT could impact plasma TAG through dietary lipid absorption, mice were given an oil meal with or without C22:6 NAT. After the oil meal, mice treated with C22:6 NAT absorbed 39% less lipid than controls, demonstrating its ability to acutely slow lipid absorption. To measure effects of chronic elevation of NATs, mice were generated with a genetic modification to prevent NAT degradation. Compared with wildtypes, these mice with high endogenously produced NATs had elevated C22:6 NAT in plasma and bile, were protected from hepatic steatosis when fed a diet containing fish oil, and displayed low plasma TAG with either lard or fish oil–based diet. To determine whether C22:6 NAT protects from hypertriglyceridemia, HFD-fed wildtype mice were treated daily with C22:6 NAT subcutaneously or in the diet for 7 days. Dietary NAT increased fecal TAG by 61% and had no effect on plasma or hepatic TAG. Mice treated subcutaneously with NAT had 11% lower plasma TAG than vehicle and trended to lower liver TAG, indicating that C22:6 NAT also works beyond the gut to lower plasma TAG. Overall, NATs are endogenous biliary lipids that increased markedly with ω-3 fatty acid supplementation in mice and humans. C22:6 NAT can acutely slow lipid absorption and chronically lower liver and plasma TAG. Therefore, C22:6 NAT may be a metabolite responsible for some of fish oil's beneficial effects on plasma TAGs.

19

GPIHBP1, the lipoprotein lipase transporter, is expressed in capillaries of gliomas

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GPIHBP1, a glycolipid-anchored protein of capillary endothelial cells, binds lipoprotein lipase (LPL) in the interstitial spaces and shuttles it to its site of action in the capillary lumen. GPIHBP1-bound LPL on the luminal surface of capillaries is essential for the margination of triglyceride-rich lipoproteins (TRLs). GPIHBP1 is expressed in capillary endothelial cells of nearly all peripheral tissues, but it is absent from capillaries of the brain (a tissue that relies on glucose for fuel). Although GPIHBP1 is absent from the normal brain, we suspected that GPIHBP1 might be expressed in gliomas, a malignancy in which capillaries are morphologically abnormal. We took advantage of monoclonal antibodies against human and mouse GPIHBP1 to investigate whether GPIHBP1 is expressed in gliomas. We found that GPIHBP1 is absent from capillaries of normal mouse and human brain but that it is easily detectable in capillary endothelial cells of mouse and human gliomas. Using NanoSIMS imaging, we found margination of TRLs along glioma capillaries. Moreover, we documented rapid uptake of TRL-derived fatty acids into gliomas. We suspect that the ability of gliomas to utilize fatty acids from lipoproteins could contribute to tumor growth.

20

Release of cholesterol-rich particles from the plasma membrane of macrophages

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Macrophages have been thought to unload surplus cholesterol through direct interactions between ABC transporters on the plasma membrane and cholesterol acceptors in the plasma (*e.g.* HDL). However, recent studies have shown that macrophages also release particles enriched in cholesterol. Thus far, the biogenesis of these cholesterol-rich particles has remained mysterious. To understand the biogenesis of macrophage particles, we examined mouse peritoneal macrophages and RAW 264.7 macrophages by scanning electron microscopy and nanoscale secondary ion mass spectrometry (NanoSIMS) imaging. By scanning EM, we observed that macrophages release enormous numbers of particles from the plasma membrane during movement of lamellipodia and filopodia. These particles remain attached to the substrate surrounding macrophages. NanoSIMS imaging revealed that the "lawn" of particles on the substrate surrounding macrophages is highly enriched in "accessible cholesterol" (detectable by a modified bacterial cytolysin, ALO-D4). The release of cholesterol-rich particles from macrophages is abolished by actin-depolymerizing agents (latrunculin A) and inhibitors of myosin II (blebbistatin). We propose that the release of accessible cholesterol-rich particles from the macrophage plasma membrane during cellular movement could be an accessory mechanism for disposing of surplus cholesterol.

21

Phospholipids as indicators of docetaxel-resistant castration-resistant prostate cancer

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The association of circulating lipids with clinical outcomes of drug-resistant castration-resistant prostate cancer (CRPC) is not fully understood. Although it is known that increases in plasma lipids correlate to decreased survival, neither the mechanisms mediating alterations in these lipids nor the correlation of resistance to treatment is well characterized. We addressed this gap in knowledge using *in vitro* models of non-cancerous, hormone-sensitive CRPC and drug-resistant cell lines combined with quantitative HPLC-ESI-Orbitrap-MS lipidomic analysis. HPLC-MS–based metabolomic analysis identified 83 features that were significantly altered in the drug-resistant cell lines as compared with the non-resistant control group. Within the 83 species, two were identified as individual plasma lipid biomarkers for diagnosis of prostate cancer from previous studies. Principal component analysis (PCA) demonstrated that drug-resistant and control cell lines were visually separated by these identified lipid biomarkers. Two of the 83 lipid classes were identified as phosphatidylcholine (PC) 18:0/18:1 and PC 18:0/18:2, both of which had a high level of abundance in docetaxel-resistant CRPC. An untargeted approach indicated that lipids corresponding to PC, phosphatidylglycerol, phosphatidic acid, and phosphatidylserine all had higher relative abundance in DR-CRPC cells (DU145-DR) compared with the parent control (DU145). These data suggest that the lipidomic profiles of prostate cancer cells lines recapitulate lipidomic profiles in the plasma of prostate cancer patients. As such, these metastasis-dependent and drug-resistant cell lines may prove useful for understanding the molecular mechanisms mediating lipid changes in prostate cancer patients.

22

Hepatic FoxO transcription factors are required for high-density lipoprotein–associated sphingosine 1-phosphate by regulating apolipoprotein M

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The FoxO family of transcription factors play an important role in mediating insulin action on glucose, lipid, and lipoprotein metabolism. Liver-specific FoxO1,3,4 knockout mice (L-FoxO1,3,4) lack all three of the FoxO transcription factors, and these mice have defects in expression of genes related to glucose production, bile acid synthesis, and high-density lipoprotein (HDL) cholesterol uptake. We have now identified apolipoprotein M (ApoM) as a novel transcriptional target of liver FoxO. ApoM is a liver-secreted apolipoprotein that is bound to HDL in the bloodstream, and it serves as a chaperone for the bioactive lipid sphingosine 1-phosphate (S1P). Several recent studies have demonstrated that S1P bound to ApoM induces unique effects compared with S1P associated with albumin. We now show that liver FoxOs are required for ApoM mRNA and protein and that ApoM is a direct transcriptional target of FoxOs. Moreover, although total plasma S1P levels are similar between control and L-FoxO1,3,4 mice, S1P is nearly absent from HDL in L-FoxO1,3,4 mice and is instead increased in the lipoprotein-depleted fraction (LPD). We also studied in a separate model of insulin resistance and hyperglycemia, db/db mice, whether the expression of ApoM-S1P is affected. We observed that db/db mice have low hepatic ApoM mRNA expression. Moreover, db/db mice have low levels of ApoM in HDL. There are no differences in total levels of plasma S1P between db/db and their heterozygous controls. However, consistent with their low levels of ApoM, db/db mice showed a reduction of S1P in the HDL fraction and an increase of S1P in the LPD fraction. Furthermore, we have studied the levels of S1P in insulin-sensitive/resistant human subjects and observed that there are no differences in total levels of plasma S1P between the subjects, but the insulin-resistant subjects showed a reduction of S1P in the HDL fraction and an increase of S1P in the LPD fraction. In conclusion, FoxO transcription factors are novel regulators of the ApoM–S1P pathway. These findings indicate a link between hepatic insulin action and HDL function.

23

Ectopic olfactory receptor regulates energy metabolism and adiposity

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Olfactory receptors (ORs) are the largest mammalian protein superfamily and are ectopically expressed in extranasal tissues. The physiological functionality of ectopic ORs and their potential application for drug development has been suggested (Lee, S. J., Depoortere, I., and Hatt, H. (2019) *Nat. Rev. Drug Discov.* **18,** 116–138), and we investigated the functionalities of ectopic OR in liver, muscle, and adipose tissues. In microarray analysis, Olfr544 was the highest expressed OR in liver, skeletal muscle, and white adipose tissues, and activation of Olfr544 by its ligand azelaic acid (AzA) specifically induced protein kinase A–dependent lipolysis in adipocytes, mitochondrial biogenesis in myocytes, and fatty acid oxidation and ketogenesis in hepatocytes the effects of which were negated by Olfr544 gene knockdown. AzA also revealed similar metabolic effects in human hepatocytes and primary adipocytes compared with those in mouse cells. Six weeks of oral administration of AzA significantly reduced adiposity in high-fat diet (HFD)-fed C57/BL6J mice. AzA stimulated multiple biological events in metabolic tissues by CREB-dependent mechanisms; thus, Ppara and fatty acid oxidation gene expressions were induced in the liver, and Ppargc1a and Ucp1 gene expressions were induced in brown adipose tissue. AzA stimulated the CREB–PGC-1α–ERK1/2 signaling axis and induced mitochondrial DNA content in mouse skeletal muscle. The insulin sensitivity index and ketone body levels increased after administering AzA. Indirect calorimetry demonstrated that AzA increased fat utilization in energy production, increasing the fatty acid oxidation rate, whereas total energy expenditure was unaltered. AzA showed similar anti-obesogenic effects in HFD-fed ob/ob mice. However, these metabolic alterations by AzA were completely abrogated in HFD-fed Olfr544-deficient mice, which demonstrated that the metabolic functions of AzA are Olfr544-dependent. Thus, ectopically expressed olfactory receptor 544 is a novel receptor that orchestrates the metabolic interplay among the liver, muscle, and adipose tissues, mobilizing stored fats from adipose tissue and shifting the fuel preference toward fats in the liver, muscle, and brown adipose tissue.

24

Neuronal IDOL regulates systemic energy balance through the VLDL receptor

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Liver X receptors (LXRs) limit the cellular uptake of lipids through transcription of the E3 ubiquitin ligase “inducible degrader of the LDL receptor” (IDOL), which targets lipoprotein receptors for lysosomal degradation. We previously demonstrated that the LXR–IDOL pathway exerts species-specific effects on levels of lipoproteins through control of LDL receptor (LDLR) protein levels. However, the broader contributions of IDOL to systemic metabolism are unknown. Here, we show that global loss of IDOL in mice is protective against the development of diet-induced obesity and metabolic dysfunction. Unexpectedly, analysis of a series of tissue-specific knockout mice revealed that IDOL affects energy balance, not through its actions in metabolic tissues but through its actions in the CNS. Single-cell RNA sequencing analysis combined with conditional knockout mouse studies demonstrated that IDOL deletion in neurons is responsible for the metabolic protection. Furthermore, we identify VLDL receptor (VLDLR) rather than LDLR as the key mediator of IDOL effects on systemic metabolism. These studies identify a role for the CNS IDOL–VLDLR pathway in energy balance and susceptibility to diet-induced obesity.

25

The role of statins in pulmonary alveolar proteinosis

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Pulmonary alveolar proteinosis (PAP) is a rare lung syndrome with no cure that is characterized by the accumulation of surfactant within the alveoli. Autoimmune PAP is associated with autoantibodies against GM-CSF that impair surfactant catabolism by alveolar macrophages. Recent studies revealed that GM-CSF regulates cholesterol clearance in alveolar macrophages. Other studies demonstrated that PAP alveolar macrophages have abnormal expression of the ATP-binding cassette transporters A1 and G1 (ABCA1 and ABCG1, respectively) and peroxisome proliferator-activated receptor γ, important mediators in cholesterol transport in macrophages. These data suggest that impairment in cholesterol homeostasis underlies the pathogenesis of PAP. This revelation has led to the discovery of new potential therapeutic agents that target cholesterol homeostasis for PAP. We identified the first patient with autoimmune PAP who had clinical resolution of her disease after being started on rosuvastatin for concurrent hyperlipidemia. Furthermore, we demonstrated that statin reduced levels of cholesterol in alveolar macrophages in both PAP patients and mice. In this study, we wanted to assess the efficacy and understand the mechanism of statin therapy in PAP patients. We performed retrospective chart reviews and examined the patients' clinical course before and after being on statin therapy. We identified two other autoimmune PAP patients who had improvement in their lung disease after being started on statin treatment for their comorbid conditions. To evaluate the mechanism, we isolated alveolar macrophages from PAP mice and measured downstream targets of GM-CSF signaling. Statin up-regulates *Abca1* and *Abcg1* in murine PAP alveolar macrophages, which suggests that statin could be improving the disease process by enhancing cholesterol efflux. Additionally, we isolated alveolar macrophages from PAP patients and treated them *ex vivo* with statins. Preliminary results demonstrate that there are differences in the response to statins depending on the patients' clinical course, suggesting that not all PAP patients may respond equivalently to statins. This could potentially be used as a novel modality to test the efficacy of specific therapies in the clinical setting to identify the best treatment for a given autoimmune PAP patient, enabling clinicians to practice personalized medicine for this patient population.

26

A haploid mammalian genetic screening approach to identify key determinants of Lp(a) uptake into cells

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Elevated circulating levels of the atherogenic lipoprotein Lp(a) are an independent risk factor for development of cardiovascular disease. Despite this recognition, the molecular and cellular pathways governing Lp(a) cellular uptake and degradation are not well understood, and in particular, the identity of the receptor(s) that mediates uptake into the cell remains controversial. To identify new genes and elucidate cellular mechanisms that govern Lp(a) uptake we have recently developed a functional genetic screen based on the use of a haploid mammalian system. This approach makes use of a unique mammalian haploid cell line which, coupled to a gene-trapping strategy, allows establishment of genotype–phenotype associations. We have recently developed a screening approach to evaluate the uptake of fluorescently labeled Lp(a) in these cells. This resulted in the identification of several potential genes that are required for the uptake of Lp(a), which we are currently studying. Collectively, our study identified several candidate genes and demonstrates the feasibility of applying mammalian haploid genetic screens as a tool to study Lp(a) uptake and metabolism. More generally, this highlights the potential of haploid mammalian genetics to study lipid metabolism.

27

Mutation in Sortilin identified in an Amish population results in hypercholesterolemia in humans and mice

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We used genetic linkage in an Amish population to identify a key residue in the Sortilin protein that is associated with increased LDL-C in humans. Our linkage study identified, for the first time, coding SNPs in the *SORT1* gene associated with physiological function. Sortilin (*SORT1*) is a member of the vacuolar protein sorting 10 (Vps10) family of receptors that play a role in post-Golgi protein trafficking and sorting. We identified a SNP (rs141749679) that changes a lysine (K) to a glutamate (E) at the highly-conserved residue 269 in the luminal cargo-binding domain of Sortilin (K269E), which is associated with increased LDL-C. We used CRISPR/Cas9 to generate a whole-body knock-in mouse expressing the Sortilin K269E variant. In response to Western-style diet feeding, male mice harboring the Sortilin K269E variant have dramatically elevated LDL compared to wild-type (WT) mice, phenocopying the humans. We found that the increased LDL in the mice is due to decreased LDL clearance, and that the mice have mislocalized hepatic LDLR. The Sortilin K269E mice also have increased fasting plasma insulin. Performing an oral glucose tolerance test (oGTT) in the Sortilin K269E mice shows that they have a dramatically increased insulin response but normal glucose tolerance, indicating insulin resistance. We found that the increased insulin response is due to both increased insulin secretion and decreased insulin clearance, as determined by measuring C-peptide and calculating the C-peptide to insulin molar ratio during the oGTT. Similar to the LDLR, immunohistochemistry for the insulin receptor in livers of Sortilin K269E mice shows altered receptor localization, which could explain the decreased insulin clearance. The Sortilin K269E mice have additional metabolic phenotypes including increased plasma ketones, gluconeogenesis, adiposity, and hepatic steatosis. Interestingly, for many of these phenotypes, whole-body Sortilin knock out (KO) mice have effects in the opposite direction of the Sortilin K269E mice, suggesting that Sortilin K269E might be a gain-of-function variant. We are using the Sortilin K269E mouse model to further the understanding of the function of Sortilin metabolism and the interplay between tissues to elicit metabolic phenotypes.

28

Lysosomal acid lipase activities negatively correlated with cholesterol-esterification rates in statin users and were lower with common functional variant LIPA T16P carriers in heterozygous FH

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Lysosomal acid lipase (LAL), coded by LIPA gene, hydrolyzes cholesterol ester (CE) from LDL and VLDL taken up by LDL receptors. Cooperation of LDL receptor and LAL is essential for metabolic utilization of LDL. LIPA is identified as a susceptibility gene for CAD in many GWAS studies. However, little is known about the relationship between physiological LAL activity and CAD risks. We investigated the role of LAL activity among a CAD high-risk group. Physiological LAL activities were determined with a fluorometric assay method using dried blood spots. A total of 425 CAD high-risk patients (male, 221; age, 57 ± 16 years; established CAD, 30%; DM, 39%; hetero-FH, 22%) were investigated. 59% of them were assessed on statin therapy. Genetic analysis of LIPA gene was performed in 376 CAD high-risk group patients, including 188 genetically determined heterozygous FH. LAL activities showed negative correlation with CE ratios only on statin therapy ($p < 0.05$). LAL activities were 24% higher in statin users ($p < 0.0001$). In lipid parameters with statin users, LAL activities showed correlation with LDL-C (R2: 8%, $p < 0.05$), apoC2, and apoC3 in FH but was not significant in non-FH. From analysis of LIPA gene, common T16P variant carriers showed lower LAL activities in FH subjects ($p < 0.05$). Then we investigated the effects of T16P variants, and T16P variants showed lower pretreatment LDL-C ($p < 0.05$) in genetically confirmed heterozygous FH. In conclusion, physiological LAL activities significantly correlated with CE ratios and CAD risks in statin users. LAL may play more roles in the cytosolic cholesterol depletion state such as HMG-CoA reductase inhibition or reduced cholesterol uptake with LDL-R. Roles of LAL should be clarified, especially in CAD high-risk patients.

29

Mitochondrial fatty acid synthesis is a master regulator of oxidative phosphorylation

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Mitochondria are often dubbed "the powerhouse of the cell;" however, this gross oversimplification has arguably led to a paucity of knowledge concerning the many other functions of these incredibly diverse organelles. Fatty acid synthesis (FAS) is an essential cellular process, but it is a little-known fact even among mitochondrial experts that eukaryotes actually harbor two distinct FAS systems: one in the cytoplasm and one in the mitochondria. Although mitochondrial FAS (mtFAS) was discovered decades ago, its only function was long thought to be the production of lipoic acid, an important lipid cofactor for several mitochondrial dehydrogenases. We now appreciate that mtFAS plays a far wider role in mitochondrial physiology as a nutrient-sensitive pathway whereby acetyl-CoA regulates its own metabolism. Specifically, in yeast, the acylated form of the mitochondrial acyl carrier protein (ACP) physically interacts with a family of (mostly) late-stage electron transport chain complex assembly factors, the LYR proteins, to facilitate OXPHOS complex assembly and iron–sulfur cluster biogenesis. When ACP is deacylated, ETC complexes fail to assemble, providing the cell with an elegant mechanism linking the TCA cycle, OXPHOS, and mtFAS via the substrate of all three processes: acetyl-CoA. The ACP-LYR network is largely conserved in mammals where CRISPR mutants of three mtFAS genes all show reduced levels of protein lipoylation, assembled ETC complexes, and mitochondrial respiration. Proteomics and metabolomics data provide intriguing insights into the consequences of mtFAS depletion and highlight promising future directions toward further understanding the role of this pathway in cellular metabolism.

30

Human milk oligosaccharides and their anti-inflammatory properties can attenuate cardiometabolic disease

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Cardiovascular disease (CVD) is often associated with atherosclerosis, a chronic inflammatory disorder. One therapeutic approach aims to stop the infiltration of macrophages into arteries and thereby reduce inflammation and foam cell formation. However, available therapeutics targeting inflammation lack expected efficiency and are associated with adverse effects, high costs, and unfavorable administration routes such as injections. We have taken a rather unusual drug discovery approach and looked at what is already working well in "Mother Nature." Human breast milk contains an arsenal of bioactive components that are beneficial for the baby and are, per definition, safe for oral consumption. In our research, we focused on the potential anti-inflammatory properties of human milk oligosaccharides (HMOs). We hypothesize that specific HMOs reduce inflammation in the context of atherosclerosis and benefit adults with CVD. HMOs are known immune system modulators, and our screening of individual HMOs identified one specific HMO to effectively reduce LPS-induced activation of murine and human macrophages by accelerating the resolution phase of inflammation. The HMO also attenuated the activation state of human endothelial cells. To test the translational aspect of our data, we used a mouse model of atherosclerosis and treated Western-type diet–fed $Ldlr^{-/-}$ mice orally with HMO for 6 weeks. We observed no differences in body weight, food intake, blood glucose, and fatty acid levels. Interestingly, HMO treatment led to a significant decrease in plasma triglyceride and cholesterol levels. This reduction was not due to impaired absorption or decreased VLDL production; however, increased lipid uptake into the liver and a slight increase in plasma lipase activity could be observed. In line with our hypothesis, HMO intervention reduced inflammation, shown by systemically decreased plasma TNFα levels and decreased hepatic Tnfα expression. Importantly, HMO treatment resulted in a significant reduction of atherosclerotic lesion size and plaque formation in the aortic valve. Moreover, remaining plaques seemed to be stabilized as evidenced by reduced necrotic core area. Together, our results suggest that specific HMOs could be safe and effective therapeutics to attenuate atherosclerosis through lipid reduction combined with attenuation of inflammation and thus reduce cardiovascular disease risk.

31

Transmembrane protein 55B regulates low-density lipoprotein receptor lysosomal decay through phosphatidylinositol 4,5-bisphosphate

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Hepatic low-density lipoprotein receptors (LDLRs) play an important role in regulating cholesterol levels and are the primary pharmaceutical target for the prevention of cardiovascular disease (CVD). LDLR releases LDL in endosomes after internalization and then is either recycled to the cell surface or transported to the lysosome for decay. We reported that transmembrane protein 55B (TMEM55B) regulates cellular cholesterol metabolism by modulating LDLR protein decay. TMEM55B is a phosphatase that hydrolyzes phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) and has been shown to affect lysosome function. Consistent with its reported function, we found that TMEM55B knockdown in HepG2 cells significantly increased, whereas overexpression decreased, levels of $PI(4,5)P_2$. Knockdown also increased $PI(4,5)P_2$ colocalization with LAMP1, a lysosome marker. TMEM55B knockdown significantly increased lysosome staining (detected by elevated LAMP1 and LysoTracker staining), decreased LDLR protein levels, and reduced LDLR–lysosome colocalization. Consistent with our previous report that TMEM55B knockdown reduced LDL uptake, we found that TMEM55B overexpression increased LDL uptake in HepG2 cells. Notably, incubation of TMEM55B-overexpressing HepG2 cells with exogenous $PI(4,5)P_2$ reduced LDL uptake to the level of control cells. Given the importance of lysosomes in LDLR protein decay, we next tested whether TMEM55B regulates LDLR through lysosomes. Under three different conditions of impaired lysosome function (incubation with NH_4Cl or knockdown of the lysosomal proteins LAMP1 or RAB7), there was no effect of TMEM55B knockdown on LDLR protein levels. TMEM55B knockdown did not alter protein levels of known regulators of LDLR decay, including PCSK9, IDOL, or COMMD1, nor did it alter levels of transferrin receptor and LRP1, which undergo endocytic recycling similar to that of LDLR. Finally, although there was no change in the level of RAB11, a marker of recycling endosomes, LDLR–RAB11 colocalization was reduced by 50% upon TMEM55B knockdown, consistent with greater LDLR trafficking to the lysosome. Together, these findings suggest that TMEM55B regulates LDLR lysosomal protein decay through $PI(4,5)P_2$-mediated effects. Hepatic $PI(4,5)P_2$ may thus be a promising target for lowering plasma LDLC and cardiovascular disease risk.

32

Immune cell–adipocyte cross-talk regulates thermogenesis

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Immune cells in adipose tissue are vital constituents of the adipose microenvironment that influence both local and systemic lipid metabolism. Although immune cell populations have been extensively studied in the setting of obesity and adipose inflammation, their roles in lipid mobilization and thermogenesis remain poorly understood. We previously reported that hematopoietically produced interleukin-10 (IL10) acts directly on adipocytes to inhibit thermogenesis in response to adrenergic signals and that ablation of IL10 improves insulin sensitivity, protects against diet-induced obesity, and elicits the browning of white adipose tissue. To better understand immune–adipocyte cross-talk in the setting of thermogenesis, we used a three-pronged approach that involved (i) characterization of adipose tissue immune cells by single-cell RNA sequencing (scRNA-Seq), (ii) creating IL10-non-responsive adipocytes by genetically deleting IL10R from adipocytes (AdIL10RKO), and (iii) determining the identity and genetic makeup of adipocytes of AdIL10RKO mice by RNA-Seq, ATAC-Seq, and scRNA-Seq of mature adipocytes. By using this combinatorial approach, we found that adrenergic stimulation causes an increase in adaptive immune cell populations that interact with adipocytes via the IL10–IL10R axis and suppresses thermogenesis by altering the chromatin accessibility of thermogenic genes. These findings suggest that distinct immune cell populations within adipose tissue limit thermogenesis via IL10 signaling. They also implicate adaptive immune cell–adipocyte communication in the maintenance of adipose identity and function.

33

Exploiting altered enhancer landscapes to decode pathogenic changes in gene expression of diverse hepatic macrophages

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Kupffer cells have specialized roles supporting the environment of the liver during homeostasis and disease. However, the key regulatory elements governing these behaviors are unknown. Using scRNA-Seq, we found diversification of Kupffer cells and recruitment of additional macrophage subtypes during nonalcoholic steatohepatitis (NASH). A significant source of macrophage heterogeneity during NASH was traced to Cx3cr1-expressing monocytes. Furthermore, macrophage subsets were localized in distinct niches, suggesting environmental specification as a key determinant of macrophage heterogeneity. We profiled chromatin accessibility of the major NASH-associated macrophage populations to identify transcription factors governing their environmental specification. These results predict greater NFκB, RUNX, and AP1 activity in recruited hepatic macrophages compared with Kupffer cells. Surprisingly, we found minimal significant chromatin accessibility changes comparing Kupffer cells from healthy mice with mice with NASH, even though several thousand genes were differentially expressed. Instead, NASH led to altered chromatin activity, as measured by H3K27ac ChIP-Seq, at Kupffer cell enhancer regions. A binding element for liver X receptor (LXR) was the top transcription factor motif identified in Kupffer cell enhancers with reduced activity during NASH. Furthermore, LXRα was required to maintain expression of a unique gene signature defining healthy Kupffer cells. Thus, our studies identify gene-regulatory events controlling diverse hepatic macrophages during homeostasis and NASH.

34

Identification of vesicle-trafficking factor p115 as a novel ACSL4-interacting protein and its function in proteasome-mediated degradation of ACSL4

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Long-chain acyl-CoA synthetase 4 (ACSL4) is an ACSL family member that exhibits unique substrate preference for arachidonic acid (AA) and that has a functional role in hepatic lipid metabolism. Within this enzyme family, ACSL4 is the prominent isoform showing elevated expression in NAFLD. Our previous studies demonstrated a marked effect of AA on ACSL4 protein stability in hepatic cells by accelerating its degradation via the ubiquitin-proteasomal pathway (UPP). To further characterize this novel substrate-induced degradation mechanism, we applied proteomic approaches coupled with LC-MS/MS to identify ACSL4-interacting proteins in HepG2 cells under basal condition and in response to AA treatment. This work led to the identification of four ACSL4-interacting proteins, including vesicle trafficking factor p115 and HSPA8, BAG2, and 26S protease regulatory subunit 4 that function in proteasomal degradation. Importantly, we found that the interaction of p115 with ACSL4 was enhanced greatly by AA. In contrast to AA, palmitic acid, oleic acid, or EPA had no effect. Utilizing adenovirus-mediated gene knockdown (Ad-shP115), we observed a higher ACSL4 protein level when p115 was depleted in hepatic cells transfected with Ad-shP115 compared with control cells (Ad-shU6-control). Experiments using AA in combination with p115 knockdown suggested that p115 may have an additive effect in AA-dependent ACSL4 degradation. Furthermore, the presence of bafilomycin A, a lysosome inhibitor, did not hinder AA-mediated ACSL4 degradation in control cells or in p115-depleted cells, providing additional evidence for a role of p115 in facilitating ACSL4 degradation via UPP. The intracellular vesicle-trafficking factor p115 has well-known functions in ER–Golgi trafficking and Golgi biogenesis. Thus far, p115-interacting proteins are mostly involved in vesicle trafficking; p115 has not been reported to interact with proteins involved in UPP or with enzymes in FA metabolism. Because AA specifically induces ACSL4 degradation via UPP, our study provides a novel insight into the connection between ER–Golgi trafficking and proteasomal degradation machinery through p115 as a critical mediator. Understanding the regulatory mechanism of ACSL4 stability in liver cells will provide us with new tools that we can use to target ACSL4 activity in hepatic disorders, such as NAFLD and NASH.

35

FURIN inhibition reduces vascular remodeling and atherosclerotic lesion progression in mice

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Atherosclerotic coronary artery disease (CAD) is the leading cause of death worldwide, and current treatment options are insufficient. Using systems-level network cluster analyses on a large CAD case–control cohort, we previously identified proprotein convertase subtilisin/kexin family member 3 (FURIN) as a member of several CAD-associated pathways. The objective of this study was to determine the role of FURIN in atherosclerosis. *In vitro*, FURIN inhibitor treatment resulted in reduced monocyte migration and reduced macrophage and vascular endothelial cell inflammatory and cytokine gene expression. *In vivo*, administration of an irreversible inhibitor of FURIN, α-1-PDX, to hyperlipidemic $Ldlr^{-/-}$ mice resulted in lower atherosclerotic lesion area and a specific reduction in severe lesions. Significantly lower lesional macrophage and collagen areas as well as systemic inflammatory markers were observed. Matrix metallopeptidase 2 (MMP2), an effector of endothelial function and atherosclerotic lesion progression and a FURIN substrate, was significantly reduced in the aorta of inhibitor-treated mice. To determine FURIN's role in vascular endothelial function, we administered α-1-PDX to $Apoe^{-/-}$ mice harboring a wire injury in the common carotid artery. We observed significantly decreased carotid intimal thickness and lower plaque cellularity, smooth muscle cell, macrophage, and inflammatory marker content, suggesting protection against vascular remodeling. Overexpression of FURIN in this model resulted in a significant 67% increase in intimal plaque thickness, confirming that FURIN levels directly correlate with atherosclerosis. In conclusion, we show that systemic inhibition of FURIN in mice decreases vascular remodeling and atherosclerosis. FURIN-mediated modulation of MMP2 activity may contribute to the atheroprotection observed in these mice.

36

Small nucleolar RNA U17 rewires cellular metabolism and regulates lipotoxicity

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Excess fatty acids promote oxidative stress and cell death in a process called lipotoxicity. Our group conducted a promoter trap mutagenesis screen in Chinese hamster ovary cells and discovered that loss-of-function mutations in the small nucleolar RNA hosting gene 3 (Snhg3) locus caused resistance to palmitate-induced oxidative stress and apoptosis. These mutants are also protected from cell death caused by hydrogen peroxide, indicating a broad role for the Snhg3 locus in oxidative stress. Snhg3 encodes an lncRNA, SNHG3, and the intronic U17A/B small nucleolar RNAs (snoRNAs). Targeted knockdown of the different RNAs from this locus indicates that the U17 snoRNAs, but not the lincRNA, are critical for lipotoxicity. Snhg3 mutants show broad alterations in cellular metabolism with decreased lactate efflux and increased mitochondrial oxidation of glucose, glutamine, and pyruvate, promoting a shift away from glycolytic metabolism and toward oxidative phosphorylation. This metabolic rewiring occurs in conjunction with an up-regulation of the NADPH–glutathione axis, suggesting that U17 snoRNAs promote resistance to metabolic stress by modulating the cellular redox environment. *In vivo*, administration of U17 snoRNA–specific LNAs augments hepatic NADPH, reduces oxidized glutathione, and up-regulates transcripts involved in oxidation/reduction processes. Together, these findings establish an important role for snoRNA U17s in regulating cell metabolism and responses to metabolic stress.

37

Regulation of mitochondrial respiration and lipidome in cardiomyocytes following persistent administration of the β-agonist isoproterenol

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Our recent study performed on non-failing and failing murine hearts using MS-based shotgun lipidomics indicated that during the development of chronic heart failure a continuous rise in systemic adrenergic activity led to significant modifications of the cardiac lipidome. In the present study, we investigated whole-lipidome changes of primary cardiomyocytes (PCMs) isolated from mice during the development of isoproterenol (ISO)-induced cardiomyopathy. 8-week-old male 129sv mice were subcutaneously injected with ISO/vehicle for 4 consecutive days. Afterward, mice were sacrificed, hearts were removed, and PCMs were isolated from the cardiac tissue using a Langendorff perfusion apparatus and collagenase type 2 digestion procedure. After Ca^{2+} reintroduction, cardiomyocytes were subjected to shotgun lipidomic analysis using a Q Exactive mass spectrometer in quadrupole-orbitrap configuration. Afterward, lipidomics-tailored bioinformatics analysis was performed. Preliminary results revealed that 4-day ISO treatment induces cardiac lipidome changes in mice. Using an initial unadjusted pairwise comparison (Mann–Whitney *U* test) we observed significant increases in the abundance of specific phosphatidylethanolamines (16:1–18:1, 18:0–18:2) and phosphatidylcholines (16:1–18:2, 20:1–20:4) in the ISO-treated group as well as a significant reduction of specific triacylglycerol (56:5, 54:6) and cardiolipins (CLs; 72:8, 72:9) when compared with controls. CLs are localized in the inner membrane of the mitochondria where they provide structural and functional support to proteins of the electron transport chain. Altered CL profiles have been reported to result in mitochondrial dysfunction, which has been linked to various cardiovascular diseases. Our preliminary data obtained from primary isolated cardiomyocytes have shown that 4-day stimulation with ISO leads to elevation in oxygen consumption rates by electron transport as evaluated by the Seahorse XF analyzer. Data also indicated an increase in glycolytic activity accompanied by increased expression levels of UCP3. In summary, our preliminary data indicate for the first time a regulation of specific lipid species, including mitochondrial CLs, in PCMs during the development of ISO-induced cardiomyopathy. This was accompanied by regulation of mitochondrial respiration following chronic ISO stimulation. Together, these data suggest that chronic adrenergic stimulation of cardiomyocytes affects the mitochondrial lipidome, resulting in mitochondrial dysregulation, suggesting an involvement of cellular lipid changes in the pathogenesis of the disease.

38

Evaluating the role of genetic variation in the risk of dyslipidemia in the South African population

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Dyslipidemia, a disorder of lipoprotein metabolism, is a commonly encountered clinical condition that has been identified as a major risk factor for cardiovascular diseases. Dyslipidemia has been shown to have a strong genetic component. Apolipoprotein E (apoE) is a key regulator of plasma lipid levels; thus, any genetic variation in this gene is likely to be of concern. ApoE exists in three major isoforms, apoE2, apoE3, and apoE4. ApoE2 has been linked to type III hyperlipoproteinemia, whereas apoE4 is linked to an increased risk for atherosclerosis. Determination of apoE isoform can be achieved by genotyping the polymorphisms rs429358T>C and rs7412C>T. In this study we genotyped a cohort of South African dyslipidemic patients to determine the prevalence of the rs429358T>C and rs7412C>T polymorphisms to identify patients at risk for developing dyslipidemia based on apoE risk allele profiles. Participants (n = 245) were recruited from Chris-Hani Baragwaneth hospital and consisted of 165 dyslipidemic black South Africans and 80 controls. The samples were genotyped for the rs429358T>C and rs7412C>T SNPs by PCR-RFLP method using AflIII and HaeII restriction enzymes, respectively, to determine each variant. ApoE isoform genotypes were determined using rs429358T>C and rs7412C>T haplotypes. The minor allele frequencies for rs429358T>C and rs7412C>T in the control group were 0.23 and 0.24, respectively, and 0.27 and 0.15 in the dyslipidemia cohort, respectively. The allele frequencies for apoE2, apoE3, and apoE4 were 0.20, 0.60, and 0.19 in the control group and 0.11, 0.63, and 0.24 in the dyslipidemia cohort, respectively. There were differences in the distribution of the apoE isoform variants between controls and samples (p = 0.034). In conclusion, we report the differences in the distribution of apoE variants between dyslipidemic and control patients. ApoE may be used as a biomarker for dyslipidemia in this South African population.

39

Increased fat oxidation and metabolic rewiring in a murine model of Marfanoid progeroid lipodystrophy syndrome

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Marfanoid progeroid lipodystrophy syndrome (MPLS) is an ultra-rare genetic condition caused by C-terminal truncating mutations in the fibrillin-1 (FBN1) gene. MPLS is related to Marfan syndrome and characterized by intrauterine growth retardation, post-natal failure to thrive, aortic bulb dilatation, and craniofacial, skeletal, and ocular abnormalities. However, one of the distinguishing characteristics of MPLS not observed in Marfan syndrome is the presence of congenital generalized lipodystrophy, which remarkably occurs with normal fasting glucose levels, insulin sensitivity, and plasma triglyceride levels (O'Neill, B., Simha, V., Kotha, V., and Garg, A. (2007) *Am. J. Med. Genet. A* **143A,** 1421–1430). Fibrillin-1 is an extracellular matrix (ECM) protein that oligomerizes to form microfibrils that have both structural and signaling roles in multiple tissues. To study the pathophysiology of MPLS we generated a mouse model with C-terminal truncation of the Fbn1 gene (Fbn1DC/+). Both male and female Fbn1DC/+ mice recapitulate the human phenotype, exhibiting a reduced growth rate and generalized lipodystrophy, with an almost complete absence of visceral and subcutaneous white adipose tissue but conservation of brown adipose tissue. Metabolic studies showed that Fbn1DC/+ mice maintain normal glucose tolerance and serum triglycerides but have an increased metabolic rate and a decreased respiratory exchange ratio (RER), the latter indicating a higher ratio of fat to carbohydrate utilization as an energy substrate. Metabolomic analyses on liver and brown adipose tissues (BAT) from Fbn1DC/+ and control mice showed signs of increased fatty acid β-oxidation in Fbn1DC/+ BAT and decreased lipogenesis in Fbn1DC/+ female livers, with phosphatidylcholines showing an increase in Fbn1DC/+ BAT and a decrease in Fbn1DC/+ livers, compared with controls. Additionally, both Fbn1DC/+ liver and brown adipose tissues showed signs of increased collagen and proteoglycan turnover, including significant increases in betaine levels compared with control tissues, which is likely a result of ECM disruption due to fibrillin-1 structural dysfunction. These results suggest that increased brown adipose β-oxidation and metabolic rate may be an important mechanism underlying the lipodystrophic and metabolic phenotype of MPLS. Changes in phosphatidylcholine and betaine levels may potentially contribute to this process and/or to a dysregulation of adipose tissue development, with future studies aimed at addressing these hypotheses.

40

Lipin 1 is required for hepatic adaptation to fasting/feeding and maintenance of mRNA splicing patterns

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Lipid synthesis and storage are critical for metabolic homeostasis, and disturbances in this process may contribute to obesity and cardiometabolic disease. The lipin proteins are phosphatidate phosphatase (PAP) enzymes required for lipid biosynthesis and also act as transcriptional co-regulators. The daily transition between fasting and feeding leads to several metabolic adaptations, including utilization of glucose as a preferred fuel substrate during the fed state and utilization of lipids during the fasted state. Lpin1$^{-/-}$ mice have aberrant metabolic adaptations to the fasting/feeding transition. RNA sequencing and lipidomic analyses indicated that Lpin1$^{-/-}$ liver experiences impaired fatty acid oxidation during fasting and decreased cholesterol biosynthesis during the fed state. A screen of lipin 1–interacting proteins in hepatocytes revealed that lipin 1 interacts with several spliceosome components, particularly members of the U2 snRNP. Furthermore, chromatin abundance of U2 snRNA was significantly increased in the Lpin1$^{-/-}$ liver, consistent with dysregulation of spliceosome function. RNA sequencing at high coverage revealed aberrant mRNA splicing patterns in liver of Lpin1$^{-/-}$ mice, with distinct effects in the fasted and refed states. We conclude that lipin 1 plays a role in the adaptation of hepatic lipid metabolism to fasting/feeding, and this may be influenced by effects on mRNA splicing.

41

Dissociation of the triglyceride and phospholipid transfer activities of a vertebrate microsomal triglyceride transfer protein

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The microsomal triglyceride transfer protein complex (MTP) is essential for the assembly of apolipoprotein B (ApoB)-containing lipoproteins in the intestine and liver. As the apolipoprotein is translated and translocated into the lumen of the endoplasmic reticulum, MTP physically interacts with and transfers lipids to ApoB to form primordial lipoproteins. *In vitro*, vertebrate MTP can transfer a variety of lipid species between vesicles, including triglycerides and phospholipids. In contrast, invertebrate MTP transfers phospholipids but not triglycerides. Comparisons of primary sequences and predicted structures of invertebrate and vertebrate MTP orthologues suggest that acquisition of triglyceride transfer during evolution was not the result of a few simple amino acid substitutions but due to many changes in the domains that form the large lipid-containing cavity of MTP. However, through forward genetic screening, we have discovered a zebrafish mutant (mttpc655) expressing a single C-terminal missense mutation (G863V) that challenges this hypothesis. *In vitro*, the G863V Mttp protein is defective at transferring triglycerides but unexpectedly retains phospholipid transfer activity. Similarly, mutating the conserved glycine in the human MTTP protein (G865V) also eliminates triglyceride but not phospholipid transfer. The G863V mutation reduces the production and size of ApoB-containing lipoproteins *in vivo* and results in the abnormal accumulation of cytoplasmic lipid droplets in the yolk syncytial layer of the developing zebrafish embryo. However, it only has mild effects on lipid malabsorption in the intestine and no consequence on growth. In contrast, zebrafish mutants bearing the previously identified mttpstl (L475P mutation) exhibit gross intestinal lipid accumulation and defective growth due to a severe deficiency in lipoprotein production. *In vitro*, the L475P mutation blocks transfer of both triglycerides and phospholipids. Thus, the G863V point mutation provides the first evidence that the triglyceride and phospholipid transfer functions of a vertebrate MTP protein can be biochemically separated and argues that the C-terminal α-helical region is more essential for triglyceride transfer activity than was previously appreciated. Significantly, these data suggest that selective inhibition of the triglyceride transfer activity of MTP may be an effective strategy for treating hyperlipidemia while preventing the gastrointestinal side effects associated with broad inhibition of MTP activity.

42

Endocytosis adaptor Dab2 in lipid metabolism

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Disabled-2 (Dab2) is a widely expressed clathrin- and cargo-binding endocytic adaptor protein, and cell biology studies indicated that Dab2 plays a role in cellular trafficking of a number of transmembrane receptors and signaling proteins. Dab2 is essential for early embryogenesis, but a conditional knockout using Sox2-Cre is able to bypass the requirement for early development and generate Dab2-null mice. Studying Dab2-null mice provides insights into the physiology and pathology of Dab2 endocytic functions. Dab2 plays key roles in lipid metabolism: regulation of cholesterol homeostasis and fatty acid storage in differentiation of adipocytes. Low-density lipoproteins (LDL) receptor is a key cargo for Dab2 as an endocytic adaptor. LDL clearance from the circulation by LDL receptor (LDLR)-mediated endocytosis in hepatic and peripheral tissues and subsequent feedback regulation of endogenous synthesis of cholesterol are a key determinant of the serum LDL level. In our genetic analysis of mutant mice, we found that deletion of Dab2 only slightly affected serum cholesterol levels. However, elimination of both dab2 and arh, another key LDLR endocytic adaptor, resulted in profound hypercholesterolemia similar to that resulting from ldlr homozygous deletion. This indicates that Arh and Dab2 are two key endocytic adaptors for LDL receptor. We found that the Dab2-deficient mice were grossly normal, and the most noticeable phenotype is their resistance to high-calorie diet–induced obesity. Interestingly, the reduced adiposity of Dab2-null mice was present in juvenile but not in mature mice. In Dab2-deficient mice, the size of adipocytes was enlarged to the same extent as the wildtypes, but the number was reduced. A population of Dab2-expressing cells residing in adipose vascular structure as an adipocyte progenitor cell population is present in juveniles but depleted in mature animals. We speculate that this Dab2-marked preadipocyte population undergoes differentiation induced by excessive calorie intake and accounts for the higher capability in adipogenesis of younger animals.

43

Transcriptional regulation of sulfur metabolism by phospholipid methylation

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Phosphatidylethanolamine (PE) is one of the most abundant phospholipids in cell membranes. In addition to the fundamental biology of PE in membrane biogenesis, emerging evidence reveals that membrane PE levels are associated with pathologies, including nonalcoholic fatty liver disease, Alzheimer's disease, Parkinson's disease, and certain infectious diseases. Our recent study demonstrates that methylation of PE for the synthesis of phosphatidylcholine mediates an unforeseen membrane-to-chromatin communication. Under nutrient starvation, we found that PE methylation consumes substantial amounts of cellular *S*-adenosylmethionine (SAM) and causes SAM depletion, which results in hypomethylation of histones and activation of sulfur metabolism (MET) genes to replenish cellular SAM levels. Although methylation of histones directly fuels sulfur metabolism through turnover of SAM, surprisingly, it does not account for transcriptional activation of MET genes. Interestingly, we found that deubiquitinylation of a MET transcriptional factor is defective in the PE methylation–deficient mutant, consistent with defective activation of MET genes found in this mutant. These findings suggest that activation of MET genes in response to SAM depletion caused by PE methylation involves a SAM-sensitive ubiquitination system governing the activity of transcriptional factors and is independent of histone methylation status. Therefore, the synthesis of phospholipids can elicit metabolic and transcriptional signals for environmental adaptation that can be pathological bases for many related diseases.

44

Haploid mammalian genetic screens empower novel gene identification in lipid metabolism and atherosclerosis

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Disturbed lipid metabolism is a key determinant of atherosclerosis development and progression. To identify new genes and elucidate cellular mechanisms that govern lipid homeostatic pathways, we have recently developed functional genetic screens based on the use of a haploid mammalian system. In a unique mammalian haploid cell line, we employ this approach to follow the fates of native and tagged endogenous proteins associated with lipid-regulating pathways. By applying this experimental system to cholesterol biosynthesis and uptake, we have been able to characterize novel regulators of the rate-limiting enzymes in cholesterol biosynthesis, HMGCR and SQLE. Furthermore, the ability to integrate results from multiple screens has allowed us to also identify novel (pan)-regulators of the SREBP pathway. Two of these novel regulators, MARCH6 and SPRING, are being studied *in vitro* and in mouse models, and results from these studies will be presented. Our findings further highlight the potential of haploid mammalian genetics to study lipid metabolism.

45

Regulation of lipid metabolism by the heterogenous nuclear ribonucleoprotein RALY

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Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a family of RNA-binding proteins with critical roles in RNA metabolism, including regulation of transcription, splicing, and nuclear export. Although multiple lines of evidence link hnRNPs to neurodegenerative diseases and cancer, their role in metabolic control remains unexplored. In this work, we outline a role for hnRNPs in regulatory circuits controlling sterol homoeostasis. Liver-specific deletion of the hnRNP RALY alters hepatic lipid content and serum cholesterol level. Gene expression analysis comparing L-*Raly* knockouts with controls shows specific enrichment of metabolic pathways. ChIP-Seq and unbiased chromatin interrogation reveal insights into preferential binding patterns of RALY, its cooperative interactions, and mode of action in regulating gene expression. Our work suggests that hnRNPs play important roles in transcriptional metabolic control.

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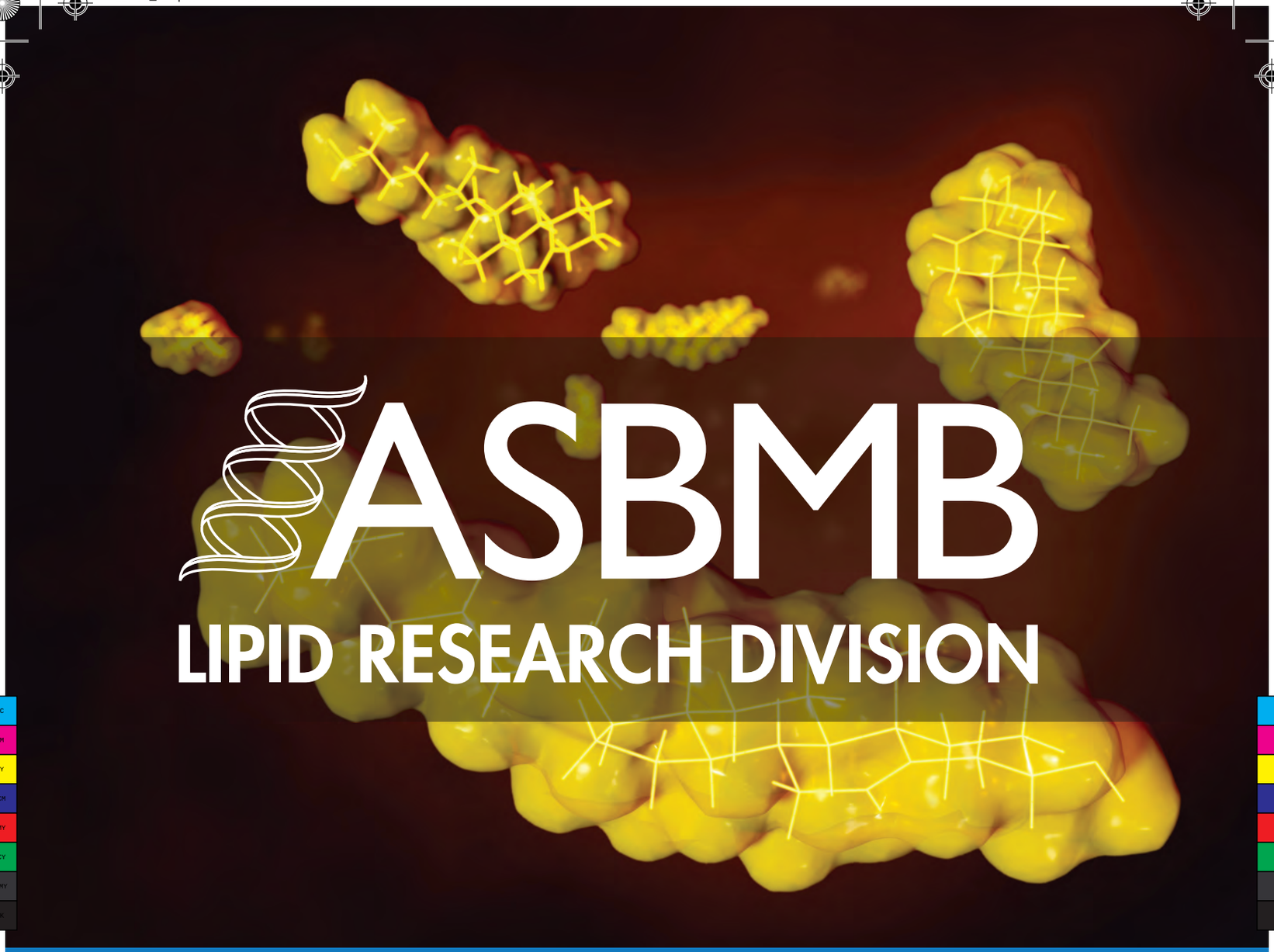

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