DEUEL CONFERENCE ON LIPIDS

March 1 — 4, 2016
Napa, 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.

Thank You to Our Generous Sponsors

ASBMB
American Society for Biochemistry and Molecular Biology

AstraZeneca

Avanti®
Polar Lipids, Inc.

Bristol-Myers Squibb

IONIS®
PHARMACEUTICALS

JLR Journal of Lipid Research

MERCK

Pfizer

REGENERON

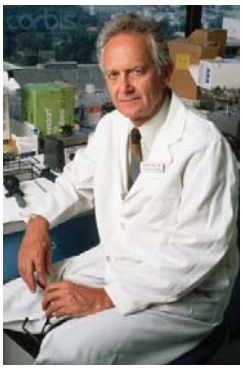
Table of Contents

2016 DEUEL Conference on Lipids

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

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

THE HAVEL LECTURE



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

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

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

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

2016 HAVEL AWARD LECTURE

Sir Stephen O'Rahilly,
University of Cambridge
"Obesity and insulin resistance; lessons from human genetics"

PAST HAVEL AWARDEES

2015 
Thomas Sudhof,
Stanford University
"Brown & Goldstein-inspired science off field: lipid membrane fusion at the synapse"

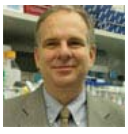
2014 
Rudolf Zechner, University of Graz
"Lipolysis - more than just the breakdown of fat"

2013 
Rick Lifton, Yale University
"From human genetics to validated therapeutic targets"

2012 
Gokhan Hotamisligil,
Harvard University
"Inflammation, endoplasmic reticulum stress and lipids: emerging networks regulating metabolism"

2011 
Christopher K. Glass,
University of California, San Diego
"Oxysterol regulation of macrophage gene expression"

2010 
David J. Mangelsdorf,
University of Texas Southwestern Medical Center
"Nuclear receptor control of lipid metabolism"

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

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

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

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

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

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

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

2002 

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

Schedule of Events

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

Meeting Program

The Deuel Conference on Lipids, March 1–4, 2016

The Silverado Resort and Spa, Napa, Calif.

Chair: Christopher K. Glass, University of California, San Diego

Tuesday, March 1

3:00 – 5:30 Meeting Registration

5:30 – 7:30 Opening Reception and Dinner

7:30 – 8:30 The Havel Lecture
"Obesity and insulin resistance; lessons from human genetics"
Sir Stephen O'Rahilly, University of Cambridge

Wednesday, March 2

Session 1: Cellular Homeostasis, 8:45– 11:45am
Session Chair: **Alan Attie**

8:45 – 9:15 **"The life and times of PPAR-delta in neurons: From normal function to neurodegenerative disease accomplice to therapeutic target"**
Al La Spada, University of California, San Diego

9:15 – 9:45 The Journal of Lipid Research Lecture
"The non-coding RNA battle for cholesterol homeostasis: miRNAs & LncRNAs duel it out"
Kathryn Moore, New York University

9:45 – 10:15 **"Growth by the mTOR pathway"**
David Sabatini, Massachusetts Institute of Technology

10:15 – 10:30 Coffee break

10:30 – 11:00 **"Time-restricted feeding - a new paradigm to understand the mechanism of metabolic disease"**
Satchin Panda, The Salk Institute

11:00 – 11:15 "**Control of hepatic lipid metabolism by an LXR-responsive Long Non-coding RNA"**
Tamer Sallam, University of California, Los Angeles, HHMI

11:15 – 11:30 "**Rpl13a snoRNAs are key mediators of lipotoxic cardiomyopathy**"
Jiyeon Lee, Washington University, St. Louis

11:30 – 11:45 **"Fatostatin blocks ER-to-Golgi transport of SCAP but inhibits cell growth in a SCAP-independent manner"**
Peter Espenshade, Johns Hopkins University School of Medicine

11:45 – 5:00 Free time

5:00 – 6:00 Poster Session 1, Napa Hall (abstracts with odd board numbers are scheduled to present, see pages 9 - 63)

Session 2: Nuclear Receptors, 7:30 – 10:00pm

Session Chair: **Jay Horton**

7:30 – 8:00 **"Gut feelings: how intestinal FXR controls fatty acid and cholesterol metabolism "**
Ron Evans, Salk Institute

8:00 – 8:30 **"Phospholipid ligands: sculpting nuclear lipid signaling via metabolic nuclear receptors"**
Holly Ingraham, University of California, San Francisco

8:30 - 8:45 Coffee break

8:45 – 9:15 **"Epigenomic repression of hepatic autophagy by an SHP-LSD1 complex"**
J. Kim Kemper, University of Illinois at Urbana-Champaign

9:15 – 9:45 **"Control of lipid metabolism by histone deacetylase 3"**
Mitch Lazar, University of Pennsylvania, Philadelphia

9:45 – 10:00 "**The anti-lipogenic effect of TTC39B deficiency is LXR-dependent and protects from steatohepatitis**"
Joanne Hsieh, Columbia University

Thursday, March 5

Session 3: Immunity, 8:45– 11:45am

Session Chair: **Alan Tall**

8:45 – 9:15 **"Multiple crosstalk points between host metabolism and antiviral innate immune response"**
Genhong Cheng, University of California, Los Angeles

9:15 – 9:45 **"Residents and passengers: biology of macrophages in a layered myeloid system"**
Frederic Geissman, Memorial Sloan Kettering Cancer Center

9:45 – 10:15 **"Lipid signaling molecules in immunity"**
Jason Cyster, University of California, San Francisco

10:15 – 10:30 Coffee break

10:30 – 11:00 **"Fighting atherosclerosis with sugar - a novel treatment option for atherosclerosis prevention and regression?"**
Eicke Latz, Universitätsklinikum

11:00 – 11:15 "**Identification of a cholesterol metabolic-type I interferon inflammatory circuit"**
Autumn York, University of California, Los Angeles

11:15 – 11:30 "**Hepatic PPP1R3B deletion promotes hepatic insulin resistance, and increases susceptibility to hepatic steatosis on a high-sucrose diet fed model**"
Minal Mehta, University of Pennsylvania

11:30 – 11:45 **"Cardiomyopathy but not brown adipose tissue dysfunction causes cold sensitivity in mice globally lacking ATGL"**
Renate Schreiber , University of Graz

11:45 – 5:00 Free time

5:00 – 6:00 Poster Session 2, Napa Hall (abstracts with even board numbers are scheduled to present, see pages 9 - 63)

Session 4: Metabolic Disease, 7:30 – 10:00pm

Session Chair: **Jean Schaffer**

7:30 – 8:00 **"The human KO project: from concept to data"**
Sek Kathiresan, Harvard Medical School

8:00 – 8:30 "**Epigenetic reader proteins as regulators of cardiometabolic transcriptional programs**"
Jorge Plutzky, Brigham and Women's Hospital

8:30 – 8:45 Coffee break

8:45 – 9:15 **"Tissue-specific actions of the metabolic hormone FGF21"**
Steve Kliewer, University of Texas Southwestern Medical Center

9:15 – 9:45 **"Nonalcoholic liver hepatic steatosis: a systems genetics approach"**
Jake Lusis, University of California, Los Angeles

9:45- 10:00 **"Development of cell-translation therapy in severe familial hypercholesterolemia undergoing ldl-apheresis therapy"**
Masahiro Koseki, Osaka University

Friday, March 6

Session 5: Better Living Through Chemistry, 8:45 – 11:00am

Session Chair: **Stephen Young**

8:45 – 9:15 **"Mapping lipid metabolic pathways in human disease by integrated chemical proteomics and metabolomics"**
Ben Cravatt, The Scripps Research Institute

9:15 – 9:45 **"Endogenous bioactive lipids with beneficial metabolic effects"**
Alan Saghatelian, The Salk Institute

9:45 – 10:15 **"Antisense therapies for rare diseases"**
Frank Bennett, Ionis Pharmaceuticals

10:15 – 10:30 **"Global analysis of plasma lipids identifies liver-derived acyl-carnitines as a fuel source for brown fat thermogenesis"**
Judith Simcox, University of Utah

10:30 – 10:45 "**ApoC-III modulates clearance of triglyceride-rich lipoproteins in mice through low density lipoprotein family receptors**"
Philip Gordts, University of California, San Diego

10:45 – 11:00 "**Isotopomer spectral analysis of cholesterol biosynthesis in vivo reveals multiple tissue-specific, flux-dependent pathways**"
Matthew Mitsche, University of Texas Southwestern Medical Center

11:00 Closing

Poster Presentations

1

Transcription Factor Dynamics Identify a Circadian Code for Fat Cell Differentiation

Zahra Bahrami-Nejad[1], Michael L. Zhao[1], Sabine von Schie[1], Karen E. Tkach[1], Mingyu Chung[1], Mary N. Teruel[1]

[1]Department of Chemical and Systems Biology, Stanford University, Stanford, CA

Adipogenesis, the process by which precursor cells differentiate into fat cells (adipocytes), is strongly driven by glucocorticoid signaling. Studies in mice and humans show that mammals have oscillating glucocorticoid levels that peak during waking and that persistent increases in glucocorticoids due to stress, cancer, or genetic predisposition correlate with increased adipogenesis and obesity. This raises the intriguing question of whether the circadian timing of glucocorticoid signaling controls the rate of adipogenesis. Here we used live single-cell analysis of two *in vitro* models of adipogenesis to test the hypothesis that pulsatile circadian glucocorticoid signals are rejected by the differentiation control system, whereas identical averaged but persistent glucocorticoid signals are rejected. We stimulated the cells with different pulse protocols of glucocorticoid and found that preadipocytes undergo only minimal differentiation for circadian 12-h-long glucocorticoid inputs, whereas prolonged glucocorticoid inputs longer than 12 h mediate strong adipocyte differentiation. This filtering out of short stimuli could not be overcome by raising stimulus doses, arguing that the adipogenesis signaling network is built to only trigger the differentiation of low numbers of preadipocytes for normal circadian glucocorticoid inputs. Using live-cell imaging of PPARG, the master transcriptional driver of adipogenesis, endogenously tagged with YFP, we found that pulsatile glucocorticoid stimuli fail to increase the level of PPARG to a critical level needed for its autoactivation by positive feedback. Above this threshold, PPARG levels become independent of the glucocorticoid input. The filtering of glucocorticoid inputs occurs at the level of early, upstream regulators of the adipogenesis network and results from a transcriptional delay between CEBPB, which is directly controlled by glucocorticoids, and the activation of PPARG and induction of CEBPA, which can replace CEBPB when differentiation is proceeding. Thus, our study provides evidence that adipocyte differentiation is controlled by temporal filtering of circadian glucocorticoid input, explaining how aging, stress, Cushing disease, overeating, and other conditions may be linked to obesity.

2

Reduction in ApoB following a Hypocaloric Diet Is Associated with Amelioration of Insulin Resistance and Postprandial Hypertriglyceridemia

Simon Bissonnette[1,2], Valerie Lamantia[1,2], Yannick Cyr[1,2], Jennifer Rene[2], Hanny Wassef[2], Remi Rabasa-Lhoret[1,2], May Faraj[1,2]

[1]University of Montreal, Montreal, QC, Canada; [2]Institut de Recherches Cliniques de Montréal, Montreal, QC, Canada

There is a large intersubject variability in the improvement of cardiometabolic risk factors in response to a hypocaloric diet-induced weight loss in obese subjects. We recently reported that, compared with obese subjects with normal plasma apoB, subjects with high apoB have higher glucose-induced insulin secretion, insulin resistance, postprandial hypertriglyceridemia, and white adipose tissue dysfunction independent of body composition. In the present study, we hypothesized that the improvement in cardiometabolic risk factors after a hypocaloric diet is dependent on the reduction in plasma apoB. Fifty-nine subjects were evaluated following 6 months of hypocaloric diet (33 women, 26 men, 58 ± 6 years, 32.6 ± 4.6 kg/m^2, 0.34-1.8 g/liter apoB, free of chronic disease). Insulin secretion was assessed during a 1-h intravenous glucose tolerance test (0.3 g of dextrose/kg), whereas insulin sensitivity was assessed following a 3-h hyperinsulinemic euglycemic clamp. Subjects in the highest quartile of baseline plasma apoB who reduced plasma apoB (range: −0.26 to −0.02 g/liter) following the intervention had an improvement in insulin sensitivity measured as AUC plasma glucose/insulin during the IVGTT (+ 32%, $p < 0.001$) and glucose infusion rate/insulin (+48%, $p = 0.002$). On the other hand, despite equal weight loss (~6 %, not significant), subjects in the lowest apoB quartile who increased or had no change in apoB (range: 0.02-0.31 g/liter) had no change in these parameters. Moreover, postprandial plasma fat clearance was evaluated in a subpopulation of 23 subjects following the ingestion of a high fat meal. The reduction in plasma apoB was associated with a reduction in total ($p < 0.001$, $R^2 = 0.50$) and incremental ($p = 0.007$, $R^2 = 0.28$) decrease in AUC of postprandial plasma triglycerides over 6 h, independent of weight loss. Reduction in plasma apoB predicts the amelioration in insulin resistance and postprandial hypertriglyceridemia following a hypocaloric diet independent of weight loss. We hypothesize that the hyperapoB phenotype may be a key therapeutic target to reduce obesity-associated cardiometabolic risks maximally by hypocaloric interventions.

3

Time-restricted Feeding Is a Preventative and Therapeutic Intervention against Diverse Nutritional Challenges

Amandine Chaix[1], Amir Zarrinpar[1,2], Phuong Miu[1], Satchidananda Panda[1]

[1]Salk Institute for Biological Studies, La Jolla, CA; [2]Division of Gastroenterology, University of California, San Diego, La Jolla, CA

Because current therapeutics for obesity are limited and only offer modest improvements, novel interventions are needed. Preventing obesity with time-restricted feeding (TRF; 8-9 h of food access in the active phase) is promising, yet its therapeutic applicability against preexisting obesity, diverse dietary conditions, and less stringent eating patterns is unknown. Here we tested TRF in mice under diverse nutritional challenges. We show that TRF attenuated metabolic diseases arising from a variety of obesogenic diets and that benefits were proportional to the fasting duration. Furthermore, protective effects were maintained even when TRF was temporarily interrupted by *ad libitum* access to food during weekends, a regimen particularly relevant to the human lifestyle. Finally, TRF stabilized and reversed the progression of metabolic diseases in mice with preexisting obesity and type II diabetes. We establish clinically relevant parameters of TRF for preventing and treating obesity and metabolic disorders, including type II diabetes, hepatic steatosis, and hypercholesterolemia.

4

Interactions of Angiopoietin-like Proteins with GPIHBP1-LPL Complexes

Xun Chi[1], Hannah W. Shows[1], Alexander J. Hjelmaas[1], Emily C. Britt[1], Shwetha K. Shetty[1], Emily K. Malcolm[1], Brandon S. Davies[1]

[1]University of Iowa, Iowa City, IA

Triglycerides derived from diet are packaged into lipoproteins for delivery to peripheral tissues. The release of fatty acids from plasma triglycerides for tissue uptake is critically dependent on the enzyme lipoprotein lipase (LPL). Hydrolysis of plasma triglycerides by LPL can be disrupted by the proteins from the angiopoietin-like family (ANGPTLs). ANGPTL3 and ANGPTL4 have been shown to inactivate LPL *in vitro*. However, *in vivo* functional LPL is complexed to glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1) on the surface of capillary endothelial cells. GPIHBP1 is responsible for trafficking LPL across capillary endothelial cells and anchors LPL to the capillary wall during lipolysis. Our goal is to investigate the interactions of ANGPTL3 and ANGPTL4 with LPL-GPIHBP1 complexes on the surface of endothelial cells. We found that ANGPTL4 was capable of binding and inactivating LPL complexed to GPIHBP1 on the surface of endothelial cells. Once inactivated, LPL dissociated from GPIHBP1. ANGPTL3 could also inactivate LPL complexed to GPIHBP1 but only at supraphysiologic concentrations. LPL inactivated by either ANGPTL3 or ANGPTL4 was incapable of binding GPIHBP1. ANGPTL4 was capable of binding, but not inactivating, LPL at 4|°C, suggesting that binding alone was not sufficient for the inhibitory activity of ANGPTL4. We were unable to detect binding of ANGPTL3 to LPL, suggesting that additional factors may facilitate binding. We observed that while the N-terminal coiled-coil domain of ANGPTL4 by itself and full-length ANGPTL4 both bound with similar affinities to LPL, the N-terminal fragment was more potent in inactivating both free and GPIHBP1-bound LPL. The N-terminal fragment of ANGPTL3 also appears to be a more potent inhibitor of LPL.

5

Long Term Persistent Activation of the Innate Immune System in a Hypercholesterolemic Environment: Trained Memory Induction in Myeloid (Precursor) Cells?

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

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

Efforts to reverse the pathologic consequences of vulnerable atherosclerotic plaques are often impeded by the complex pro-inflammatory environment within the plaque, which might be provoked by a long term (epigenetic) reprogramming of myeloid cells. Indeed, epigenetic reprogramming and the induction of a "trained innate memory" in myeloid cells have recently been encouraged by several studies. These changes probably evolved as an ancient mechanism to protect against pathogens because it confers nonspecific protection from secondary infections. However, dysregulated processes of immunological imprinting mediated by trained innate immunity may contribute to the exaggerated immune responses in inflammatory diseases, such as atherosclerosis. Given the above hypothesis, we sought to investigate whether a hyperlipidemic environment can considerably modify myeloid subsets in atherosclerosis-prone mouse models (*Ldlr*$^{-/-}$, *Ldlr*$^{-/-}$/*ApoA-I*$^{-/-}$), which in turn could evoke a condition of continuous immune cell activation. Therefore, mice were fed 1) a standard chow diet, 2) a high fat/high cholesterol diet (HFD) for 4 weeks, or 3) an HFD for 4 weeks followed by a 4-week resting period ("trained innate memory" induction). As expected, circulating cholesterol as well as pro-inflammatory cytokine levels (e.g. IL-1b, IL-6, TNF-α) peaked by 4 weeks of diet feeding in both mouse genotypes and returned to baseline in the HFD/rested animals. To test for evidence of HFD-induced memory build-up, bone marrow as well as splenic myeloid cells were extracted from the three feeding groups and stimulated *ex vivo* with a panel of Toll-like receptor ligands. In contrast to the chow diet group, stimulated cells from the HFD/rested animals gave as much response as or even greater response than stimulated cells from the 4-week HFD-fed animals. Interestingly, sequencing of mRNA isolated from flow-sorted bone marrow myeloid precursor subsets revealed a more pronounced gene induction of many pro-inflammatory genes involved in innate immune signaling and antigenic stimulation in both the HFD and the HFD/rested animals. These data support the concept of a diet-induced epigenetic reprogramming already on the level of myeloid progenitor subsets within the bone marrow, which may induce a long term hyperactive state of diverse peripheral innate immune cell subsets.

6

Cellular and Molecular Analysis of Macrophages Associated with Adipose Tissues

Lucile Crozet[1,2,3], Nathanael J. Spann[4], Katrin Kierdorf[3], Hannah Garner[3], Elisa Gomez Perdiguero[3], Christopher K. Glass[4], Frederic Geissmann[2, 3]

[1]Memorial Sloan Kettering Cancer Center, New York, NY; [2]Weill Cornell Graduate School of Biomedical Sciences, New York, NY; [3]Center for Molecular and Cellular Biology of Inflammation, King's College, London, UK; [4]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA

As dietary lipid intake has massively increased during the last decades, the rate of obesity has become a major concern worldwide. Lipid-rich diets are associated with chronic inflammation, type II diabetes, cardiovascular diseases, and cancer. Myeloid cells, and macrophages in particular, have been proposed to contribute to the pathogenesis of lipid-rich diet-associated diseases, but the mechanisms involved remain unclear. We aim to characterize the population(s) of macrophages associated with adipose tissues and to characterize their possible roles in metabolism, in naive animals, during the course of a lipid-rich diet that causes glucose intolerance and insulin resistance and following the return to a normal diet and metabolism. We use fate mapping models to separate adipose tissue macrophages into prospective subsets based on origin, phenotype, and dynamics under control and high fat diet. The dynamics and transcriptional responses of subsets are further investigated using flow cytometry, histology, and RNA sequencing. Depending on results, further studies will aim at characterizing the mechanisms that control the distinct responses of macrophage subsets and the consequences of their manipulation for mouse metabolism during and following a lipid-rich diet.

7

White Adipose Tissue ApoC-I: Relation to White Adipose Tissue Dysfunction and Delayed Fat Clearance in Humans

Yannick Cyr[1,2], Hanny Wassef[1,2], Simon Bissonnette[1,2], Valerie Lamantia[1,2], Jean Davignon[1,2], May Faraj[1,2]

[1]Institut de Recherches Cliniques de Montréal, Montreal, QC, Canada; [2]Faculty of Medicine, University of Montreal, Montreal, QC, Canada

White adipose tissue (WAT) dysfunction is at the root of delayed triglyceride-rich lipoprotein (TRL) clearance. We demonstrated that apolipoprotein C-I (apoC-I), a lipoprotein lipase (LPL) inhibitor, is secreted by human adipocytes and WAT and is associated with delayed TRL clearance. Therefore, we hypothesized that WAT-secreted apoC-I associates with WAT dysfunction. A cohort of 39 overweight and obese men and women (postmenopausal) were examined. The secretion of WAT apoC-I over 4 h averaged 86.9 ± 31.4 pmol/g in women (n = 28) and 74.1 ± 36.6 pmol/g in men (n = 11), with no sex differences. Subjects were grouped based on median WAT-apoC-I secretion per sex. Following a [^{13}C]triolein-labeled fat tolerance test meal, high WAT secretion subjects had reduced TRL clearance over 6 h assessed as higher $AUC_{6|h}$ of plasma apolipoprotein B48 (46.1 ± 4.9 *versus* 26.9 ± 3.6, p = 0.003) and ^{13}C-labeled triglycerides (328.7 ± 55.1 *versus* 200.5 ± 41.6, p = 0.04). These subjects also had reduced WAT function, defined as reduced hydrolysis and storage of a synthetic [^{3}H]triolein-labeled TRL substrate by WAT *ex vivo* (i.e. *in situ* LPL activity, 3.15 ± 0.47 *versus* 5.09 ± 0.80, p = 0.04). Adjusting for BMI or fat mass did not eliminate these associations. Finally, VLDL-extracted apoC-I directly reduced the hydrolysis and storage of the [^{3}H]TRL in murine adipocytes, in a concentration- and time-dependent manner suggesting a direct role of apoC-I in the clinical observations. WAT-apoC-I secretion in obese subjects is associated with WAT dysfunction and delayed dietary TRL and fat clearance, which may be secondary to direct inhibition of WAT LPL activity. We propose apoC-I as a potential target to reduce cardiometabolic risk.

8

Angiopoietin-like 4 (ANGPTL4) Promotes Intracellular Degradation of Lipoprotein Lipase in Adipocytes

Wieneke Dijk[1], Anne P. Beigneux[2], Mikael Larsson[2], André Bensadoun[3], Stephen G. Young[2,4], Sander Kersten[1]

[1]Nutrition, Metabolism, and Genomics Group, Human Nutrition, Wageningen University, Wageningen, The Netherlands; [2]Department of Medicine, David Geffen School of Medicine, UCLA, Los Angeles, CA; [3]Division of Nutritional Sciences, Cornell University, Ithaca, NY; [4]Department of Human Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA

Lipoprotein lipase (LPL) hydrolyzes triglycerides in triglyceride-rich lipoproteins along the capillaries of heart, skeletal muscle, and adipose tissue. The activity of LPL is a major determinant of plasma triglyceride levels. LPL activity is repressed by angiopoietin-like 4 (ANGPTL4), but the mechanisms have not been fully elucidated. Our objective was to study the cellular location and mechanism for LPL inhibition by ANGPTL4. We performed cell culture studies in transfected cells, *ex vivo* studies, and *in vivo* studies with *Angptl4*$^{-/-}$ mice. Co-transfection of CHO pgsA-745 cells with ANGPTL4 and LPL reduced intracellular LPL protein levels, indicating that ANGPTL4 has the capability of inhibiting LPL processing. This conclusion was further supported by studies of primary adipocytes and adipose tissue explants from wild-type and *Angptl4*$^{-/-}$ mice. The absence of ANGPTL4 in mouse adipocytes resulted in an accumulation of the mature glycosylated form of LPL and increased secretion of LPL. Blocking endoplasmic reticulum (ER)-Golgi transport abolished differences in LPL abundance between wild-type and *Angptl4*$^{-/-}$ adipocytes, suggesting that ANGPTL4 acts upon LPL after LPL processing in the ER. Finally, physiological changes in adipose tissue ANGPTL4 expression during fasting and cold exposure resulted in inverse changes in the amount of mature glycosylated LPL in wild-type but not *Angptl4*$^{-/-}$ mice. ANGPTL4 promotes loss of intracellular LPL by stimulating LPL degradation after LPL processing in the ER. Our data provide novel insight into the mechanisms of regulation of plasma triglyceride levels.

9

Fatostatin Blocks ER-to-Golgi Transport of SCAP but Inhibits Cell Growth in a SCAP-independent Manner

Wei Shao[1], Carolyn Machamer[1], Peter Espenshade[1]

[1]Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD

Sterol regulatory element-binding protein (SREBP) transcription factors are central regulators of cellular lipid homeostasis and activate expression of genes required for fatty acid, triglyceride, and cholesterol synthesis and uptake. The sterol sensor SREBP cleavage-activating protein (SCAP) plays an essential role in SREBP activation by mediating ER-to-Golgi transport of SREBP. Once in the Golgi, membrane-bound SREBPs are activated by sequential cleavage by the Site-1 and Site-2 proteases. Recent studies have shown a requirement of the SREBP pathway for tumor growth (e.g. in glioblastoma). Thus, SREBP pathway inhibitors are potential cancer therapeutics. SCAP is required for activation of all SREBP family members, making it an ideal target for SREBP pathway drug development. Fatostatin is a chemical inhibitor reported to bind SCAP directly and to block ER exit of SCAP. Although the mechanism of fatostatin inhibition is incompletely understood, the drug has been applied to preclinical models for metabolic diseases and cancer. In this study, we investigated further the mechanism of fatostatin action on the SREBP pathway. We provide evidence that fatostatin blocks ER-to-Golgi transport of SCAP, and that inhibition is independent of INSIG proteins, which function to retain SCAP in the ER in response to elevated sterols. Both fatostatin and PF-429242, an inhibitor of Site-1 protease, potently inhibit cell growth, probably by inhibiting SREBP activation. Unexpectedly, exogenous lipids restored cell proliferation in the presence of PF-429242 but failed to rescue fatostatin-treated cells. Furthermore, fatostatin inhibits cell growth in cells lacking SCAP. Finally, using a classic VSVG maturation assay, we demonstrate that fatostatin delays ER-to-Golgi transport of VSVG. In summary, although fatostatin inhibits ER exit of SCAP, fatostatin additionally inhibits cell proliferation through both lipid-independent and SCAP-independent mechanisms, possibly by general inhibition of ER-to-Golgi transport.

10

AIBP-regulated Notch Signaling Limits Retinal Angiogenesis

Renfang Mao[1], Felicidad Almazan[2], Yury Miller[2], Longhou Fang[1]

[1]Center for Cardiovascular Regeneration, Houston Methodist Research Institute, Houston, TX; [2]Department of Medicine, University of California, San Diego, La Jolla, CA

Angiogenesis, a process of blood vessel formation, is essential for embryonic development, tissue homeostasis, and repair as well as disease progression. Our recent study has unveiled a connection of apoA-I-binding protein (AIBP)-regulated cholesterol metabolism with angiogenesis. Mechanistically, AIBP accelerates cholesterol efflux from endothelial cells (ECs), which reduces lipid rafts and disrupts VEGFR2 signaling, thereby limiting angiogenesis. We extended our studies in zebrafish by generating global AIBP knock-out mice, which are viable and fertile. Here we explored the role of AIBP in developmental angiogenesis using the postnatal murine retina model. Retinal vessels characteristically begin to sprout from the optic disc on postnatal day 1 (P1) and reach the retinal margin on P7. AIBP depletion resulted in augmented angiogenesis, as evidenced by increased numbers of branching points, greater circumferential vasculature length, and elevated numbers of tips cells. Mechanistically, we found that loss of AIBP in the murine retinas impairs Notch signaling, which is essential for proper angiogenesis. In human ECs, recombinant AIBP treatment enhances JAG1-stimulated Notch signaling. Our studies uncover a new mechanism by which AIBP-mediated Notch orchestrates angiogenesis.

11

Lipin 1 Acetylation Status and Deacetylation by Histone Deacetylase 1 Regulates Its Localization, Stability, and Activity

Sara L. Collier[1], George G. Schweitzer[1], Brian N. Finck[1]

[1]Washington University School of Medicine, St. Louis, MO

Lipin 1 is a bifunctional, intracellular protein that regulates lipid metabolism as a phosphatidic acid phosphohydrolase (PAP) enzyme and by acting as a transcriptional regulatory protein. Lipin 1 intracellular localization has been previously shown to be affected by post-translational modifications, including phosphorylation and sumoylation. To evaluate whether lipin 1 is also regulated by acetylation, we treated cells expressing lipin 1 with the deacetylase inhibitor tricostatin A (TSA) and examined its acetylation status after immunoprecipitation. TSA treatment robustly increased lipin 1 acetylation and overall lipin 1 protein abundance due to increased protein stability. Four general classes of protein deacetylases are acknowledged (Class I, II, III (also known as sirtuins), and IV). Class-specific histone deacetylase (HDAC) inhibitors as well as genetic inactivation or activation of specific HDACs were used to identify the HDAC that regulated lipin 1 acetylation. We determined that HDAC1, which is a Class I HDAC that predominantly resides in the nucleus, is critical for controlling lipin 1 acetylation. Specifically, HDAC1 physically interacted with lipin 1, and overexpression or knockdown of HDAC1 led to reduced or increased lipin 1 acetylation, respectively. Lysine 752, which lies within a canonical haloacid dehalogenase domain believed to be critical for lipin 1-mediated PAP activity, was identified as an important site of acetylation. Mutation of lysine 752 to inhibit acetylation led to cytoplasmic localization and decreased protein half-life. Altogether, these data suggest that acetylation of lipin 1 is an important post-translational modification of lipin 1 that regulates its localization, stability, and potentially its activity.

12

ApoC-III Modulates Clearance of Triglyceride-rich Lipoproteins in Mice through Low Density Lipoprotein Family Receptors

Philip L. Gordts[1], Bastian Ramms[1,2], Ni-Huiping Son[3], Richard G. Lee[4], Debapriya Basu[3], Mark Graham[4], Rosanne M. Crooke[4], Ira J. Goldberg[3], Joseph L. Witztum[1], Jeffrey D. Esko[2]

[1]Department of Medicine, University of California, San Diego, La Jolla, CA; [2]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA; [3]Division of Endocrinology, Diabetes, and Metabolism, NYU Langone Medical Center, New York, NY; [4]Ionis Pharmaceuticals, Inc., Carlsbad, CA

Hypertriglyceridemia is an independent risk factor for cardiovascular disease, and plasma triglycerides (TGs) correlate strongly with plasma apolipoprotein C-III (apoC-III) levels. ApoC-III antisense oligonucleotides (ASOs) reduce plasma TGs in primates and in mice, but the underlying mechanism of action remains controversial. We show that a murine specific apoC-III ASO reduces fasting TG levels through a mechanism dependent on low density lipoprotein receptors (LDLRs) and LDL receptor-related protein 1 (LRP1). ApoC-III lowered plasma TGs in mice lacking lipoprotein lipase (LPL), hepatic heparan sulfate proteoglycan receptors (HSPGs), LDLR, and LRP1 and in animals with combined deletion of HSPG receptors and LDLR or LRP1. However, the apoC-III ASO did not lower TG levels in mice lacking both LDLR and LRP1. The ability of apoC-III ASO to lower plasma TGs through LDLR and LRP1 also occurred in mice fed a high fat diet, in post-prandial clearance studies, in lipoprotein lipase-deficient mice, and when apoC-III-rich or apoC-III-depleted lipoproteins were injected into mice. ASO reduction of apoC-III had no effect on VLDL secretion, heparin-induced TG reduction, and uptake of lipids into heart and skeletal muscle. Our data show that apoC-III inhibits turnover of TG-rich lipoproteins primarily through a hepatic clearance mechanism mediated by the LDLR/LRP1 axis.

13

N-Acyl Taurines Regulate Lipids and Metabolism in the Gut and Liver

Trisha J. Grevengoed[1], Matthew P. Gillum[1]

[1]University of Copenhagen, Copenhagen, Denmark

N-Acyl taurines (NATs) are endogenous, circulating molecules that are highly regulated but with little known about the biological function. When lacking the enzyme that degrades NATs and another class of lipids, mice accumulate up to 100-fold more NATs than controls, develop fatty liver, and display severe insulin resistance, indicating a potential important role for NATs in regulation of metabolic state. This work aims to determine the function of NATs in the gut and liver. NATs are amphipathic with a hydrophobic acyl chain and hydrophilic taurine regions. NATs were excellent detergents with critical micelle concentrations 2-fold lower than SDS and the ability to solubilize lipids in aqueous solutions at up to 12-fold lower concentrations than bile acids. Acyl chain was important in determining the properties of NATs, because *N*-oleoyl taurine was able to facilitate palmitate solubilization in water and denature proteins, whereas *N*-arachidonyl taurine could do neither. In an *in vitro* supersaturated simulated bile assay, *N*-oleoyl taurine decreased formation of cholesterol crystals by 20% and dissolved 28% of preformed crystals, potentially implicating these as a treatment for cholesterol-based gallstones. Oral administration of *N*-oleoyl taurine increased early intestinal triacylglycerol (TAG) absorption by 32%, further demonstrating the importance of NATs in bile. NATs are also bioactive molecules that can alter hepatic metabolism. Subcutaneous treatment with *N*-oleoyl taurine for 2 weeks increased gluconeogenic gene expression 2-fold and decreased liver TAG by 28% without altering fasting plasma glucose or insulin or food intake. In conclusion, NATs are a previously overlooked component of bile that are more effective than cholesterol-based bile acids at improving solubility of lipids and can diminish cholesterol crystal formation. Therefore, NATs may be a treatment option for gallstones or lipid absorption disorders, but the effects of orally administered NAT on whole-body metabolism will need to be characterized first.

14

The Role of PCSK9 in Nephrotic Syndrome-associated Hypercholesterolemia

Mary E. Haas[1], Amy E. Levenson[1], Xiaowei Sun[1], Joseph M. Rutkowski[2], Valerie A. Schumacher[1], Phillip E. Scherer[2], David J. Salant[3], Sudha B. Biddinger[1]

[1]Boston Children's Hospital, Harvard Medical School, Boston, MA; [2]Touchstone Diabetes Center, University of Texas Southwestern Medical Center, Dallas, TX; [3]Boston University Medical Center, Boston, MA

In nephrotic syndrome, damage to the podocytes of the kidney produces severe hypercholesterolemia. However, the underlying mechanisms remain poorly understood, and novel treatments are urgently needed. Proprotein convertase subtilisin/kexin type 9 (PCSK9) has emerged as an important inducer of hypercholesterolemia and therapeutic target. Here, we tested the role of PCSK9 in mediating the hypercholesterolemia of nephrotic syndrome. Both nephrotoxic serum (NTS), which induces immune-mediated damage of the podocyte, and selective ablation of the podocyte in podocyte apoptosis through targeted activation of caspase-8 (Pod-ATTAC) mice produced hypercholesterolemia and a 7-16-fold induction in plasma PCSK9. Conversely, patients with nephrotic syndrome showed a decrease in plasma PCSK9 and plasma cholesterol upon the remission of their disease. The induction in plasma PCSK9 appeared to be due to increased expression and secretion of PCSK9, predominantly but not exclusively from the hepatocyte, coupled with decreased clearance. Interestingly, although NTS treatment induced hypercholesterolemia in PCSK9-KO mice, plasma cholesterol levels were nonetheless 60% lower in NTS-treated PCSK9-KO *versus* NTS-treated wild-type mice. Therefore, podocyte damage triggers marked inductions in plasma PCSK9, and knock-out of PCSK9 reduces cholesterol in a mouse model of nephrotic syndrome. These data suggest that PCSK9 inhibitors may be beneficial in patients with nephrotic syndrome-associated hypercholesterolemia.

15

The Retention Adaptor Protein PID1 Regulates Insulin-mediated Lipid and Glucose Disposal

Kirstin Albers[1], Britta Hoffzimmer[1], Lucia M. Krott[1], Hartwig Schmale[1], Philip L. Gordts[2], Joerg Heeren[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]University of California, San Diego, La Jolla, CA

Obesity as a consequence of excess weight gain is characterized by insulin resistance associated with impaired glucose and lipoprotein disposal into metabolically active organs, such as adipose tissues and liver. These metabolic alterations cause hyperglycemia and dyslipidemia, both contributing to the development of type 2 diabetes and atherosclerosis. The phosphotyrosine-interacting domain-containing protein 1 (PID1) has been identified as an adaptor protein for the LDL receptor-related protein 1 (LRP1). This lipoprotein receptor is important for the rapid clearance of pro-atherogenic lipoprotein remnants into the liver and was recently shown to be part of the insulin-responsive glucose transporter 4 (GLUT4) storage vesicles in muscle and adipose tissues. Here, we investigated the functional consequences of PID1 deficiency for LRP1 localization and function *in vitro* and *in vivo*. In the absence of PID1, hepatic LRP1 as well as LRP1 and GLUT4 in muscle and adipose tissue were sorted to the plasma membrane even without insulin signaling. In wild-type cells, insulin-mediated phosphorylation of the distal LRP1-NP*XYXX*L domain disrupts the LRP1-PID1 interaction, initiating translocation and fusion of recycling endosomes with the plasma membrane. In mice fed a diabetogenic diet, PID1 deficiency lead to constitutive plasma membrane localization of both LRP1 and GLUT4, improving systemic glucose and lipid homeostasis. In conclusion, PID1 serves as an insulin-regulated retention adaptor protein keeping LRP1 and GLUT4 in perinuclear recycling endosomes under basal, fasting conditions. Notably, loss of PID1 corrects for insulin resistance-associated metabolic alterations, emphasizing its pivotal role in the regulation of systemic energy metabolism.

16

Anabolic Energy Uptake Processes into Brown Fat during Catabolic Conditions of Adaptive Thermogenesis Are Dependent on Organ-specific Insulin Signaling

Markus Heine[1], Alexander Fischer[1], Nils Mangels[1], Renate Schreiber[2], Rudi Zechner[2], Ludger Scheja[1], Jörg Heeren[1]

[1]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]University of Graz, Graz, Austria

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

17

The Anti-lipogenic Effect of TTC39B Deficiency Is LXR-dependent and Protects from Steatohepatitis

Joanne Hsieh[1], Masahiro Koseki[2], Matthew M. Molusky[1], Kristin M. McCabe[1], Daniel J. Rader[3], Alan R. Tall[1]

[1]Division of Molecular Medicine, Department of Medicine, Columbia University, New York, NY; [2]Division of Cardiovascular Medicine, Osaka University Graduate School of Medicine, Osaka, Japan; [3]Division of Translational Medicine and Human Genetics, University of Pennsylvania, Philadelphia, PA

In human GWAS, HDL cholesterol and total cholesterol levels were associated with SNPs in *TTC39B* (*T39*), a novel locus with an eQTL in the liver. T39 encodes a protein of unknown function. Whole-body T39 knock-out mice had modestly elevated HDL cholesterol levels on chow and high cholesterol diets. When fed the high fat/high cholesterol/bile salt (HF/HC/BS) diet, the whole-body T39 knock-out mice were remarkably protected from steatohepatitis and death. The HDL increase was associated with LXR target gene up-regulation, such as *Abca1*, *Abcg5/8*, and *Idol*, and a posttranscriptional increase in LXR protein in the enterocytes of $T39^{-/-}$ mice. The livers of $T39^{-/-}$ mice fed the HF/HC/BS diet had a similar posttranscriptional LXR activation, and the LXRα-deficient background reversed the differences in serum ALT and hepatic gene expression between $T39^{+/+}$ and $T39^{-/-}$ mice. In the livers of $T39^{-/-}$ mice fed the HF/HC/BS diet, there was significantly decreased polyubiquitination of endogenous LXRα, which inhibited proteasome-dependent LXRα turnover, as determined by pulse chase studies in primary hepatocytes. *VillinCre(+)T39*$^{fl/fl}$ and *AlbuminCre(+)T39*$^{fl/fl}$ mice were generated to delete T39 in enterocytes and hepatocytes, respectively. Only the hepatic T39 knock-out mice were protected from HF/HC/BS diet-induced mortality, and their livers had fewer infiltrating inflammatory cells, less hepatocellular ballooning, and minimal fibrosis compared with the livers of intestinal knockouts and floxed controls, which displayed prominent features of NASH. Hepatic T39 deficiency had only a modest effect on dietary cholesterol absorption, suggesting that decreased cholesterol or oxysterol accumulation was not the main protective factor. There was a dramatic reduction in SREBP-1c processing and consequently lipogenesis in T39-deficient livers, which was attributable to *Insig2a* up-regulation and increased polyunsaturated fatty acid-containing phosphatidylcholine species in microsomes. In the postprandial state, hepatic T39 deficiency was associated with a blunted induction of lipogenic genes that are both SREBP-1 and LXR targets, including *Scd1*, *Elovl5*, *Fasn*, *Acc*α, and, in particular, *Pnpla3*. In summary, T39 deficiency reduces LXR ubiquitination and preserves endogenous LXR but uncouples LXR from its lipogenic activities. By promoting cholesterol removal and inhibiting hepatic lipogenesis, T39 inhibition could represent a therapeutic approach for both atherosclerosis and steatohepatitis.

18

Investigating the Role of the Mevalonate-Isoprenoid Arm of the Cholesterol Pathway in Modulating Viral Replication

Wei Yuan Hsieh[1], Peter Ghazal[1]

[1]Division of Infection and Pathway Medicine, University of Edinburgh, Edinburgh, UK

Mammalian innate immunity against foreign pathogens involves the secretion of interferon by pathogen-sensing cells. The activation of interferon-stimulated genes (ISGs) by interferon leads to the remodeling of the intracellular environment, thus providing resistance to viral infection. We previously showed that activation and secretion of 25-hydroxycholesterol (25-HC) by macrophages participate in interferon-mediated antiviral response. 25-HC exerts an antiviral property partially through the suppression of the sterol biosynthesis pathway. However, the dependency of viral replication on the sterol pathway has not been fully elucidated. The work presented here examines redundant and non-redundant controls of the sterol biosynthesis pathway in regulating viral replication. We show that altering lipid conditions affects murine cytomegalovirus (MCMV) replication through changes in the mevalonate-isoprenoid arm. Direct inhibition of this arm by siRNA or statins significantly inhibits MCMV replication. Importantly, the antiviral effect of statins is associated with changes in the prenylation state of particular host proteins. Last, we explore the multilayer inhibitory effects of statin, 25-HC, and ganciclovir on MCMV replication. Results presented here would further support the development of selective combinatorial antiviral strategy through regulating host metabolic pathways.

19

Opposing Roles for C/EBPβ in Regulating Adipogenesis and Inflammation

Devon Hunerdosse[1], Mary Teruel[1]

[1]Stanford University, Stanford, CA

Individuals with insulin resistance (IR) are predisposed to the development of type 2 diabetes and cardiovascular disease. Growing evidence points to a correlative and causative relationship between IR and systemic, low grade inflammation of insulin-responsive tissues, including adipose tissue. Adipose tissue is a complex endocrine organ and a major site of glucose disposal and lipid metabolism and a regulator of whole body metabolism, and defective adipogenesis is a prominent feature of IR. The development of functional adipocytes (adipogenesis) requires C/EBPβ, an early transcription factor that up-regulates the expression of PPARγ, the master regulator of adipogenesis. During healthy adipogenesis, these two factors exist in a positive feedback loop. However, under an inflammatory (IR) state, this loop is broken, and PPARγ levels and adipogenesis are decreased despite high expression of C/EBPβ. Understanding how C/EBPβ, which is highly post-translationally modified, switches from being adipogenic (promoting insulin sensitivity) to inflammatory (promoting IR) could be a promising new way to treat IR, but the mechanisms underlying this switch are poorly understood. Here, we present evidence for alternate genomic binding of C/EBPβ in preadipocytes under inflammatory conditions, partially revealing a mechanism through which C/EBPβ activity is changed under IR, thereby reducing adipogenesis. To begin to elucidate the nature behind this alternate activity, we tested the extent to which phosphorylation status of C/EBPβ can affect cellular phenotypes. Using inducible phosphomutant OP9 preadipocytes, we show that phosphorylation of C/EBPβ at Ser-184 can significantly impact insulin responsiveness. These data can be integrated to begin to form a comprehensive model by which C/EBPβ activity impacts 1) adipogenic capacity during inflammation and 2) insulin sensitivity, potentially controlled through post-translational modification.

20

Contribution of Accelerated Degradation to Feedback Regulation of HMG-CoA Reductase and Cholesterol Metabolism in Liver

Seonghwan Hwang[1], Isamu Z. Hartman[1], Leona N. Calhoun[1], Kristina Garland[1], Gennipher A. Young[1], Matthew A. Mitsche[1], Jeffrey McDonald[1], Luke Engelking[1], Russell A. DeBose-Boyd[1]

[1]Department of Molecular Genetics, University of Texas Southwestern Medical Center, Dallas, TX

HMG-CoA reductase (HMGCR) produces mevalonate, a key intermediate in synthesis of sterol and non-sterol isoprenoids. These end-products of mevalonate metabolism exert feedback regulation on HMGCR through multiple mechanisms, including inhibition of transcription and translation as well as acceleration of protein degradation. The transcriptional component of the HMGCR regulatory system has been scrutinized in livers of mice through analyses of various transgenic and knock-out animals, whereas post-translational regulation of HMGCR in animal livers has not been studied in detail. This is due to a lack of tools to directly measure these parameters *in vivo*.

The current study is aimed at elucidating the role of sterol-accelerated degradation of HMGCR in overall regulation of HMGCR protein and cholesterol metabolism in mouse liver. To achieve this, we generated two mouse models: 1) transgenic mice expressing the membrane domain of HMGCR, which is necessary and sufficient for sterol-regulated degradation of HMGCR in cultured cells, and 2) knock-in mice expressing mutant HMGCR that is resistant to sterol-induced ubiquitination. These models were subjected to various feeding regimens known to modulate the Insig and/or Scap-SREBP pathway, key players in feedback regulation of HMGCR. Cholesterol feeding accelerated degradation of HMGCR in the liver of transgenic animals, whereas deprivation of sterol by lovastatin feeding suppressed degradation of HMGCR. Ubiquitination-resistant HMGCR accumulated in the liver and other tissues, despite the reduction in its transcript level. Defective ubiquitination of HMGCR led to reduction in levels of nuclear SREBP-2 and mRNAs of SREBP-2 target genes that are involved in cholesterol synthesis (e.g. HMG-CoA synthase, squalene synthase, etc.) in the liver and other tissues, whereas hepatic levels of cholesterol and cholesteryl ester were elevated. Overall, these findings indicate that degradation of HMGCR plays significant role in the *in vivo* regulation of the enzyme and cholesterol homeostasis.

21

APOC3 A43T Variant Promotes ApoC-III Catabolism and Accelerates TG-rich Lipoprotein Clearance in Mice and Humans

Sumeet A. Khetarpal[1], John S. Millar[1], Amritha Varshini[1], Cecilia Vitali[1], Xuemei Zeng[2], Paolo Zanoni[1], Zhiyuan Sun[2], David Nguyen[1], James T. McParland[1], Mary G. McCoy[1], Pradeep Natarajan[3], Marina Cuchel[1], Leland Mayne[1], S. Walter Englander[1], Sissel Lund-Katz[1], Michael C. Phillips[1], Nathan A. Yates[2], Sekar Kathiresan[3], Daniel J. Rader[1]

[1]University of Pennsylvania, Philadelphia, PA; [2]University of Pittsburgh, Pittsburgh, PA; [3]Harvard Medical School, Boston, MA

Humans with loss-of-function (LoF) variants in *APOC3*, the gene encoding apolipoprotein C-III (apoC-III), have significantly reduced plasma triglycerides (TGs) and protection from coronary disease. These findings suggest that apoC-III may be a viable therapeutic target for decreasing vascular risk through TG reduction and that elucidation of the protective mechanism of *APOC3* LoF variants would inform such strategies. We report here the protective mechanism of the *APOC3* A43T missense variant, one of four recently identified CAD-protective variants. By genotyping >8,000 human participants with low TGs, we identified 17 *APOC3* A43T carriers and phenotyped six carriers and 54 matched controls. A43T heterozygotes demonstrate a significant reduction in apoC-III levels relative to non-carriers (50% reduction, $p < 0.05$), resulting in decreased plasma TG (50% reduction, $p < 0.05$). We generated viral vectors expressing WT or A43T apoC-III and expressed these in humanized mouse models to further explore the mechanism of reduced apoC-III levels due to the A43T variant. Mice expressing human CETP and the apoC-III A43T variant exhibit reduced plasma apoC-III (50% reduction, $p < 0.0001$) despite equal hepatic expression and secretion relative to controls expressing WT human apoC-III. These mice also exhibit reduced plasma TG and VLDL-C and increased HDL-C relative to WT-expressing mice, fully recapitulating the protective lipoprotein profile of the human A43T carriers. Radioisotope-labeled apoC-III turnover studies showed that the A43T mutation causes a >3-fold higher apoC-III clearance rate *in vivo* ($p < 0.0001$) due to defective integration into lipoprotein particles and accelerated renal catabolism (40% increase, $p < 0.01$). This results in increased lipoprotein lipase (LPL) activity (27% increase, $p < 0.01$) and faster chylomicron-TG clearance (97% increase, $p < 0.01$) *in vivo*. We are currently performing analogous studies of WT *versus* A43T apoC-III turnover and VLDL clearance in human *APOC3* A43T carriers. Collectively, our results support the rationale for therapeutic efforts to target circulating apoC-III through disruption of its binding to lipoproteins, mirroring the genetics-driven approaches for targeting *PCSK9* that have recently yielded novel therapies.

22

Nat1 Deficiency Is Associated with Mitochondrial Dysfunction and Exercise Intolerance in Mice

Indumathi Chennamsetty[1], Michael Coronado[1], Kévin Contrepois[1], Mark Keller[2], Ivan Carcamo-Orive[1], Mohsen Fathzadeh[1], John Sandin[1], Giovanni Fajardo[1], Gerald Reaven[1], Alan Attie[2], Daniel Bernstein[1], Thomas Quertermous[1], Joshua W. Knowles[1]

[1]Stanford University, Stanford, CA; [2]University of Wisconsin, Madison, WI

We recently identified human *N*-acetyltransferase 2 (*NAT2*) as a new candidate gene for insulin resistance (IR) using a genome-wide association approach followed by *in vitro* and *in vivo* validation. However, the cellular mechanism linking *NAT2* to IR remains unclear. Here we show that *Nat1* (the mouse ortholog of *NAT2*) is significantly co-regulated with mitochondrial pathway genes, including key regulators of mitochondrial function. In differentiated 3T3-L1 adipocytes, RNA interference-mediated silencing of *Nat1* led to mitochondrial dysfunction characterized by increased production of intracellular reactive oxygen species and superoxide as well as decreased mitochondrial membrane potential, biogenesis, and mass. Furthermore, *Nat1* knockdown promoted mitochondrial fragmentation and decreased cellular respiration and ATP generation. *Nat1*-deficient mice not only had marked changes in metabolomic and lipidomic profile consistent with impaired mitochondrial function and an insulin resistance phenotype but also had a significant decrease in exercise capacity without changes in cardiac function. Collectively, our results suggest that *Nat1* deficiency results in mitochondrial dysfunction, which may be the mechanistic link between this gene and insulin resistance.

23

Association of HyperapoB with Insulin Resistance and Hyperinsulinemia Is Dependent on Reduced Fat Clearance *in Vivo* and *ex Vivo* in White Adipose Tissue in Obese Subjects

Valerie Lamantia[1,2,3], Simon Bissonnette[1,2,3], Nathalie Saint-Pierre[2], Yannick Cyr[1,2,3], Hanny Wassef[1,2,3], Remi Rabasa-Lhoret[1,2,3], May Faraj[1,2,3]

[1]University of Montreal, Montreal, QC, Canada; [2]Institut de Recherches Cliniques de Montréal, Montreal, QC, Canada; [3]Montreal Diabetes Research Center, Montreal, QC, Canada

The existence of elevated plasma apolipoprotein B (apoB)-lipoproteins predicts type 2 diabetes in humans; however, the underlying mechanisms are not fully elucidated. Delayed fat clearance is known to promote prediabetes characterized by lipotoxicity, insulin resistance, and compensatory hyperinsulinemia. We recently reported that apoB-lipoproteins associate with and directly reduce the clearance of triglyceride (TG)-rich lipoproteins (TRLs) by white adipose tissue (WAT) in women. Thus, we hypothesized that the association of hyperapoB with insulin resistance and compensatory hyperinsulinemia is dependent on reduced fat clearance *in vivo* and *ex vivo* in WAT. Insulin secretion and sensitivity were examined in 13 men and 17 postmenopausal women ($\geq$27 kg/m^2, 45-74 years, normoglycemic) using the 1-h intravenous glucose tolerance (IVGTT) test followed by 3-h hyperinsulinemia clamp. One week later, the clearance of TRL was examined *in vivo* following ingestion of [^{13}C]triolein-labeled high fat meal (600 kcal/m^2, 68% fat) and *ex vivo* by incubating fasting WAT with synthetic [^{3}H]triolein-labeled TRL (1.27 mmol/liter TG) for 4 h. Plasma apoB (0.5-1.7 g/liter) correlated positively with delayed postprandial plasma clearance of TRL, assessed from total TG (r = 0.70), dietary [^{13}C]TG (r = 0.47), and apoB48 (r = 0.76). It also associated positively with hyperinsulinemia (second phase: r = 0.41) and negatively with insulin sensitivity (glucose infusion rate: r = –0.38) and WAT function (WAT ^{3}H-lipids: r = –0.52). Moreover, delayed postprandial plasma clearance of [^{13}C]TG was also associated with hyperinsulinemia (second phase: r = 0.37) and reduced insulin sensitivity (r = –0.39). Adjusting the association of plasma apoB with insulin sensitivity and secretion for $AUC_{6|h}$ plasma [^{13}C]TG or *ex vivo* WAT function eliminated significance, whereas adjusting for adiposity had no effect. Association of hyperapoB with insulin resistance and hyperinsulinemia in normoglycemic obese subjects is dependent on reduced fat clearance *in vivo* and *ex vivo* in WAT, independent of obesity.

24

Rpl13a snoRNAs Are Key Mediators of Lipotoxic Cardiomyopathy

Jiyeon Lee[1], Christopher Holley[1], Alexis Harris[1], Renate Schreiber[2], Rudolf Zechner[2], Jean Schaffer[1]

[1]Diabetic Cardiovascular Disease Center, Washington University, St. Louis, MO; [2]Institute of Molecular Biosciences, University of Graz, Graz, Austria

Lipid accumulation in non-adipose tissues is the *sine qua non* of lipid overload states such as obesity and type 2 diabetes. Furthermore, ectopic lipid accumulation has been proposed to contribute to organ dysfunction that underlies diabetic complications. However, the molecular mechanisms through which excess lipid damages tissues such as the heart are not well understood. A genetic screen performed in our laboratory identified small nucleolar RNAs (snoRNAs) from the *Rpl13a* locus as important mediators of lipid-induced cell death in cultured fibroblasts. To understand the contribution of these non-coding RNAs to the pathophysiology of lipid overload, we generated a mouse model in which homologous recombination was used to knock out the four snoRNAs encoded within the introns of the *Rpl13a* gene. *RpL13a*-snoless mice are viable, born in normal Mendelian ratios, and fertile. Quantitative PCR (qRT-PCR) and Western blotting analyses revealed that tissues from *Rpl13a*-snoless animals have no expression of the four *Rpl13a*-snoRNAs but preserved expression of the Rpl13a mRNA and protein. To test whether loss of function of the snoRNAs could protect against the pathophysiology of cardiac lipotoxicity, we crossed *Rpl13a*-snoless mice, which have normal cardiac function, with a model of lipotoxic cardiomyopathy in which long-chain acyl-CoA synthetase is overexpressed in cardiac myocytes to drive excessive lipid uptake (MHC-ACS). Loss of the snoRNAs prevented cardiac hypertrophy and preserved systolic function in the MHC-ACS model and provided a survival advantage. Tissue analyses at 3-4 weeks of age, prior to the onset of heart failure, suggested that loss of the snoRNAs leads to alterations in mitochondrial structure and lipid metabolism. We extended our findings in a cross of the *Rpl13a*-snoless mice with mice lacking adipose triglyceride lipase (*Atgl*), another model of cardiac lipotoxicity. Similar to our findings with the MHC-ACS model, $Atgl^{-/-}$ mice with loss of the *Rpl13a* snoRNAs demonstrated improved cardiac function. Together, our findings establish a new role for snoRNAs in the pathogenesis of cardiac lipotoxicity.

25

Loss of the Nuclear Receptor LXRs in Sensory Neurons Modifies Neuregulin-1 Gene Expression and Myelin Structure

Gustavo Untiveros[1], Chaitanya Gavini[1], David Mangelsdorf[2], Laurent Gautron[2], Joel Elmquist[2], Virginie Mansuy-Aubert[1]

[1]Loyola University Chicago, Chicago, IL; [2]University of Texas Southwestern Medical Center, Dallas, TX

Peripheral nerve injury causes significant morbidity and reduced quality of life from weakness, sensory loss, and neuropathic pain. In the United States, 6% of the elderly population suffers from peripheral neuropathy, which can be caused by inherited or inflammatory disorders or by metabolic diseases, such as diabetes. Patients suffer from chronic pain or loss of sensation that can result in amputation. Recent clinical evidence from obese and diabetic patients strongly suggests that a modification of circulating lipid levels and/or quality is linked with the development and progression of neuropathy. However, little is known about how lipids damage or regulate peripheral sensory neurons and contribute to the pathogenesis of neuropathy. Recently, we combined unique tissue-specific LXR knock-out mice generated using the Cre line Nav1.8-Cre and double-floxed allele line LXRs (LXRfl (control) and LXRNav1.8)) and *ex vivo* approaches to identify a previously unknown role for the nuclear receptor LXRs as a lipid sensor in sensory neuron. Our novel results showed that sciatic nerves of mice lacking LXRs in sensory neurons overexpressed myelin structural genes and exhibited an aberrant myelin phenotype compared with control mice. Remarkably, this phenotype is exacerbated when mice were fed a Western-style diet and was associated with decreased neuregulin-1 (nrg1) mRNA levels in dorsal root ganglia (DRG). We hypothesize that LXRs in DRG sensory neurons operate as a sensor to modulate Schwann cell function via NRG1-dependent pathway(s). Accordingly, we predict that LXR pathways are important for maintaining peripheral nerve functions in various dietary settings. We predict that our findings will lay the groundwork for therapies aimed at correcting the ensuing impairment in peripheral neurons induced by metabolic disorders, such as diabetic neuropathies.

26

Hepatic ATGL Regulates Lipid Metabolism through Altered Autophagy/Lipophagy

Aishwarya Sathyanarayan[1], Mara T. Mashek[1], Douglas G. Mashek[1]

[1]University of Minnesota, Minneapolis, MN

Adipose triglyceride lipase (ATGL) catalyzes the rate-limiting step of triacylglycerol hydrolysis in multiple tissues. Our laboratory has previously shown that hepatic ATGL preferentially channels hydrolyzed fatty acids to β-oxidation with a concomitant induction of peroxisome proliferator-activated receptor-α. Additionally, our work describes a novel signaling axis involving cAMP/PKA, ATGL-catalyzed lipolysis, and sirtuin1 (SIRT1) activation that governs transcriptional regulation of oxidative metabolism and mitochondrial biogenesis. Recent studies have also shown that SIRT1 promotes autophagy, which also contributes to lipid droplet catabolism (lipophagy). Thus, the objective of these studies is to determine the effects of ATGL on autophagy and lipophagy in the liver. Hepatic ATGL overexpression promoted the expression of autophagy genes, whereas ATGL knockdown had the opposite effect. ATGL promoted autophagic flux and increased the association of lipid droplets with autophagosomes and lysosomes, indicative of increased lipophagy. Chemical or genetic inhibition of autophagy or lipophagy specifically (via lysosomal acid lipase) reduced lipid droplet catabolism and blocked the effects of ATGL on triacylglycerol turnover and oxidation of the hydrolyzed fatty acids. Finally, ATGL required SIRT1 to govern its effects on autophagy/lipophagy, as evidenced by studies showing that ATGL overexpression in liver-specific SIRT1 KO mice was unable to induce autophagy/lipophagy. Thus, these studies show that ATGL-mediated lipolysis controls hepatic lipid droplet metabolism via induction of autophagy/lipophagy.

27

Eicosapentaenoic Acid (EPA) Inhibited Cholesterol Domain Formation and Lipid Oxidation While Preserving Bilayer Width in Model Membranes Exposed to Oxidative Stress or High Cholesterol Levels

Richard P. Mason[1,2], Samuel C. R. Sherratt[2], Robert F. Jacob[1]

[1]Elucida Research LLC, Beverly, MA; [2]Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Atherosclerotic membranes are characterized by elevated cholesterol and lipid oxidation levels, which adversely affect structure and function, including changes in fluidity, width, and cholesterol crystalline domain formation. Marine-derived ω-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6), may differentially influence membrane structure and fluidity due to differences in chain length and saturation. In this study, we tested physiologically relevant doses of EPA, other triglyceride-lowering agents (fenofibrate, niacin, and gemfibrozil), and DHA for these potential effects in model membranes exposed to high glucose or cholesterol levels. Changes in membrane lipid oxidation and fluidity were measured by quantitating lipid hydroperoxide (LOOH) formation and anisotropic fluctuations in the fluorescent probe, diphenylhexatriene (DPH), respectively. Small angle x-ray scattering was used to measure changes in membrane width and cholesterol domain formation. In membranes prepared at relatively low cholesterol levels (0.6 cholesterol/phospholipid mole ratio), exposure to glucose (200 mg/dl) for 72 h increased LOOH by 3000% and induced cholesterol domain formation but did not alter membrane width. Fenofibrate, niacin, and gemfibrozil did not alter glucose-induced changes in LOOH and cholesterol domain levels but reduced membrane width by 8-11% ($p < 0.01$). In contrast, EPA reduced LOOH by 91% ($p < 0.001$) and completely inhibited cholesterol domain formation ($p < 0.05$) while preserving membrane width. DHA reduced LOOH by 87% ($p < 0.001$). These results were consistent across various experimental conditions. In membranes not subjected to oxidation, increasing cholesterol to 50 mol % increased DPH apparent rotational correlation time (ARCT) 5.8-fold. EPA had no significant effect on membrane fluidity; however, DHA decreased ARCT by 20% ($p < 0.05$) and in a dose-dependent manner. Increasing the experimental temperature decreased membrane width (from 56.0 to 54.2 Å) but had no effect on domain formation. At all temperature levels, EPA increased membrane width by 5-7% ($p < 0.05$) with no effect on domain formation, whereas DHA increased cholesterol domain formation with no effect on membrane width. These data suggest that EPA and DHA inhibit lipid oxidation through a potent antioxidant mechanism but have otherwise distinct effects on membrane structure and fluidity that may ultimately result in differential effects on membrane and cell function under disease-like conditions.

28

Hepatic PPP1R3B Deletion Promotes Hepatic Insulin Resistance and Increases Susceptibility to Hepatic Steatosis on a High Sucrose Diet-fed Model

Minal Mehta[1], Daniel J. Rader[1], Nicholas J. Hand[1], Jeffrey T. Billheimer[1]

[1]Department of Human Genetics, University of Pennsylvania, Philadelphia, PA

Hepatic regulation of glucose and lipid metabolism is integral for the maintenance of nutrient homeostasis, and dysregulation in these pathways causes many critical metabolic disorders, such as diabetes and insulin resistance. The PPP1R3B locus has been linked with genome-wide significance to total, high density, and low density cholesterol (TC, HDL-C, and LDL-C, respectively) in the large scale Global Lipids Genetics Consortium association study. The gene encodes a regulatory subunit, classically known as G_L, of a protein phosphatase (PP1) that regulates glycogen synthase in liver. To test the hypothesis that PPP1R3B itself is causative of the lipid phenotype, we generated and characterized liver-specific PPP1R3B (PPP1R3B LSKO) knock-out mice. PPP1R3B LSKO mice have pronounced reduction in hepatic glycogen stores, significant reduction in glycogen synthase protein levels and activity, impairment in glucose disposal, and progressive, increased hepatic triglycerides on a standard chow diet. Given the key role of glucose in the pathogenesis of hepatic insulin resistance, we hypothesized that PPP1R3B LSKO mice would be susceptible to sucrose-induced hepatic insulin resistance and hepatic steatosis. In order to examine the hypothesis, we fed WT and PPP1R3B LSKO mice a high carbohydrate (66% sucrose) diet for 12 weeks. Consistent with decreased liver glycogen pool, 4-h fasting glucose concentrations were significantly lower in PPP1R3B LSKO mice compared with controls (PPP1R3B LSKO, 100 $\pm$ 5 mg/dl; control, 150 $\pm$ 10 mg/dl; $p < 0.05$). Glucose disposal after overnight fasting was significantly impaired (PPP1R3B LSKO AUC, 53,000 $\pm$ 5,000; control AUC, 33,000 $\pm$ 5,000; $p < 0.0005$). PPP1R3B LSKO mice were severely insulin-resistant: insulin-stimulated glucose clearance after a 6-h fast is significantly impaired (PPP1R3B LSKO AUC, 10,000 $\pm$ 500; control AUC, 5,900 $\pm$ 500; $p < 0.0005$). Plasma total cholesterol and HDL-C levels were significantly higher in PPP1R3B LSKO mice compared with control mice (PPP1R3B LSKO, 170 $\pm$ 20 mg/dl (TC) and 130 $\pm$ 20 mg/dl (HDL-C); control, 130 $\pm$ 20 mg/dl (TC) and 100 $\pm$ 20 mg/dl (HDL-C)). Hepatic triglycerides were significantly increased (PPP1R3B LSKO, 80 $\pm$ 20; control, 34 $\pm$ 20 mg/g; $p < 0.05$). The increased hepatic fat could be attributed to increased conversion of excessive carbohydrates for synthesis and storage of triglycerides, processes that are precursors to susceptibility of hepatic steatosis. These data suggest that genetic ablation of PPP1R3B perturbs nutrient homeostasis, leading to insulin resistance, elevated plasma lipids, and hepatic fat accumulation, which are exacerbated by dietary carbohydrate overload. This is consistent with PPP1R3B being the causative gene of the human genome-wide association with plasma lipid traits.

29

Isotopomer Spectral Analysis of Cholesterol Biosynthesis *in Vivo* Reveals Multiple Tissue-specific, Flux-dependent Pathways

Matthew A. Mitsche[1], Jeffrey G. McDonald[1], Helen H. Hobbs[1,2], Jonathan C. Cohen[1]

[1]University of Texas Southwestern Medical Center, Dallas, TX; [2]Howard Hughes Medical Institute, Dallas, TX

The final stage of cholesterol biosynthesis involves the conversion of lanosterol to cholesterol. Two alternative pathways, known as the Bloch and Kandutsch-Russell (K-R) pathways, have been proposed for this conversion. To assess the relative utilization of these pathways *in vivo*, we used LC-MS/MS methods developed by the Lipid MAPS Consortium, deuterium water (D_2O) incorporation, and isotopic spectral analysis to measure flux of post-squalene cholesterol biosynthetic intermediates in mouse tissues and cultured cells. Surprisingly, no tissue utilized the K-R pathway. Rather, we identified a hybrid between the two pathways that we called the modified K-R (MK-R) pathway. Proportional flux through the Bloch pathway varied widely, from 8% (preputial gland) to 97% (testes). Tissues with a higher biosynthesis rate had a greater utilization of the Bloch pathway, with the exception of ectodermal tissues (skin and preputial). The tissue-specific pathways utilized *in vivo* were retained in cell cultures. Sterol depletion in cultured cells increased flux through the Bloch pathway without affecting the flux through the MK-R pathway. Overexpression of desmosterol reductase (DHCR24) enhanced usage of the MK-R pathway; however, DHCR24 protein or expression levels only loosely correlated with utilization of the Bloch pathway in mouse tissues. We also provide evidence that DHCR24 is the rate-limiting enzyme for the conversion of zymosterol $\rightarrow$ cholesterol, but not lanosterol $\rightarrow$ cholesterol. Thus, relative use of the Bloch and MK-R pathways is highly variable, tissue-specific, flux-dependent, and epigenetically fixed. Maintenance of two intersecting pathways permits production of diverse bioactive sterols that can be regulated independently of cholesterol.

30

snoRNA U17 Is an Unexpected Regulator of Mitochondrial Cholesterol Trafficking and Steroidogenesis

Sarah Jinn[1], Arthur Sletten[1], Jessie Zhang[1], Lubav Ezerskiy[1], Jean Schaffer[1], Daniel Ory[1]

[1]Diabetic Cardiovascular Disease Center, Washington University, St. Louis, MO

Cholesterol is required for the growth and viability of mammalian cells and is an obligate precursor for steroid hormone synthesis. Using a loss-of-function screen for mutants with defects in intracellular cholesterol trafficking, a Chinese hamster ovary cell mutant with haploinsufficiency of the U17 snoRNA was isolated. U17 is a H/ACA snoRNA, for which a function other than ribosomal processing has not previously been identified. Through expression profiling, we identified hypoxia-up-regulated mitochondrial movement regulator (HUMMR) mRNA as a target that is negatively regulated by U17 snoRNA. Interaction between U17 snoRNA and HUMMR mRNA is mediated through an m1/m2 antisense motif, which is required for correction of the cholesterol trafficking defect in the mutant cells. Up-regulation of HUMMR in U17 snoRNA-deficient cells promoted formation of ER-mitochondrial contacts, decreasing esterification of cholesterol and facilitating cholesterol trafficking to mitochondria. We found that U17 snoRNA and HUMMR are inversely associated and developmentally regulated in steroidogenic tissues. Using antisense oligonucleotide-mediated knockdown of U17 snoRNA, we show that U17 and HUMMR regulate mitochondrial synthesis of steroids *in vivo*, suggesting that the U17 snoRNA-HUMMR pathway may serve a previously unrecognized physiological role in gonadal tissue maturation. To extend these findings, we have generated and are now characterizing mouse models of conditional overexpression and deletion of U17 snoRNA to explore how modulation of U17 snoRNA expression may impact fertility.

31

HDAC3 and SCAP Control Mutually Reinforcing Pathways of Hepatic Lipid Metabolism

Romeo Papazyan[1], Zheng Sun[1], Paul M. Titchenell[1], Erika Briggs[1], Mitchell A. Lazar[1]

[1]University of Pennsylvania, Philadelphia, PA

HDAC3 functions as a transcriptional repressor of lipogenic gene expression in the liver. Previously, we showed that conditional and liver-specific knockout of HDAC3 leads to fatty liver formation in the absence of increased LXR, ChREBP, and SREBP expression and increased SREBP cleavage. Based on these observations and the fact that SREBP and HDAC3 do not extensively overlap in their chromatin binding, we hypothesize that HDAC3 and SCAP control independent pathways of hepatic lipid metabolism. To test this, we generated a double-floxed HDAC3/SCAP C57Bl/6 mouse strain that allows us to simultaneously knock out HDAC3, SCAP, and SREBPs in adult mouse livers. Remarkably, HDAC3/SCAP double knock-out mice develop severe liver damage and die within 3 weeks of the conditional knockout. Metabolic gene expression and metabolomic profiling reveal errors in lipid handling as well as an increase in inflammatory signaling that precede the liver damage. Intriguingly, SCAP is dominant over HDAC3 with respect to expression of genes involved in *de novo* lipogenesis, yet the double knock-out mice develop hepatic steatosis on a high fat diet. Together, our data show that HDAC3 and SCAP control symbiotic gene expression programs that are essential for liver function and normal hepatic lipid metabolism.

32

Insights into the Structural Basis of Function of Cholesteryl Ester Transfer Protein

Gang (Gary) Ren[1]

[1]**Lawrence Berkeley National Laboratory, Berkeley, CA**

Cholesteryl ester transfer protein (CETP) decreases the level of atheroprotective high density lipoprotein cholesterol (HDL-C) while elevating the level of atherogenic low density lipoprotein cholesterol (LDL-C). CETP inhibitors are a new class of therapies for dyslipidemia, and several CETP inhibitors have been evaluated in large scale clinical trials for treating cardiovascular diseases (CVDs). However, three clinical trial studies have failed at the Phase III stage, reflecting the fact that the detailed molecular mechanisms of CETP interaction with lipoprotein and transfer cholesteryl esters (CEs) are poorly understood. Here, we examined the CETP incubated with various lipoproteins using electron microscopy and discovered that CETP bridges a ternary complex with its N-terminal β-barrel domain penetrating into HDL and its C-terminal domain interacting with LDL or very low density lipoprotein (VLDL) (*Nat. Chem. Biol*. (2012)
8: 342-349). By using HDL-like particles, we found that CETP binds to HDL via hydrophobic interactions rather than protein-protein interactions (*Sci. Rep*. (2015)
5: 8741). By using three of these agents (torcetrapib, dalcetrapib, and anacetrapib), we found that none of the inhibitors altered the CETP structure or binary complex conformation of CETP-lipoprotein. However, inhibitors did increase the binding ratios of binary complexes while reducing the binding ratios of ternary complexes, HDL-CETP-LDL. Some inhibitors exhibited high inhibition efficiency, decreasing the rate of HDL fusion more effectively than others. To understand the detailed mechanism, we performed molecular dynamics simulations to explore the structural features of CETP in aqueous solution, in the lipoprotein binding state, and in the inhibitor binding condition. Results show that the CETP distal portion flexibility of the N-terminal β-barrel domain is considerably greater in solution than in crystal. The distal end of the C-terminal β-barrel domain expanded, whereas the hydrophilic surface increased more than the hydrophobic surface (*Proteins* (2013)
81: 415-425). A formed tunnel is sufficiently large to mediate the transfer of a CE molecule through CETP with a predicted transfer rate comparable with physiological measurements. Analyses of the interactions and energies between the CE and CETP tunnel during transfer indicated several residues that might regulate CETP function during CE transfer. This study provides insight into the CE transfer mechanism for future development of CETP inhibitors.

33

Lipid Emulsions, Rich in *n*-3 or *n*-9 Fatty Acids, Reverse the Progression of Parenteral Nutrition-induced Hepatic Steatosis in Mice

Kuan-Hsun Huang[1], Philip B. Smith[2], Andrew D. Patterson[2,3], A. Catharine Ross[1]

[1]Department of Nutritional Sciences, Pennsylvania State University, State College, PA; [2]Center for Molecular Toxicology, Pennsylvania State University, State College, PA; [3]Department of Veterinary and Biomedical Sciences, Pennsylvania State University, State College, PA

Hepatic steatosis occurs in the early stage of the parenteral nutrition (PN)-associated liver disease. Previously, we reported that Intralipid®, a lipid emulsion (LE) rich in *n*-6 fatty acids (FAs), reduced hepatic triacylglycerol (TG) accumulation and markers of inflammation, once lipid accumulation had begun. However, it remains unclear whether other LEs (Omegaven®, rich in *n*-3 FA, and ClinOleic®, rich in *n*-9 FA) can reverse hepatic steatosis. Here, we compared three LEs (Intralipid®, Omegaven®, and ClinOleic®) for their ability to reverse hepatic TG accumulation after the onset of steatosis. Male C57BL/6 mice, 8-9/group, were fed chow for 5 weeks (reference group) or a PN diet (Clinimix-E® with vitamins and minerals plus 3% Intralipid® for sufficient essential FA) for 2.5 weeks; tissues were collected from the PN2.5 group to establish that hepatic steatosis had developed. The remaining mice were randomized into 4 groups: continuation of PN alone to week 5 (PN5) or change to PN with 13.5% (en-%) of either Omegaven® (fish oil LE, FOLE), ClinOleic® (olive oil LE, OOLE), or Intralipid® (soybean oil LE, SOLE) and fed for another 2.5 weeks (to the end of week 5). Transcripts of genes associated with lipogenesis and lipid mobilization and liver total FA composition were measured. PN5 mice had worsened values *versus* PN2.5 mice. At week 5, FOLE and OOLE reduced TG *versus* PN2.5 and PN5 ($p < 0.001$); SOLE lowered hepatic TG vs. PN5 ($p < 0.01$). FOLE mice had the lowest hepatic TG, which did not differ from chow-fed mice, and the lowest transcripts for lipogenesis-associated genes and *Srepbf*, *Ppar*-α, and *Ppar*-γ ($p < 0.001$). FOLE, OOLE, and SOLE lowered hepatic palmitic, palmitoleic, and vaccenic acids, whereas FOLE resulted in the lowest concentration of arachidonic acid but the highest concentration of docosahexaenoic acid and eicosapentaenoic acid *versus* PN2.5 and PN5 mice ($P < 0.001$). Genes associated with lipid mobilization were also reduced by FOLE, including *Acox1*, *Cpt-1*, *Acad1*, and *Mttp*, all $p < 0.0001$, whereas OOLE and SOLE lowered *Acox1* ($p < 0.001$). Overall, the inclusion of lipid into the PN diet in the form of 13.5 en-% LE, especially as FOLE, reversed the progression of hepatic lipid accumulation in mice with preexisting PN diet-induced hepatic steatosis.

34

Control of Hepatic Lipid Metabolism by an LXR-responsive Long Non-coding RNA

Tamer Sallam[1,2], Marius Jones[1], Thomas Gilliland[1], Li Zhang[1], Xiahui Wu[1,2], Ascia Eskin[3], Jaspreet Sandhu[1], David Casero[1], Thomas Vallim[2], Cynthia Hong[1], Melanie Katz[4], Richard Lee[4], Julian Whitelegge[5], Peter Tontonoz[1]

[1]Department of Pathology and Laboratory Medicine, Howard Hughes Medical Institute, UCLA, Los Angeles, CA; [2]Division of Cardiology, Department of Medicine, UCLA, Los Angeles, CA; [3]Department of Human Genetics, UCLA, Los Angeles, CA; [4]Ionis Pharmaceuticals, Carlsbad, CA; [5]Pasarow Mass Spectrometry Laboratory, NPI-Semel Institute, UCLA, Los Angeles, CA

The liver X receptors (LXRs) are key modulators of cellular responses to cholesterol overload. The molecular mechanisms that integrate LXRs with other sterol regulatory pathways are incompletely understood. In this work, we show that ligand activation of LXRs in liver not only promotes cholesterol efflux but also simultaneously inhibits cholesterol biosynthesis. We further identify the novel long non-coding RNA *LeXis* as an unexpected mediator of this effect. *LeXis* is robustly induced in mouse liver in response to Western diet feeding or pharmacologic LXR activation. Tissue-specific overexpression or deletion of *LeXis* affects the expression of cholesterol biosynthetic genes and the levels of cholesterol in the liver and plasma. *LeXis* interacts with and gates the DNA binding of the heterogeneous ribonucleoprotein Raly, a transcriptional coactivator that is required for the maximal expression of cholesterologenic genes. These studies outline a regulatory role for a non-coding RNA in lipid metabolism and advance our understanding of the mechanisms orchestrating sterol homeostasis.

35

Cardiomyopathy but Not Brown Adipose Tissue Dysfunction Causes Cold Sensitivity in Mice Globally Lacking ATGL

Renate Schreiber[1], Clemens Diwoky[1], Matthias Rosenwald[2], Christian Wolfrum[2], Guenter Haemmerle[1], Rudolf Zechner[1]

[1]Institute of Molecular Biosciences, University of Graz, Graz, Austria; [2]Institute of Food, Nutrition, and Health, ETH Zurich, Schwerzenbach, Switzerland

Thermoregulation in response to cold makes high metabolic demands on an organism. The key site of heat production is brown adipose tissue (BAT) that is packed with mitochondria containing uncoupling protein 1 (UCP-1). UCP-1 dissociates mitochondrial respiration from ATP synthesis to produce heat by the combustion of available energy substrates, such as glucose and fatty acids (FAs). FAs are mobilized from intracellular triacylglycerol (TG) stores, a process that is initiated by adipose triglyceride lipase (ATGL). Mice systemically lacking ATGL (AKO) have impaired lipolysis in BAT, massively increased BAT mass, and reduced UCP-1 transcript levels. Moreover, AKO mice are cold-intolerant, suggesting that ATGL in BAT is critical for thermogenesis.

In this study, we delineated the tissue-specific role of ATGL in thermoregulation. Genetic deletion of ATGL solely in BAT (BAKO), led to reduced lipolysis and hypertrophy in BAT. In contrast to AKO, BAKO mice had unaltered transcript levels of thermogenic genes, UCP-1 protein levels, or mitochondrial function. Importantly, however, BAKO mice showed normal cold tolerance, demonstrating that a BAT-specific loss of ATGL does not impair thermogenesis. Body temperature is maintained by a coordinated physiological interplay between various tissues. The cardiovascular system is particularly important to distribute heat and substrates. Because AKO mice exhibit a progressive cardiomyopathy, we hypothesized that cardiac insufficiency causes cold intolerance in these mice. To prove our hypothesis, we first exposed 6- and 10-week-old AKO and heart-specific AKO mice (HAKO) to cold. At the age of 6 weeks, AKO and HAKO mice were cold-tolerant. At the age of 10 weeks, however, both mouse models became extremely hypothermic. This phenotype strongly correlated with decreasing ejection fraction with age and suggests that AKO hearts were unable to meet the increased metabolic demand during cold. Remarkably, when we restored ATGL expression in the heart of AKO mice (AKO/cTg) and thus cardiac function, these mice maintained their body temperature in response to cold, although their BAT phenotype is comparable with that of AKO mice. In summary, our study demonstrates that ATGL in BAT is not limiting for thermogenesis *in vivo* but reinforces a currently underestimated role of cardiac function in thermogenesis.

36

Geranylgeranyl-regulated Transport of the Prenyltransferase UBIAD1 between ER and Golgi Membranes

Marc M. Schumacher[1], Dong-Jae Jun[1], Joachim Seemann[2], Russell A. DeBose-Boyd[1]

[1]Department of Molecular Genetics University of Texas Southwestern Medical Center, Dallas, TX; [2]Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX

UbiA prenyltransferase domain-containing protein-1 (UBIAD1) utilizes the nonsterol isoprenoid geranylgeranyl pyrophosphate (GGpp) to synthesize the vitamin K2 subtype menaquinone-4 (MK-4). A potential link between synthesis of MK-4 and cholesterol was provided by the recent observation that sterols trigger binding of UBIAD1 to 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting enzyme in synthesis of cholesterol and essential nonsterol isoprenoids. Sterol-regulated ubiquitination is obligatory for accelerated degradation of reductase from endoplasmic reticulum (ER) membranes, which constitutes one of several mechanisms that govern feedback regulation of reductase. Geranylgeraniol (GGOH), the alcohol derivative of GGpp, inhibits sterol-induced binding of UBIAD1 to reductase. Inhibition of this binding allows for maximal ER-associated degradation (ERAD) of reductase and permits transport of UBIAD1 from ER to Golgi. Here, we characterize geranylgeranyl-regulated transport of UBIAD1. Our results show that the addition of GGOH to isoprenoid-deprived cells stimulates transport of UBIAD1 from the ER to the medial-trans region of the Golgi. Retrograde transport of UBIAD1 from Golgi to ER is rapidly stimulated by depletion of isoprenoids. Moreover, the prenyltransferase accumulates in the ER of isoprenoid-replete cells when export from the organelle is inhibited. These findings suggest a model in which UBIAD1 continuously cycles between ER and Golgi, becoming sequestered in ER when the prenyltransferase senses a decline in membrane-embedded GGpp derived from *de novo* synthesis or phosphorylation of exogenous GGOH. This novel sensing mechanism allows for stringent control of intracellular transport of UBIAD1, which may directly contribute to control of reductase ERAD as well as synthesis of MK-4 and cholesterol.

37

Role of SVIP in the Biogenesis of ER-derived VLDL Transport Vesicle

Samata Tiwari[1], Shaila Siddiqi[1], Olga Zhelyabovska[1], Shadab A. Siddiqi[1]

[1]Biomedical Sciences, College of Medicine, University of Central Florida, Orlando, FL

Nascent very low density lipoprotein (VLDL) particles depart the endoplasmic reticulum (ER) in a distinct vesicle, the VLDL transport vesicle (VTV), which exports them to the Golgi for their maturation. Our data have shown that the biogenesis of the VTV from the ER membranes requires proteins in addition to coat complex II (COPII) proteins. Our proteomic analysis of the VTVs revealed that a 9-kDa protein, small VCP-interacting protein (SVIP) is uniquely present in these specialized vesicles. Our biochemical and morphological analyses revealed that SVIP is concentrated in the VTV but not in other ER-derived vesicles. Our confocal microscopy and co-immunoprecipitation data indicated that SVIP specifically interacts with VLDL protein, apolipoprotein B100, and COPII proteins. In this study, we investigated the role of SVIP protein in the biogenesis of VTV and VLDL secretion from the liver. To examine the role of SVIP, we either blocked the SVIP protein using specific antibodies or silenced SVIP by siRNA in hepatocytes and performed an *in vitro* ER-budding assay. We show that both blocking and knockdown of SVIP abrogate the VTV formation from the ER membranes. Additionally, we show that knockdown of SVIP significantly reduces VLDL secretion, suggesting a physiological role of SVIP in intracellular VLDL trafficking and secretion. We conclude that SVIP regulates the VTV biogenesis and thus its transport to the Golgi and plays an important role in VLDL secretion by the liver.

38

Global Analysis of Plasma Lipids Identifies Liver-derived Acyl-carnitines as a Fuel Source for Brown Fat Thermogenesis

Judith Simcox[1,2], Marzia Pascuali[3,4], James Cox[1], Sanghoon Lee[1,2], Ian Huck[5], Udayan Apte[5], Nicola Longo[3,4], Claudio J. Villanueva[1,2]

[1]Department of Biochemistry, University of Utah, Salt Lake City, UT; [2]University of Utah School of Medicine, Salt Lake City, UT; [3]Department of Pathology, University of Utah, Salt Lake City, UT; [4]Department of Pediatrics, University of Utah, Salt Lake City, UT; [5]Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS

Activation of brown adipose tissue (BAT) thermogenesis during cold exposure is a highly energy-demanding process. The metabolic fuel that drives thermogenesis relies in part on peripheral sources of energy. Using shotgun lipidomics, we identified plasma acyl-carnitines as a fuel source for BAT thermogenesis during cold exposure. Plasma long-chain acyl-carnitines (LCACs) are elevated in response to the cold and with B3-adrenergic receptor activation. Transgenic mice lacking BAT have a similar increase in LCACs with B3-adrenergic receptor activation, suggesting that this LCAC production is not BAT-dependent. We determined that the liver is a source of LCAC synthesis in response to cold exposure. Upon cold stimulation, B3-adrenergic receptors are activated in white adipocytes, which signals for the release of free fatty acids that enter the circulation. These free fatty acids activate hepatic HNF4a, which subsequently up-regulates transcription of CPT1 and OCTN2, genes that encode for enzymes involved in LCAC synthesis and release, respectively. Knockout of HNF4a in the liver decreases circulating LCAC, and the mice exhibit sensitivity to cold. Furthermore, stable isotope labeling of palmitoyl-carnitine confirms that brown adipocytes are able to use LCAC as a fuel source in response to B3-adrenergic receptor activation. Finally, a bolus of L-carnitine, which leads to elevated LCACs, is able to rescue cold sensitivity in old mice. Our data highlight an elegant mechanism where white adipose tissue provides FFAs for hepatic carnitilation to produce plasma LCAC as a fuel source for BAT thermogenesis.

39

Evaluating the Role of Genetic Variation in the Risk of Dyslipidemia in the South African Diabetic Population

Donald Moshen Tanyanyiwa[1,2], Jonathan Evans[2], Keleabetswe Mpye[2], Collet Dandara[2]

[1]Chemical Pathology, University of the Witwatersrand, Johannesburg, South Africa; [2]Division of Human Genetics, University of Cape Town, Cape Town, South Africa

Dyslipidemia, a disorder of lipoprotein metabolism, is commonly encountered and has been identified as a major risk factor for cardiovascular diseases. Dyslipidemia has been shown to have a strong genetic component. The absence of dyslipidemia in other patients with similar levels of glycemic control and economic background seen in the Diabetic POCT Clinic prompted an interest to explore possible genetic influence. 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. The three isoforms differ from one another at one or two amino acid residues. ApoE2 has been linked to type III hyperlipoproteinemia, whereas apoE4 has been linked to increased plasma cholesterol levels and an increased risk for atherosclerosis. The determination of ApoE isoform genotype can be achieved by the genotyping of the polymorphisms rs429358 (C112R) and rs7412 (R158C). In this study, we genotype a cohort of South African dyslipidemic patients to determine the prevalence of the rs429358 and rs7412 polymorphisms to identify patients at risk for developing dyslipidemia based on risk allele profiles. Participants (n = 245) were recruited from a cohort of 165 dyslipidemic South Africans and 80 controls attending Baragwanath hospital from whom a blood sample was obtained for DNA extraction. The samples were genotyped for the rs429358 and rs7412 SNPs by the PCR-RFLP method using the AflIII and HaeII restriction enzymes, respectively, to determine each variant. ApoE isoform genotypes were determined using rs429358 and rs7412 haplotypes. The minor allele frequencies (MAFs) for rs429358 and rs7412 in the control group were 0.23 and 0.24, respectively, and were 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 dyslipidemia cohort, respectively. There were statistically significant differences in the distribution of the ApoE variants. We report significant differences in the distribution of ApoE variants between diabetic dyslipidemic and control patients. ApoE appears to be a possible biomarker for dyslipidemia in this South African population.

40

AGPAT2 Is Required for *in Vitro* Brown Adipogenesis but Not for *in Vivo* Preadipocyte Abundance or Differentiation

Pablo J. Tapia[1], Victor A. Cortes[1]

[1]Department of Nutrition, Diabetes, and Metabolism, Pontificia Universidad Católica de Chile, Santiago, Chile

Mutations in the *AGPAT2* gene cause congenital generalized lipodystrophy, a disease characterized by absence of body fat and insulin resistance. Post-weaning *Agpat2*$^{-/-}$ mice completely lack adipose tissue, although they have a normal mass of interscapular brown adipose tissue (iBAT) at birth. This study aimed to determine the mechanisms of lipodystrophy in *Agpat2*$^{-/-}$ mice. iBAT preadipocytes were isolated from *Agpat2*$^{-/-}$ and *Agpat2*$^{+/+}$ newborn mice. Adipogenesis was induced with 5 μM rosiglitazone, 0.5 mM IBMX, 125 nM indomethacin, 500 nM dexamethasone, 1 nM T3, 20 nM insulin (DMEM/F-12, 10% FBS). Proteins were assessed by immunoblotting or confocal immunofluorescence; mRNA was quantified by qRT-PCR. To track preadipocytes, *Agpat2*$^{-/-}$ mice expressing EGFP under regulation of the Zfp423 promotor (Zfp423-EGFP) were generated. iBAT slices were stained with hematoxylin-eosin and Oil Red O.
Differentiated *Agpat2*$^{-/-}$ iBAT preadipocytes had fewer and smaller lipid droplets. Perilipin-1 total levels were lower, but it had normal subcellular distribution. Adipogenic markers PPARγ and C/EBPβ were normal in *Agpat2*$^{-/-}$ cells; however, brown adipocyte marker UCP1 failed to rise, whereas PGC1α remained low. Histologically, *Agpat2*$^{-/-}$ *Zfp423*$^{+}$ mouse iBAT had a normal abundance of preadipocytes at the E18.5 and P0.5 stages. Similarly, newborn *Agpat2*$^{-/-}$ mice (P0.5) had normal iBAT structure and neutral lipid levels. Progressive destruction of iBAT was noted in the following days, with increased levels of cleaved caspase-3 and -7 and increased levels of caspase-1 and IL-1β. Accordingly, UCP1 was progressively decreased. Total loss of iBAT was completed at P7.5. Our conclusions were as follows. 1) *In vitro*, *Agpat2* is required for preadipocyte brown adipogenic differentiation because its lack determines lesser neutral lipid accumulation and failed induction of brown adipocyte markers UCP1 and PGC1α. Nonetheless, AGPAT2 appears to be more critical for later phases of brown adipogenesis because general adipogenic regulators C/EBPβ and PPARγ were unaltered in *Agpat2*$^{-/-}$ cells. 2) *In vivo*, AGPAT2 is not required for preadipocyte differentiation because no differences were noted in the abundance of ZFP423-positive cells or in the morphology and lipid content of iBAT in *Agpat2*$^{-/-}$ mice (E18.5 and P0.5). Lipodystrophy in *Agpat2*$^{-/-}$ mice resulted from postnatal adipose tissue destruction associated with apoptosis and pyroptosis activation.

41

Identification and Characterization of PCSK9 Binding to Lipoprotein (a)

Hagai Tavori[1], Ilaria Giunzioni[1], Barton P. Duell[1], Deanna Plubell[1], Calvin Yeang[2], Sotirios Tsimikas[2], Sergio Fazio[1]

[1]Knight Cardiovascular Institute, Oregon Health and Science University, Portland, OR; [2]Department of Medicine, University of California, San Diego, La Jolla, CA

Lipoprotein (a) (Lp(a)) is an LDL particle that includes a protein, apo(a), bound to apoB. PCSK9 inhibition therapy reduces LDL-C and Lp(a) levels. Because PCSK9 binds to LDL particles, we hypothesized that it can also be found associated with Lp(a) in plasma. We aimed at characterizing the interaction between PCSK9 and Lp(a) *in vitro* in mice and in 39 subjects with high Lp(a) levels (39-320 mg/dl). Using three different approaches, we demonstrate that PCSK9 is physically associated with Lp(a) *in vivo*: 1) reducing and non-reducing Western blotting analyses of Lp(a) fractions isolated by iodixanol-based ultracentrifugation; 2) immunoprecipitation studies of human and Lp(a) transgenic mouse plasma; and 3) an ELISA method to detect and quantify Lp(a)-bound PCSK9. Interestingly, PCSK9 did not associate with Lp(a) *in vitro*. We further show that PCSK9 association with Lp(a) does not depend on the particle's ability to bind oxidized phospholipid and that PCSK9 is not directly associated with apo(a). There were no differences in the levels of apoB-associated and apoB-free PCSK9 between subjects with high Lp(a) levels and subjects without detectable Lp(a). We found a preferential association of PCSK9 with Lp(a) in plasma, with a 2.1-fold higher association compared with that of PCSK9 with LDL. Finally, Lp(a)-bound PCSK9 levels directly correlated with Lp(a) levels (after adjusting for age, sex, and lipid-lowering medications) but not with total plasma PCSK9 levels, suggesting that this may become a biomarker for cardiovascular risk. In conclusion, our results support a scenario where 1) PCSK9 is found in association with Lp(a) in subjects with high Lp(a) levels; 2) PCSK9 association with Lp(a) occurs *in vivo* only; 3) the association does not depend on the presence of apo(a) or on the particle's ability to bind oxidized phospholipids; and 4) Lp(a)-bound PCSK9 is apparently "stolen" from LDL-bound PCSK9. Levels of Lp(a)-bound PCSK9 may be linked to cardiovascular disease risk prediction and to the extent of Lp(a) lowering caused by PCSK9 antibodies.

42

Competition for Chromatin Remodeler Brg1 May Mediate Transcriptional Repression by LXR

David G. Thomas[1], Alan R. Tall[1]

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

In macrophages, activation of the liver x receptor (LXR) by metabolites in the inflammatory milieu results in repression of inflammatory gene expression. The mechanism of this repression has been linked to recruitment of corepressor NCoR or SMRT by liganded LXR or alternatively the biochemical activities of LXR targets. Here, we show that repression of inflammatory gene expression by LXR persists in the presence of protein synthesis inhibition by cycloheximide, so it is unlikely that LXR targets play a substantial role in early LXR-mediated repression. Moreover, we found that transcriptional repression by LXR in the presence of cycloheximide appears specific for targets of interferon regulatory factor 3 (IRF3), which are known to be dependent on ATP-dependent chromatin remodeling for transcriptional activation. siRNA depletion of the major catalytic subunit of the chromatin remodeling complex, Brahma-related gene 1 (Brg1), resulted in abrogation of LXR-mediated repression of most inflammatory genes. The interaction of Brg1 with IRF3 and the recruitment of Brg1 to IRF3-dependent genes were diminished after activation of LXR. We propose a new mechanism in which transcriptional repression by LXR is caused by competition for the ATP-dependent chromatin remodeling complex containing Brg1.

43

Cholesterol in Remnant Lipoproteins as Measured by Different Methods

Peter P. Toth[1], Harold E. Bays[2], W Virgil Brown[3], Joanne E. Tomassini[4], Colin Wang[4], Adam B. Polis[4], Andrew M. Tershakovec[4]

[1]CGH Medical Center, Sterling, IL; [2]Louisville Metabolic and Atherosclerosis Research Center, Louisville, KY; [3]Emory University School of Medicine, Atlanta, GA; [4]Merck & Co., Inc., Kenilworth, NJ

Elevated remnant-lipoprotein cholesterol (RLP-C) levels are associated with an increased risk of ischemic heart disease (IHD). The concurrence of RLP-C measurement by different separation methods is not well described. This analysis assessed RLP-C by three commonly used measurements, including immunoseparation (IM (ApoAI and ApoB100 monoclonal antibodies)), vertical autoprofile (VAP (IDL + $VLDL_3$)), and calculated RLP-C (total cholesterol minus HDL-C minus LDL-C) methods using samples from a previously reported randomized clinical trial. This analysis assessed fasting RLP-C in hyperlipidemic patients (*n* = 2,382) treated with ezetimibe/simvastatin (E/S) (10/20 mg), E/S + niacin (N) (2 g), and N (2 g) during 24 weeks and E/S (10/20 mg) and E/S + N (2 g) during 64 weeks. RLP-C levels, change from baseline, and percentage change from baseline were evaluated by the three methods. Relationships and agreement among the three methods used in the measurement of these parameters were assessed by Pearson correlation coefficients and Bland-Altman plot, respectively. Cholesterol mass at baseline measured by the VAP and calculated methods was ~3-4 times higher than by IM; all declined with treatment by 24 weeks (Table) with little further reduction at 64 weeks (not shown). RLP-C change and percentage reduction from baseline were larger when measured by VAP *versus* calculated and IM methods. Although the three methods were moderately to strongly correlated ($r = 0.37 - 0.79$) for RLP-C levels and changes, Bland-Altman plots showed little agreement between the methods for RLP-C levels but slightly better agreement for RLP-C changes (not shown). RLP-C defined by IM, VAP, and calculated methods differs in mass and response to pharmacologic intervention. Given the relationship between RLP-C and IHD risk, standardization of methods is needed for RLP-C use in risk assessment.

Parameter	RLP-C E/S				RLP-C E/S+ERN			
	n	[mg/dL] mean(S.D.)	Change from baseline [mg/dl] mean(S.D.)	% change from baseline mean (S.D.)	*n*	[mg/dl] mean(S.D.)	Change from baseline [mg/dl] mean(S.D.)	% change from baseline mean(S.D.)
Immunoseparation								
Baseline	270	11.7(7.0)			670	11.2(5.8)		
24 wks	212	6.9(3.5)	-5.0(7.5)	-3.2(4.1)	395	5.9(4.3)	-5.2(5.8)	-4.2(4.9)
VAP								
Baseline	257	39.8(14.4)			651	40.3(13.4)		
24 wks	206	17.2(8.3)	-22.9(12.7)	-16.1(10.7)	371	13.2(9.1)	-27.7(13.9)	-29.8(20.1)
Calculated								
Baseline	271	35.7(17.1)			673	33.9(15.8)		
24 wks	214	26.9(11.8)	-8.6(14.0)	-4.0(5.6)	395	20.2(11.0)	-13.8(14.0)	-8.7(8.1)
E/S = ezetimibe (10 mg)/simvastatin (20 mg); ERN = extended-release niacin (titrated to 2 g)								

44

Mutations in ABCA8 Underlie Reduced Plasma High Density Lipoprotein Cholesterol Levels

Laia Trigueros-Motos[1], Julian vanCapelleveen[2], Linhua Zhang[3], Caryn Chai[1], Martin Kang[3], Ian Tietjen[4], Chris Radomski[4], Amina Barhdadi[5], Gopala K. Yakala[1], Uwe J. Tietge[6], Geesje Dallinge-Thie[2], Marie-Pierre Dube[5], Michael R. Hayden[1,3], Kees Hovingh[2], Roshni R. Singaraja[1]

[1]Translational Laboratories for Genetic Medicine, A*STAR, and National University of Singapore, Singapore; [2]Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; [3]Centre for Molecular Medicine, University of British Columbia, Vancouver, BC, Canada; [4]Xenon Pharmaceuticals Inc., Burnaby, BC, Canada; [5]Beaulieu-Saucier Université de Montréal Pharmacogenomics Centre, Montreal, QC, Canada; [6]Department of Pediatrics, University of Groningen, Groningen, The Netherlands

The risk of cardiovascular disease (CVD) has been inversely correlated with high density lipoprotein cholesterol (HDLc) levels, with low levels of HDLc being one of the major risk factors for CVD. However, despite intense efforts, including genetic studies and clinical trials, the causality of this relationship is still unclear, and functional studies are needed to better understand the molecular pathology underlying low HDLc levels and the association with CVD. We selected the ATP binding cassette transporter A8 (*ABCA8*) for study because variants in this gene had been previously associated with low HDLc levels in humans. We sequenced *ABCA8* in 80 low (HDLc percentile <10th) and 120 high HDLc (HDLc percentile ≥90th) individuals and identified three predicted damaging *ABCA8* variants exclusively in subjects with low HDLc: $Pro^{609} \rightarrow$ Arg (in the ATP-binding domain), E17[hyphen]2 A>G (disruption of essential splice site) and a previously identified variant $Thr^{741} \rightarrow$ Stop. Expansion and genotyping of families of the probands identified additional carriers for these variants. Compared with first-degree relative controls, heterozygous mutation carriers showed a significant 26.5% decrease in plasma HDLc levels. Hepatic overexpression of human ABCA8 in mice increased plasma HDLc levels by 23.1%, validating the association observed in humans, and also increased the first steps of reverse cholesterol transport. Wild-type ABCA8 was localized at the plasma membrane and endoplasmic reticulum, and its overexpression led to a significant 1.8-fold increase in cholesterol efflux to lipid-free APOA-I. However, P609R and T741X were only present at the ER, and their overexpression did not induce cholesterol efflux to APOA-I. We show here that ABCA8 facilitates cholesterol transport to lipid-free APOA-I, stimulates the first steps of reverse cholesterol transport, and modulates HDL cholesterol levels in humans.

45

Loss of ABCG1 Specifically in Pulmonary Type 2 Cells Results in Impaired Surfactant Lipid Metabolism and Innate Immune Responses

Thomas Quad de Aguiar Vallim[1,2], David Merriott[1,3], Joan Cheng[1], Angela Cheng[3], Angel Baldan[4], David Ford[4], Christopher N. Goulbourne[5], Katayun Barmak[5,6], Tisha Wang[1], Elizabeth J. Tarling[1,2]

[1]Department of Medicine, UCLA, Los Angeles, CA; [2]Molecular Biology Institute, UCLA, Los Angeles, CA; [3]Department of Biological Chemistry, David Geffen School of Medicine, UCLA, Los Angeles, CA; [4]Edward A. Doisy Department of Biochemistry and Molecular Biology, Saint Louis University, St Louis, MO; [5]Columbia Nano Initiative Electron Microscopy Facility, Columbia University, New York, NY; Department of Physics and Applied Mathematics, Columbia University, New York, NY

Cardiovascular disease (CVD) is the leading cause of death in the United States, and patients with pulmonary disease are at least 2-fold more likely to develop cardiovascular complications. Whole-body cholesterol balance is a complex and tightly regulated process. Indeed, cholesterol is the most highly decorated small molecule, being the subject of 13 Nobel Prizes. The sterol-transporter ATP binding cassette transporter G1 (ABCG1) is critical for maintaining normal cellular cholesterol and lipid homeostasis. After the liver and intestine, the lung is the third most active lipid-secreting organ. Pulmonary alveolar proteinosis (PAP) is a rare lung disease characterized by the accumulation of surfactant lipids and proteins in the pulmonary alveoli resulting in respiratory distress. For almost all reported cases, the underlying cause is unknown. Synthesis and secretion of surfactant in the lung is restricted to epithelial type 2 pneumocytes (T2 cells). However, subsequent clearance of surfactant from the hypophase is dependent upon both T2 cells and macrophages. Both T2 cells and macrophages express high levels of ABCG1. Further, *Abcg1*$^{-/-}$ mice develop a severe pulmonary lipidosis, characterized by elevated levels of multiple lipid species and the presence of lipid-loaded macrophages and T2 cells containing abnormal lamellar bodies. Recent advances have also implicated a role for the innate immune system in the pathogenesis of PAP. We have recently demonstrated that the lungs of *Abcg1*$^{-/-}$ mice exhibit a profound immune response associated with a local increase in innate immune natural antibody-secreting B-1 B cells and both local and circulating natural antibodies. Here we show that the lungs of mice containing ABCG1-deficient non-bone marrow-derived cells and wild-type macrophages contain elevated levels of both phospholipids and esterified cholesterol. In addition, the non-bone marrow-derived cells in these mice accumulated lamellae bodies and unesterified cholesterol and exhibited abnormal gene expression. Mice lacking ABCG1 specifically in T2 cells (*Abcg1*$^{T2-/T2-}$) also develop abnormal lamellar bodies with altered phospholipid and cholesterol homeostasis. Collectively, our results suggest that ABCG1 is critical for normal surfactant metabolism and that the pulmonary expansion of B-1 B cells and the subsequent increase in natural antibodies in *Abcg1*$^{-/-}$ mice may provide a novel model in which to study the role of the innate immune system in PAP and the subsequent risk of developing cardiovascular disease.

46

Endocytic Adaptors Arh and Dab2 Control Homeostasis of Circulatory Cholesterol

Xiang-Xi Mike Xu[1]

[1]Department of Cell Biology, Sylvester Comprehensive Cancer Center, University of Miami Miller School, Miami, FL

High serum cholesterol (hypercholesterolemia) strongly associates with cardiovascular diseases as the atherogenic low density lipoproteins (LDL) promote atheroma development in arteries (atherosclerosis), and reducing serum cholesterol has been an active pharmacological intervention. LDL clearance from the circulation by LDL receptor (LDLR)-mediated endocytosis by hepatic and peripheral tissues and subsequent feedback regulation of endogenous synthesis of cholesterol is a key determinant of serum LDL level. Human mutation analysis revealed that ARH, an LDLR endocytic adaptor, and PCSK9, a proprotein convertase binding to the LDLR, perturb LDLR function and thus impact serum cholesterol levels. In our genetic analysis of mutant mice, we found that the deletion of another LDLR endocytic adaptor, Disabled-2 (Dab2), only slightly affected the serum cholesterol level. However, elimination of both *arh* and *dab2* genes in mice resulted in profound hypercholesterolemia similar to that resulting from *ldlr* homozygous deletion. Arh is widely expressed, in hepatocytes and peripheral tissues, whereas Dab2 is expressed in a more restricted pattern. In liver, Dab2 is expressed in endothelial cells but not in hepatocytes. In the absence of both Dab2 and Arh, HMG-CoA reductase level increased to a level similar to that of *ldlr* knockout. Thus, in the absence of Arh, the function of Dab2 in liver endothelial cells regulates cholesterol synthesis in hepatocytes. We conclude that the combined roles of Arh and Dab2 are responsible for the majority of adaptor function for LDLR endocytosis and LDLR-mediated cholesterol clearance from circulation and the regulation of cholesterol synthesis.

47

Defining the Mechanism of Lipoprotein (a) (Lp(a)) Lowering by Alirocumab in Transgenic Mouse Models

Calvin Yeang[1], Phuong Miu[1], Green Simone[1], Xiaohong Yang[1], Ming-Yow Hung[2], Witztum L. Joseph[1], Sotirios Tsimikas[1]

[1]University of California, San Diego, La Jolla, CA; [2]Taipei Medical University, Taipei, Taiwan

Lp(a) consists of apo(a) covalently bound to apoB of LDL. PCSK9 monoclonal antibodies, such as alirocumab, lower Lp(a) in humans by 9-38%, but the mechanisms are not well defined. We utilized transgenic apo(a)/Lp(a) mice to assess how alirocumab lowers Lp(a) *in vivo*. C57BL/6 transgenic mice expressing apo(a) only (under control of an apoE promoter) and double transgenic Lp(a) (expressing both apo(a) and human apoB-100 (hApoB)) were treated with alirocumab or vehicle. Plasma levels of Lp(a), total apo(a) (free and true Lp(a)), and hApoB were quantified by ELISA. Hepatic mRNA levels of apo(a), hApoB, and mApoB were measured in both groups. Finally, plasma clearance of human [^{125}I]apo(a) was determined following intravenous injection to WT mice treated with alirocumab or vehicle. Alirocumab lowered total cholesterol and mApoB 29-62% ($p < 0.05$ for all). hApoB levels were unaffected, possibly due to the low affinity of hApoB for the mouse LDLR. In alirocumab-treated apo(a) and Lp(a) mice, total apo(a) levels declined by up to 42 and 41% ($p < 0.05$ for all), respectively. In alirocumab-treated Lp(a) mice, Lp(a) levels were unaffected, which we speculate is due to the excess of apo(a) (unassociated with hApoB) in plasma of these mice. Hepatic mRNA levels of apo(a), hApoB, and mApoB were not affected by alirocumab. Plasma clearance of intravenously injected [^{125}I]apo(a) was accelerated by 25% in alirocumab-treated WT mice *versus* vehicle. Alirocumab-mediated lowering of plasma apo(a) is due to enhanced clearance. Work is ongoing to identify the receptors and/or pathways involved.

48

Identification of a Cholesterol Metabolic Type I Interferon Inflammatory Circuit

Autumn G. York[1], Kevin J. Williams[1], Joseph P. Argus[1], Quan Zhou[1], Gurpreet Brar[1], Laurent Vergnes[1], Elizabeth E. Gray[2], Elizabeth J. Tarling[1], Ting-Ting Wu[1], Karen Reue[1], Daniel B. Stetson[2], Steven J. Bensinger[1]

[1]UCLA, Los Angeles, CA; [2]University of Washington, Seattle, WA

Cellular lipid requirements are achieved through a combination of biosynthesis and import programs. To date, the molecular mechanisms that regulate whether a cell preferentially scavenges or synthesizes lipids are not well understood, particularly in non-metabolic tissues, such as immune cells. Perturbations in fatty acid and cholesterol homeostasis have been observed in response to a number of viral and microbial infections, leading us to ask whether signaling through immune receptors could influence the cellular programs that regulate lipid homeostasis in macrophage. Using isotope tracer analysis, we show that type I interferon (IFN) signaling shifts the balance of lipid metabolism by increasing import and decreasing *de novo* synthesis of both cholesterol and long-chain fatty acids. Further analysis found that type I IFN-mediated alterations in lipid metabolism are regulated at both the transcriptional and enzymatic levels and are only partially dependent on *Ch25h* induction. This multi-tiered regulation led us to hypothesize that specifically limiting lipid synthesis may be a novel aspect of the type I IFN response and may be important for antiviral immunity. Indeed, genetically enforcing this metabolic shift in macrophages alone is sufficient to render mice resistant to viral challenge, demonstrating the importance of reprogramming the balance of these two metabolic pathways *in vivo*. Unexpectedly, mechanistic studies reveal that limiting flux through the cholesterol biosynthetic pathway spontaneously engages a type I IFN response in a STING-dependent manner. The up-regulation of type I IFNs was traced to a decrease in the pool size of synthesized cholesterol and could be inhibited by replenishing cells with free cholesterol. Taken together, these studies delineate a metabolic-inflammatory circuit that links perturbations in cholesterol biosynthesis with activation of innate immunity.

49

Identification of Novel Regulators of Cancer Cell Lipid Homeostasis Using High Throughput siRNA Screening

Quan D. Zhou[1], Kevin J. Williams[1], Joseph P. Argus[1], Moses Q. Wilks[2], Steven J. Bensinger[1]

[1]UCLA, Los Angeles, CA; [2]Massachusetts General Hospital, Boston, MA

Reprogramming of lipid metabolism is emerging as a hallmark of many cancers. Identification of altered metabolic pathways affords therapeutic opportunities; however, our knowledge of the molecular events driving altered lipid metabolism and the cancer metabolic phenotype is limited. To better understand the relationship between oncogenic signaling and lipid homeostasis of cancers, we have undertaken an unbiased screening approach that combines the use of high throughput gene silencing with mass spectrometry-based metabolic isotope labeling analysis. To this end, H1299 lung adenoma carcinoma cells were transiently transfected with a commercial siRNA library targeting 720 kinases in the human kinome and labeled with 50% [^{13}C]glucose to track *de novo* lipid synthesis. After 48 h of labeling, cells were harvested for quantitative measurement of long-chain fatty acids and cholesterol by GC/MS. Multidimensional data were extracted with mathematical modeling, and lipid pool size, percentage of *de novo* synthesis, and percentage of glucose contribution of each lipid were determined. Preliminary of data sets indicate a number of potential novel regulators of lipid homeostasis. Redundant siRNA activity analysis (RSA) will be employed to rank "hits" for each metabolic parameter of interest. Ongoing analysis includes clustering, PCA, and KEGG pathway analysis to develop a comprehensive picture of how signaling nodes or pathways alter lipid homeostasis. Future studies will be focused on further validating, identifying novel signaling pathways regulating lipid metabolism and potential therapeutic targets.

50

Characterization of the Adipose Triglyceride Lipase (ATGL) Regulators G0S2 and CGI-58

Lisa Wechselberger[1], Andras Boeszoermenyi[2], Roland Viertlmayr[1], Christoph J. Pilip[1], Harald M. Nagy[1], Haribabu Arthanari[2], Ines K. Cerk[1], Krishna M. Padmanabha Das[1], Martina Schweiger[1], Achim Lass[1], Robert Zimmermann[1], Rudolf Zechner[1], Klaus Zangger[1], Monika Oberer[1]

[1]University of Graz, Graz, Austria; [2]Harvard Medical School, Boston, MA

Elevated cellular levels of unesterified fatty acids (FAs) have been directly linked to lipotoxicity. Lipid droplet (LD)-associated lipases, namely adipose triglyceride lipase (ATGL), hormone-sensitive lipase, and monoacylglycerol lipase catalyze the reactions releasing diacylglycerols, monoacylglycerols, FAs, and glycerols during the fine-tuned process of intracellular lipolysis. The proteins comparative gene identification 58 (CGI-58) and G_0/G_1 switch gene 2 (G0S2) act as activator and inhibitor of ATGL, respectively. Until now, very little has been known about the molecular mechanism behind the mode of activation and inhibition. We show that both proteins interact with the N-terminal half of ATGL and that the inhibition of ATGL by G0S2 does not depend on the presence of CGI-58. Structural studies of G0S2 indicate that G0S2 is partially unfolded with a propensity to form α-helices. It appears likely that the protein adopts a stable tertiary fold solely upon interaction with a binding partner. Full-length G0S2 inhibits ATGL with an IC_{50} of 19 nM. We used N- and C-terminally truncated variants of G0S2 to identify core requirements for G0S2 to inhibit ATGL. The hydrophobic central region encompassing Tyr^{27}–Met^{43} is essential for inhibition. Kinetic analysis revealed that a G0S2-derived peptide inhibits ATGL in a non-competitive mode. C-terminally truncated ATGL mutants, which fail to localize to LDs otherwise, translocated to the LDs upon co-expression with G0S2, suggesting that G0S2 is sufficient to anchor ATGL to LD surfaces independently of the ATGL C-terminal LD binding region. We also identified a lipophilic and Trp-rich region at the N terminus of CGI-58 that is essential for proper localization of CGI-58 to LDs and for CGI-58-mediated stimulation of ATGL activity. We determined the solution structure of this region in an LD mimic-bound conformation using NMR spectroscopy. The structure reveals that CGI-58 binds to lipid droplets via a short N-terminal lipid droplet anchor motif. This motif contains a short α-helical stretch flanked by two tryptophan residues and a relatively independent third tryptophan residue surrounded by two prolines. The CGI-58 anchor motif tolerates the loss of either one of these two regions robustly but not the loss of the two regions at the same time.

51

Development of Cell Translation Therapy in Severe Familial Hypercholesterolemia Undergoing LDL Apheresis Therapy

M. Koseki[1,2], A. Saga[2,3], T. Okada[2], R. Kawase[2], K. Tomita[4], K. Osuga[5], S. Ezoe[3], Y. M. Nishida[1,2], Sakata[2], S. Yamashita[2,6]

[1]Health Care Center, Osaka University, Osaka, Japan; [2]Cardiovascular Medicine, Osaka University Graduate School of Medicine, Osaka, Japan; [3]Medical Center for Translational Research, Osaka University Hospital, Osaka, Japan; [4]Plastic Surgery, Osaka University Graduate School of Medicine, Osaka, Japan; [5]Diagnostic and Interventional Radiology, Osaka University Graduate School of Medicine, Osaka, Japan; [6]Community Medicine, Osaka University Graduate School of Medicine, Osaka, Japan

Familial hypercholesterolemia (FH) is characterized by high LDL cholesterol levels in the blood and premature cardiovascular disease. Although most heterozygous FH patients are usually treated with statin, ezetimibe, bile acid sequestrants, and monoclonal anti-PCSK9 antibody, homozygous FH patients are resistant to drug therapy except for lomitapide. Many homozygous FH patients used to be treated by LDL apheresis in Japan. However, the effect of removing LDL cholesterol is temporary and still not sufficient. As a definitive therapy, liver transplantation is one of the options to recover the LDL receptor, but the number of donors is always limited in Japan. With the increase in evidence of the safety of mesenchymal stem cells and the percutaneous transhepatic portal approach in islet transplantation, we developed a cell transplantation therapy with allogeneic adipose tissue-derived multilineage progenitor cells (ADMPCs) as an alternative treatment to liver transplantation. We have already demonstrated that xenogenic transplantation of human ADMPCs into Watanabe heritable hyperlipidemic (WHHL) rabbits via a portal vein resulted in a significant reduction in total cholesterol, and the reductions were observed within 4 weeks and maintained for 12 weeks. These results suggested that human ADMPC transplantation could correct the metabolic defects and be a novel therapy for inherited liver diseases. We generated a protocol for the first-in-human clinical trial, and it has been approved by the institutional review board and the Ministry of Health, Labor, and Welfare of Japan. We performed the first ADMPC transplantation in February 2016 and are currently collecting data.

52

Adipocyte plasma membrane associated protein (APMAP) – a nexus between extracellular matrix and the regulation of glucose homeostasis in adipocytes?

Pessentheiner AR[1], Huber K[1], Gadermaier B[1], Birner-Gruenberger R[2], Ruelicke T[3], and Bogner-Strauss JG[1]

[1]Institute of Biochemistry, Graz University of Technology, Austria; [2]Institute of Pathology, Medical University of Graz, Austria; [3]Institute of Laboratory Animal Sciences, Veterinary University of Vienna, Austria

Adipose tissue (AT) is a complex multicellular organ and the biggest caloric reservoir of the body. During positive energy balance, excess energy is stored in adipocytes in form of lipid droplets. Dynamic AT remodeling is a prerequisite for healthy expansion. Thereby, the extracellular environment of adipocytes plays an important role. Adipocytes are embedded in an extracellular matrix (ECM) that not only provides adipocytes with structural and mechanical support, but is also involved in various signaling events. Imbalances in ECM synthesis and degradation cause fibrosis which is a hallmark of AT dysfunction and tightly associated with inflammatory processes and the progression of insulin resistance. Thus, one way of identifying targets for pharmaceutical interventions against obesity and type II diabetes is a detailed understanding of the factors involved in ECM biosynthesis and remodeling.
Adipocyte plasma membrane associated protein (APMAP) is a glycosylated membrane protein. We previously showed that APMAP expression is highly upregulated during adipocyte differentiation, concomitantly translocating from the ER to the plasma membrane. Moreover, APMAP expression is crucial for adipogenesis *in vitro* since APMAP-silenced 3T3-L1 cells hardly differentiate into adipocytes. The current study investigates the physiological function of APMAP. We identified extracellular matrix components lysyloxidase-like 1 and 3 as interaction partners of APMAP. Moreover, our data indicate that APMAP regulates the expression of these ECM proteins.
Lysyloxidases and lysyloxidase-like proteins (LOX and LOXLs) mediate the crosslinking of collagens and are therefore implicated in ECM remodeling. LOX expression is connected to obesity and insulin resistance, since LOX expression is increased during progression of obesity and its inhibition decreases bodyweight and ameliorates glucose homeostasis in obese rats. Here, we show that APMAP knock-out mice tend to have reduced body weight and improved insulin sensitivity upon diet-induced obesity. Together, our data suggest that APMAP expression influences ECM remodeling thereby impacting adipocyte differentiation capacity and the progression of fibrosis.
This data places APMAP as a novel regulator of ECM components, thereby linking the properties of adipocyte ECM to the development of insulin resistance.

Author Index (name, abstract/board number)

DEUEL Board Members

CHAIR

KARIN BORNFELDT (2016)
Department of Pathology,
Diabetes and Obesity
Center of Excellence
University of Washington
South Lake Union Campus
Box 358055
815 Mercer Street
Seattle, WA 98109
Tel:(206) 543-1681
Fax: (206) 543-3567

MEMBERS

ALAN ATTIE (2018)
Department of Biochemistry
University of Wisconsin-Madison
433 Babcock Drive
Madison, WI, 53706-1544
Tel: (608) 262-1372

DAN BLOOMFIELD (2018)
Merck & Co.
126 E. Lincoln Avenue
PO BOX 2000
Rahway, NJ, 07065-0900
Tel: (732) 594-0800

ROSALIND COLEMAN (2017)
University of North Carolina
Department of Nutrition
2209 McGaven-Greenberg Bldg. CB#7461
Chapel Hill, NC 27599-7461
Tel: (919) 966-7213

BRANDON DAVIES (2020)
Department of Biochemistry
University of Iowa
Iowa City, IA 52242
Tel: (319) 335-3225

OMAR FRANCONE (2017)
Senior Director, Discovery Research
Shire HGT
300 Shire Way
Lexington, MA 02421
Tel: (781) 482-0605

JESPER GROMADA (2019)
Metabolism and Muscle Department
Regeneron Pharmaceuticals
777 Old Saw Mill River Road
Terrytown, NY 10591
Tel: (914) 847-7971

JAY HORTON (2019)
Department of Internal Medicine, Molecular Genetics
UT Southwestern Medical Center
5323 Harry Hines Blvd.
Dallas, TX 75390

FREDRIC KRAEMER (2018)
Division of Endocrinology, S005
Stanford University Medical Center
Stanford, CA 94305-5103
Tel: (650) 493 5000 x 63184

RICHARD LEHNER (2016)
Department of Pediatrics, Group on Molecular and
Cell Biology of Lipids
University of Toronto
328 Heritage Medical Research Centre
Tel: (780) 492-2963
Fax: (780) 492-3383

ROGER NEWTON (2016)
Esperion Therapeutics, Inc
46701 Commerce Center Drive
Plymouth, MI 48170
Tel: (734) 862-4841

SAMPATH PARTHASARATHY (2017)
University of Central Florida
Clayton Campus, Wellington Road
6900 Lake Nona Blvd.
Orlando, FL 32827
Tel: (407) 266-7121

JEAN SCHAFFER (2019)
Division of Biology and
Biomedical Sciences
Campus Box 8226
660 S. Euclid Ave.
St. Louis, MO 63110-1093
Tel: (314) 362-8717

ROSHNI SINGARAJA (2020)
Translational Laboratories in Genetic Medicine
National University of Singapore
Singapore
Tel: 6407 4382

MARK SLEEMAN (2017)
Monash University
Clayton Campus, Wellington Road
Clayton, Melbourne, Australia
3800
Tel: 0399052516

DEUEL Board Members

ALAN TALL (2020)
Department of Medicine
Columbia University
630 West 168th St.
New York, NY 10032
Tel: (212) 305-4899

MURIELLE VENIANT (2019)
Department of Metabolic Disorders
Amgen, Inc.
One Amgen Center Drive
Mail stop29-1-A
Thousand Oaks, CA 91320
Tel:(805) 447-8009
Fax: (805) 499-0953

STEVE WATKINS (2017)
Lipomics
3410 Industrial Boulevard, Suite 103
West Sacramento, CA 95835
Tel: (916) 371-7974

JOSEPH WITZTUM (2020)
Univ California, San Diego
Biomedical Science Building-Room 1080
9500 Gilman Drive
La Jolla, CA 92093-0682
Tel: 858-534-4347

RUDOLF ZECHNER (2019)
Institute of Molecular Biosciences
Karl Franzens Universität Graz
Heinrichstraße 31
8010 Graz
Tel: +43 316 380 1900

PROGRAM COMMITTEE CHAIRS

CHRISTOPHER K. GLASS (PROGRAM CHAIR 2016)
Professor of Cellular and Molecular Medicine Professor of Medicine
University of California, San Diego Department of Cellular &
Molecular Medicine School of Medicine
9500 Gilman Dr. Mailcode 0651
GPL Building, Room 217
La Jolla, CA 92093-0651
Tel: (858) 534-6011
Fax: (858) 822-2127

JEAN SCHAFFER (PROGRAM CHAIR, 2017)
Division of Biology and
Biomedical Sciences
Campus Box 8226
660 S. Euclid Ave.
St. Louis, MO 63110-1093
Tel.: (314) 362-8717

TREASURER/FUNDING

STEPHEN G. YOUNG
University of California, Los Angeles
Department of Medicine
Division of Cardiology
650 Charles E. Young Drive, South
A2-237 CHS Bldg.
Los Angeles, CA 90095
Tel: (310) 825-4934
Fax: (310) 206-0865

CHRISTOPHER K. GLASS
University of California San Diego
Cellular & Molecular Medicine
9500 Gilman Drive, MC 0651
GPL Room 217
La Jolla CA 92093-0651
Tel: (858) 534-6011
FAX: (858) 822-2127

LOCAL ARRANGEMEN TS

BARBARA A. GORDON
American Society for Biochemistry and Molecular Biology
11200 Rockville Pike
Suite 302
Rockville, MD 20852
Tel: (240) 283-6600
Fax: (301) 881-2080

Conference Attendees as of February 16, 2016

Christopher M Allan
University of California, Los Angeles
Los Angeles, CA USA
callan@chem.ucla.edu

Alan D Attie
University of Wisconsin
Madison, WI USA
adattie@wisc.edu

Virginie Aubert
Loyola University Chicago
Maywood, IL USA
vmansuyaubert@luc.edu

Zahra Bahrami Nejad
Stanford University
Stanford, CA USA
zbahrami@stanford.edu

Robert C Bauer
University of Pennsylvania
Philadelphia, PA USA
rcbauer@mail.med.upenn.edu

C. Frank Bennett
Ionis Pharmaceuticals
Carlsbad, CA USA
fbennett@ionisph.com

Steven Bensinger
UCLA School of Medicine
Los Angeles, CA USA
sbensinger@mednet.ucla.edu

Jean-Mathieu E Berger
University of Texas Southwestern Medical Center
Dallas, TX USA
jean-mathieu.berger@utsouthwestern.edu

Simon Bissonnette
Universite de Montreal / IRCM
Montreal, QC Canada
simon.bissonnette@umontreal.ca

Robert Blaustein
Merck & Co., Inc.
Rahway, NJ USA
robert_blaustein@merck.com

Karin E Bornfeldt
University of Washington
Seattle, WA USA
bornf@u.washington.edu

Aaron Carlin
University of California, San Diego
La Jolla, CA USA
acarlin@ucsd.edu

Amandine Chaix
Salk Institute
La Jolla, CA USA
achaix@salk.edu

Arthur Charles
University of California, San Francisco
Suasalito, CA USA
macharle@uci.edu

Farid Chehab
University of California, San Francisco
San Francisco, CA USA
chehabf@labmed2.ucsf.edu

Genhong Cheng
University of California, Los Angeles
Los Angeles, CA USA
gcheng@mednet.ucla.edu

Xun (Allison) Chi
University of Iowa
Iowa City, IA USA
xun-chi@uiowa.edu

Anette Christ
UMass Medical School
Worcester, MA USA
Anette.Christ@umassmed.edu

Victor A Cortes
Pontificia Universidad Catolica de Chile
Santiago, NO Chile
vcortesm@gmail.com

Benjamin F Cravatt
Scripps Research Institute
La Jolla, CA USA
cravatt@scripps.edu

Kate Townsend Creasy
University of California, San Francisco
San Francisco, CA USA
kate.creasy@ucsf.edu

Lucile Crozet
Memorial Sloan Kettering cancer center
New York, NY USA
crozetl@mskcc.org

Yannick Cyr
University of Montreal
Montreal, QC Canada
yannick.cyr@ircm.qc.ca

Jason G Cyster
University of California, San Francisco
San Francisco, CA USA
jason.cyster@ucsf.edu

Brandon S Davies
University of Iowa
Iowa City, IA USA
brandon-davies@uiowa.edu

Russell DeBose-Boyd
University of Texas Southwestern Medical Center
Dallas, TX USA
russell.debose-boyd@utsouthwestern.edu

Samir S Deeb
University of Washington
Seattle, WA USA
sdeeb@u.washington.edu

Helén L Dichek
University of Washington
Seattle, WA USA
hdichek@uw.edu

Wieneke Dijk
Wageningen University
Wageningen, NO Netherlands
wieneke.dijk@wur.nl

Bart Duell
Oregon Health and Science University
Portland, OR USA
duellb@ohsu.edu

Nikla Emambokus
Cell Metabolism, Cell Press
Cambridge, MA USA
nemambokus@cell.com

Jeffrey D Esko
University of California, San Diego
La Jolla, CA USA
jesko@ucsd.edu

Peter J Espenshade
Johns Hopkins Univ School of Medicine
Baltimore, MD USA
peter.espenshade@jhmi.edu

Ronald M Evans
The Salk Institute for Biological Studies
La Jolla, CA USA
evans@salk.edu

Longhou Fang
Houston Methodist Research Institute
Houston, TX USA
lhfang@houstonmethodist.org

Brian Finck
Washington University
St. Louis, MO USA
bfinck@dom.wustl.edu

Michael Fitzgerald
Massachusetts General Hospital
Boston, MA USA
mfitzgerald@ccib.mgh.harvard.edu

JoAnne M Foody
Merck & Co., Inc
Rahway, NJ USA
joanne.foody@merck.com

Philip H Frost
University of California, San Francisco
San Francisco, CA USA
philip.frost@ucsf.edu

Frederic Geissmann
Memorial Sloan Kettering Cancer Center
New York, NY USA
geissmaf@mskcc.org

Matthew P Gillum
University of Copenhagen
Copenhagen, NO Denmark
gillum@sund.ku.dk

Christopher K Glass
University of California - San Diego
La Jolla, CA USA
ckg@ucsd.edu

Philip L Gordts
University of California, San Diego
La Jolla, CA USA
pgordts@ucsd.edu

Andrew Greenberg
Tufts University
Boston, MA USA
andrew.greenberg@tufts.edu

Trisha J Grevengoed
University of Copenhagen
Copenhagen, NO Denmark
2grevengoed@sund.ku.dk

Jesper Gromada
Regeneron Pharmaceuticals
Tarrytown, NY USA
jesper.gromada@regeneron.com

Mary E Haas
Boston Children's Hospital, Harvard Medical School
Boston, MA USA
mary.haas@childrens.harvard.edu

Cuiwen He
University of California, Los Angeles
Los Angeles, CA USA
cuiwenhe@chem.ucla.edu

Joerg Heeren
University Medical Center Hamburg Eppendorf
Hamburg, NO Germany
heeren@uke.de

Markus C Heine
University Medical Center Hamburg Eppendorf
Hamburg, NO Germany
ma.heine@uke.de

Cynthia Hong
Pfizer
Boston, MA USA
cynthia.hong@pfizer.com

Jay D Horton
University of Texas Southwestern Medical Center
Dallas, TX USA
jay.horton@utsouthwestern.edu

Andrew Hsieh
Akcea Therapeutics
Cambridge, MA USA
andrew@hsieh.com

Joanne Hsieh
Columbia University
New York, NY USA
jh3265@columbia.edu

Wei Yuan (Wayne) Hsieh
University of Edinburgh
EDINBURGH, NO United Kingdom
wayne_hsieh@hotmail.com

Devon Hunerdosse
Stanford University
Stanford, CA USA
devonh@stanford.edu

Eva Hurt-Camejo
AstraZeneca
Mölndal, NO Sweden
Eva.Hurt-Camejo@astrazeneca.com

Seonghwan Hwang
UT Southwestern Medical Center
Dallas, TX USA
seonghwan.hwang@utsouthwestern.edu

Holly A Ingraham
University of California, San Francisco
San Francisco, CA USA
Holly.ingraham@ucsf.edu

Shun Ishibashi
Jichi Medical University
Shimotsuke, NO Japan
ishibash@jichi.ac.jp

John P Kane
University of California, San Francisco
San Francisco, CA USA
john.kane@ucsf.edu

Sekar Kathiresan
Massachusetts General Hospital
Boston, MA USA
skathiresan@partners.org

Jongsook K Kemper
Univ of Illinois at Urbana - Champaign
Urbana, IL USA
jongsook@illinois.edu

Sander Kersten
Wageningen University
Wageningen, NO Netherlands
sander.kersten@wur.nl

Amit V Khera
Massachusetts General Hospital
Cambridge, MA USA
Avkhera@mgh.harvard.edu

Sumeet A Khetarpal
University of Pennsylvania
Philadelphia, PA USA
sumeetak@mail.med.upenn.edu

Steven Kliewer
University of Texas Southwestern Medical Center
Dallas, TX USA
steven.kliewer@utsouthwestern.edu

Joshua Knowles
Stanford University
Stanford, CA USA
carment@stanford.edu

Masahiro Koseki
Osaka University Graduate School
Suita, NO Japan
kosekey@yahoo.co.jp

Fredric B Kraemer
Stanford University School of Medicine
Stanford, CA USA
fbk@stanford.edu

Albert La Spada
University of California, San Diego
La Jolla, CA USA
alaspada@ucsd.edu

Valerie Lamantia
Institut de recherches cliniques de Montreal
Montreal, QC Canada
valerie.lamantia@ircm.qc.ca

Mikael Larsson
University of California Los Angeles
Los Angeles, CA USA
mikael.larsson@medbio.umu.se

Mitchell A Lazar
University of Pennsylvania
Philadelphia, PA USA
lazar@mail.med.upenn.edu

Stephen D Lee
University of California, Los Angeles
Los Angeles, CA USA
sdlee@mednet.ucla.edu

Youbin Liu
CVRI, UCSF
San Francisco, CA USA
youbin.liu@ucsf.edu

Mary J Malloy
University of California, San Francisco
San Francisco, CA USA
mary.malloy@ucsf.edu

R. Preston Mason
BWH, Harvard Medical School
Beverly, MA USA
rpmason@elucidaresearch.com

Yury Miller
University of California, San Diego
La Jolla, CA USA
yumiller@ucsd.edu

Kathryn J Moore
New York University Medical Center
New York, NY USA
kathryn.moore@nyumc.org

Stephen P O'Rahilly
University of Cambridge
Cambridge, NO United Kingdom
so104@medschl.cam.ac.uk

Eicke Latz
Institute for Innate Immunity, University Hospital
Bonn, Germany
Bonn, NO Germany
eicke.latz@uni-bonn.de

Jiyeon Lee
Washington University in St. Louis
St. Louis, MO USA
nousful@hotmail.com

Richard Lehner
University of Alberta
Edmonton, AB Canada
richard.lehner@ualberta.ca

Aldons Lusis
Univeristy of California, Los Angeles
Los Angeles, CA USA
jlusis@mednet.ucla.edu

Douglas Mashek
University of Minnesota
Minneapolis, MN USA
dmashek@umn.edu

Minal Mehta
University of Pennsylvania
Philadelphia, PA USA
mimehta@mail.med.upenn.edu

Matthew A Mitsche
University of Texas Southwestern Medical Center
Dallas, TN USA
matthew.mitsche@utsouthwestern.edu

Eric J Murphy
University of North Dakota
Grand Forks, ND USA
eric.murphy@med.und.edu

Monika Oberer
University of Graz
Graz, NO Austria
m.oberer@uni-graz.at

Daniel S Ory
Washington University
St. Louis, MO USA
dory@wustl.edu

Satchidananda Panda
Salk Institute for Biological Studies
La Jolla, CA USA
panda@salk.edu

Romeo Papazyan
University of Pennsylvania
Philadelphia, PA USA
romeop@upenn.edu

Antonio Paul
Albany Medical college
Albany, NY USA
paula@mail.amc.edu

Ariane Pessentheiner
Graz University of Technology
Graz, NO Austria
ariane.pessentheiner@tugraz.at

Michael Ploug
Copenhagen University Hospital
Copenhagen, NO Denmark
m-ploug@finsenlab.dk

Jorge Plutzky
Brigham & Women's Hospital
Boston, MA USA
jplutzky@rics.bwh.harvard.edu

Christina M Priest
Univeristy of California, Los Angeles
Los Angeles, CA USA
CPriest@mednet.ucla.edu

Clive R Pullinger
University of California, San Francisco
San Francisco, CA USA
clive.pullinger@ucsf.edu

Xuchu Que
University of California, San Diego
La Jolla, CA USA
xque@ucsd.edu

Peter Reaven
Univ. of Arizona
Phoenix, AZ USA
peter.reaven@va.gov

Gang (Gary) Ren
Lawrence Berkeley National Laboratory
Berkeley, CA USA
gren@lbl.gov

A. Catharine Ross
Pennsylvania State University
University Park, PA USA
acr6@psu.edu

Kerry-Anne Rye
School of Medical Sciences, University of New South Wales
Sydney, NO Australia
karye@ozemail.com.au

David D Sabatini
Whitehead Institute
Cambridge, MA USA
sabatini@wi.mit.edu

Alan Saghatelian
Salk Institute
La Jolla, CA USA
asaghatelian@salk.edu

Tamer Sallam
Univeristy of California, Los Angeles
Los Angeles, CA USA
sallamt@gmail.com

Jean E Schaffer
Washington University School of Medicine
St Louis, MO USA
jschaff@wustl.edu

Renate Schreiber
University of Graz
Graz, NO Austria
renate.schreiber@uni-graz.at

Marc M Schumacher
University of Texas Southwestern Medical Center
Dallas, TX USA
marc.schumacher@utsouthwestern.edu

Walter A Shaw
Avanti Polar Lipids, inc.
Alabaster, AL USA
waltshaw@avantilipids.com

Wen-Jun Shen
Stanford University
Palo Alto, CA USA
wenjun@stanford.edu

Diana M Shih
Univeristy of California, Los Angeles
Los Angeles, CA USA
dshih@mednet.ucla.edu

Carol C Shoulders
William Harvey Research Institute
London, NO United Kingdom
c.shoulders@qmul.ac.uk

Shadab A Siddiqi
College of Medicine, University of Central Florida
Orlando, FL USA
Shadab.siddiqi@ucf.edu

Judith A Simcox
University of Utah
Salt Lake CIty, UT USA
judith.simcox@biochem.utah.edu

Roshni R Singaraja
National University of Singapore
Singapore, NO Singapore
roshni@cmmt.ubc.ca

William L Smith
University of Michigan
Ann Arbor, MI USA
wsmith@asbmb.org

Eveline O Stock
University of California, San Francisco
San Francisco, CA USA
eoestreicher@medicine.ucsf.edu

Alan R Tall
Columbia University Col of C and S
New York, NY USA
art1@columbia.edu

Donald M Tanyanyiwa
WITS Faculty of Health Science/ NHLS
Johannesburg, NO South Africa
donald.tanyanyiwa@wits.ac.za

Pablo J Tapia
Pontificia Universidad Catolica de Chile
Santiago, NO Chile
pjtapia@uc.cl

Elizabeth Tarling
Univeristy of California, Los Angeles
Los Angeles, CA USA
etarling@mednet.ucla.edu

Hagai Tavori
Oregon Health and Science University
Portland, OR USA
tavori@ohsu.edu

Mary Teruel
Stanford University
Stanford, CA USA
teruel1@stanford.edu

David G Thomas
Columbia University
New York, NY USA
dgt2109@columbia.edu

Joanne E Tomassini
Merck & Co., Inc.
N Waler, PA USA
joanne_tomassini@merck.com

Laia Trigueros Motos
A*STAR
Singapore, NO Singapore
Laia_Trigueros_Motos@tlgm.a-star.edu.sg

Thomas Vallim
Univeristy of California, Los Angeles
Los Angeles, CA USA
tvallim@mednet.ucla.edu

Rosemary Walzem
Texas A&M University
College Station, TX USA
rwalzem@poultry.tamu.edu

Ethan J Weiss
University of California, San Francisco
San Francisco, CA USA
ethan.weiss@ucsf.edu

Joseph L Witztum
University of California, San Diego
La Jolla, CA USA
jwitztum@ucsd.edu

Mike X Xu
University of Miami
Miami, FL USA
xxu2@med.miami.edu

Yu-Xin Xu
Massachusetts General Hospital
Boston, MA USA
YXU17@PARTNERS.ORG

Calvin Yeang
University of California, San Diego
San Diego, CA USA
cyeang@ucsd.edu

Autumn G York
Univeristy of California, Los Angeles
Los Angeles, CA USA
AGYork@gmail.com

Stephen G Young
Univeristy of California, Los Angeles
Los Angeles, CA USA
sgyoung@mednet.ucla.edu

Jessica Yue
University of Alberta
Edmonton, AB Canada
jessica.yue@ualberta.ca

Rudolf Zechner
University of Graz
Graz, NO Austria
rudolf.zechner@uni-graz.at

Quan Zhou
Univeristy of California, Los Angeles
Los Angeles, CA USA
zhouquanzq@gmail.com

Jun Zhu
University of California, San Francisco
San Francisco, CA USA
jun.zhu@ucsf.edu

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes

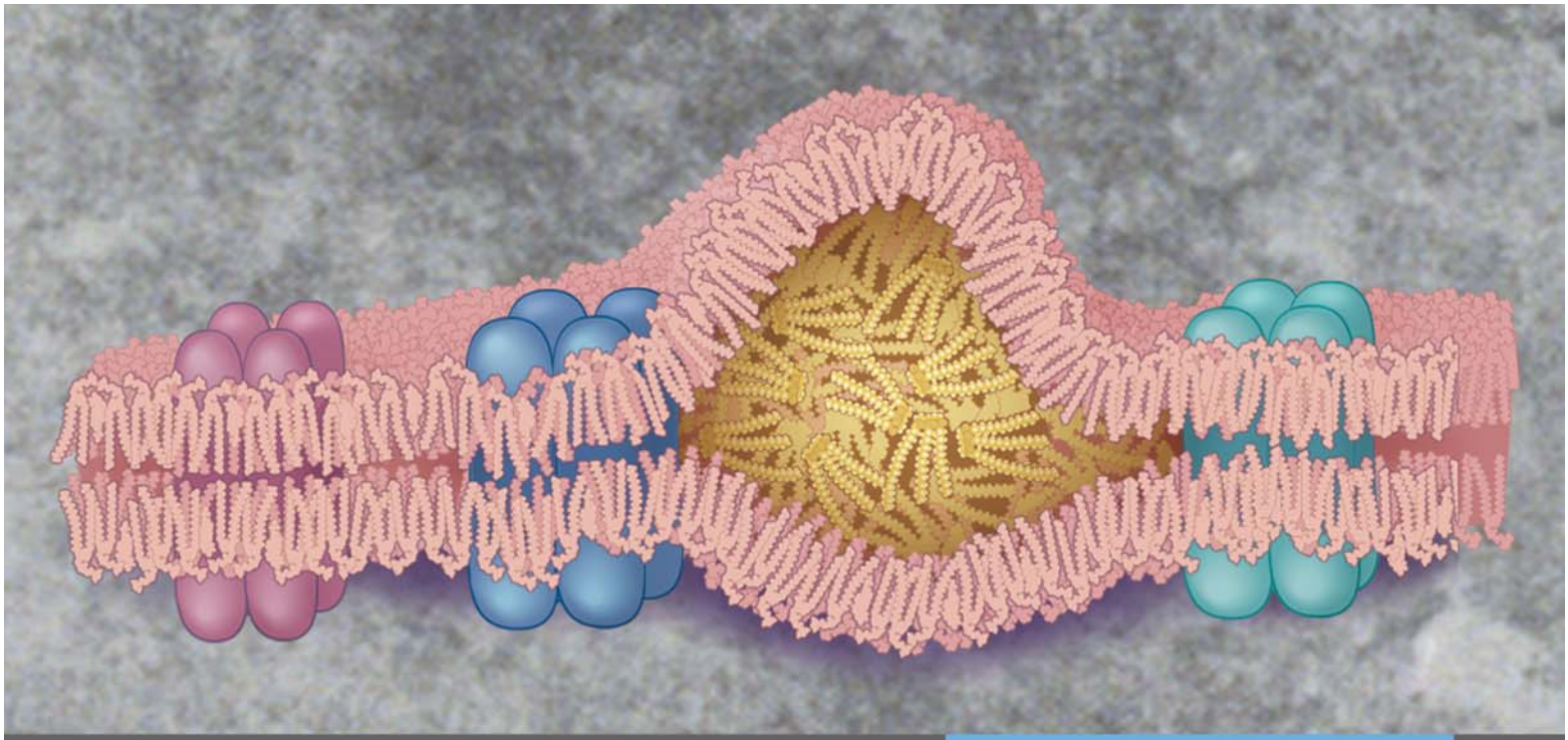
Notes

Notes

Notes

Notes

Notes



www.jlr.org

Journal of LIPID RESEARCH

Special Collections:
Thematic Review Series

- Glycosylphosphatidylinositol anchors: Biochemistry and cell biology
- Phospholipases: Central role in lipid signaling and disease
- Intestinal lipid metabolism
- Calorie restriction and ketogenic diets
- Recent advances in the treatment of lysosomal storage diseases
- Lysophospholipids and their receptors
- Structure and function of high density lipoprotein

*Visit **www.jlr.org/site/collections** to learn more.*

ASBMB
American Society for Biochemistry and Molecular Biology

DEUEL 2017

March 7 — 10, 2017

InterContinental
The Clement Monterey
Monterey, Calif.

Conference on Lipids
DEUEL