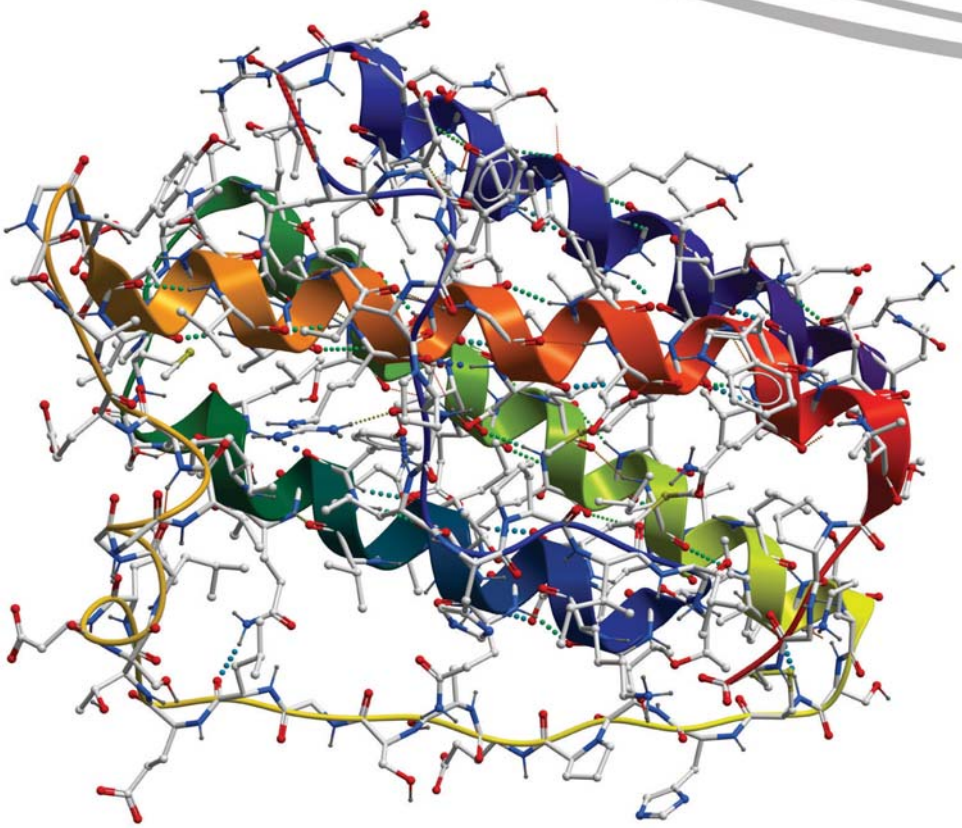
March 4–7, 2014

Coronado, CA

DEUEL CONFERENCE ON LIPIDS



Conference on Lipids DEUEL

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2014 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.

We Invite You to Join the

ASBMB Lipid Research Division

Exciting Research News

Forum Discussions

Lipid Events Calendar

www.asbmb.org/lipidcorner

The Havel Lecture

The Havel Lecture was named after Dr. Richard J. Havel because he has done more than anyone else to keep the Deuel Conference going. Richard J. Havel is known by many as "Mr. Lipoprotein, USA." He, more than any other investigator, unraveled 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. His manuscript on the ultracentrifulgal separation of lipoproteins is one of the most frequently cited papers, rivaling Lowry's paper on protein measurement. Richard Havel has published over 300 manuscripts. Their quality is attested to by 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.

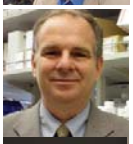
2014 - Rudolf Zechner, University of Graz
"Lipolysis - more than just the breakdown of fat"

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

2012 Gokhan Hotamisligil, Harvard University
"Inflammation, Endoplasmic Reticulum Stress and Lipids: Emerging Networks Regulating Metabolism"

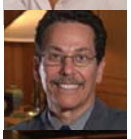
2011 Christopher K. Glass, University of California, San Diego
"Oxysterol regulation of macrophage gene expression"

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



2009 Stephen G. Young, UCLA
"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 for Biological Sciences
"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, HHMI/UTSW Medical Center
"Structure of the LDL receptor"

2004 Jeffrey M. Friedman, Rockefeller University
"The Function of Leptin in Nutrition, Weight and Physiology"

2003 Bruce Spiegelman, Harvard Medical School
"Transcriptional Control of Energy and Glucose Metabolism"

2002 Co-Lecturers Michael S. Brown & Joseph L. Goldstein, University of Texas Southwestern
"SREBPs: Master Regulators of Lipid Metabolism"

Schedule of Events

<table>
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7-8:30</td><td rowspan="2">Breakfast
7-8:30</td><td rowspan="2">Board Meeting
7-8:30</td><td rowspan="2">Breakfast
7-8:30</td></tr>
<tr><td>8 AM</td></tr>
<tr><td>9 AM</td><td rowspan="2">Session 1
8:45-10:30</td><td colspan="2" rowspan="2">Session 2
8:45-10:30</td><td rowspan="2">Session 4
8:45-10:30</td></tr>
<tr><td>10 AM</td></tr>
<tr><td></td><td></td><td>Coffee Break
10:30</td><td colspan="2">Coffee Break
10:30</td><td>Coffee Break
10:30</td></tr>
<tr><td>11 AM</td><td rowspan="4"></td><td rowspan="2">Session 1 continued
10:45-12:00</td><td colspan="2" rowspan="2">Session 2 continued
10:45-12:00</td><td rowspan="2">Session 4 continued
10:45-11:20</td></tr>
<tr><td>12 PM</td></tr>
<tr><td>1 PM</td><td rowspan="5">Free Time</td><td colspan="2" rowspan="2">Free Time</td><td rowspan="8"></td></tr>
<tr><td>2PM</td></tr>
<tr><td>3 PM</td><td rowspan="3">Registration
3–6:30</td></tr>
<tr><td>4 PM</td><td rowspan="2">Sponsor Reception
(by invitation only)
5-6:00</td></tr>
<tr><td>5 PM</td></tr>
<tr><td>6 PM</td><td rowspan="4">Opening Reception and Dinner
6:30</td><td rowspan="2">Dinner
6:00</td><td colspan="2" rowspan="2">Dinner
6:00</td></tr>
<tr><td>7 PM</td></tr>
<tr><td>8 PM</td><td rowspan="3">Havel Lecture, Wine Reception & Poster Session</td><td colspan="2" rowspan="3">Session 3
7:30-10:30</td></tr>
<tr><td>9 PM</td></tr>
<tr><td>10 PM</td></tr>
</table>

Meeting Program

The Deuel Conference on Lipids, March 4–7, 2014
Coronado Island Marriott Resort & Spa, Coronado, California

"Obesity, diabetes and atherosclerosis"

Tuesday, March 4

3:00 – 6:30 pm Registration
6:30 – 10:00 pm Opening Reception and Dinner

Wednesday, March 5

Wednesday, March 5, 8:45 AM – 12:00 Noon
Session Chairs: Karin Bornfeldt, Alan Tall

Session 1	**Obesity/Diabetes**
8:45 – 9:20	"PPAR-Gamma-FGF1 signaling from feast to famine"
	Ron Evans, Salk Institute
9:20 -9:55	"Outfoxing insulin resistance and atherosclerosis"
	Domenico Accili, Columbia University
9:55 -10:30	Introduction: Stephen Young, UCLA
	The Journal of Lipid Research Lecture: "Human obesity and insulin resistance: lessons from genetics"
	Stephen O'Rahilly, Cambridge University
10:30 - 10:45	Coffee Break
10:45 -11:05	"Regulation of metabolic flux by FoxOs during the fasting-refeeding transition"
	Rebecca A. Haeusler, Columbia University
11:05-11:25	Hepatocyte toll-like receptor 4 regulates obesity-induced inflammation and insulin resistance"
	Lin Jia, University of Texas Southwestern Medical Center
11:25 – 12:00	"mTOR signaling in metabolism and cancer"
	Brendan Manning, Harvard Medical School

Wednesday, March 5, 7:30 – 10:00 PM
Session Chair: Steve Kliewer

7:30-8:30	**The Havel Lecture:** "Lipolysis – more than just the breakdown of fat"
	Rudolf Zechner, University of Graz
8:30-10:00	Wine Reception and Poster Session

Thursday, March 6

Thursday, March 6, 8:45 AM to 12:00 Noon
Session Chairs: David Mangelsdorf, Jean Schaffer

Session 2	**Triglycerides/Droplets/Regeneration**
8:45 -9:20	**The ELife Lecture:** "Characterization of post-transcriptional regulators of lipogenesis"
	Jay Horton, University of Texas Southwestern Medical Center
9:20 -9:55	"Phases of fat cell biology of neutral lipid storage"
	Tobias Walther, Yale University
9:55-10:30	"New insights on triglyceride hydrolysis in capillaries"
	Stephen Young, UCLA
10:30- 10:45	Coffee Break

10:45 –11:05 "Fatty acid-inducible myokine ANGPTL4 governs lipid metabolic response to exercise"
Milene Catoire, Wageningen University

11:05-11:25 "Angptl4 is a regulator of lipid uptake in brown and white adipose tissue during cold exposure"
Wieneke Dijk, Wageningen University

11:25-12:00 "Lipins, lipid intermediates, and diverse roles in metabolism"
Karen Reue, UCLA

Thursday, March 6, 7:30-10:30 PM

Session Chairs: Jay Horton, Murielle Véniant

Session 3 Nuclear Receptors/Cellular Stress

7:30 – 8:05 "Metabolic actions of FGF21"
David Mangelsdorf, University of Texas Southwestern Medical Center

8:05-8:40 "Mitochondrial oxidative stress and fission in atherosclerosis"
Ira Tabas, Columbia University

8:40- 9:15 "Integration of lipid metabolism and inflammation by LXRs"
Peter Tontonoz, UCLA

9:15-9:35 "TTC39B deficiency stabilizes LXR and protects from hepatic steatosis"
Joanne Hsieh, Columbia University

9:35-9:55 "Antisense inhibition of angiopoietin-like protein 3 reduces atherosclerosis and hepatic steatosis in dyslipidemic mouse models"
Richard G. Lee, Isis Pharmaceuticals

9:55-10:30 "Small nucleolar RNAs as regulators of metabolic stress"
Jean Schaffer, Washington University in St. Louis

Friday, March 7

Friday, March 7, 8:45 to 11:20 AM

Session Chairs: Karen Reue, Steve Kliewer

Session 4 New Therapies Based On Genetics and Cell Biology

8:45 -9:20 "Regenerative and restorative functions of Type 2 immunity"
Ajay Chawla, UCSF

9:20 -9:55 "Lessons from studies in population isolates"
Alan Shuldiner, University of Maryland

9:55-10:30 "New therapeutic targets for dyslipidemia and atherosclerosis revealed through human genetics"
Daniel Rader, University of Pennsylvania

10:30- 10:45 Coffee Break

10:45 – 11:20 "Therapeutic actions of FGF21"
Murielle Véniant, Amgen

Poster Presentations

1

Structure and Dynamics of Nascent Discoidal HDL

Lei Zhang[1], Gang Ren[1]

[1]Lawrence Berkeley National Laboratory, Molecular Foundry, 1 Cyclotron Road, MS 67R2206, Berkeley, CA

During reverse cholesterol transport (RCT), high density lipoprotein (HDL) conveys excess cholesterol from peripheral tissues to the liver and steroidogenic organs. It is through HDL's mediation of RCT that a majority of HDL's ability to reverse or prevent the onset of cardiovascular disease is derived. As HDL proceeds through RCT, it forms a variety of subclasses, dependent on the HDL particle's lipid cargo status. To drive the process of RCT, each subclass of HDL has a unique set of biological properties (receptor affinity, enzyme activation capabilities, etc.), most of which are derived from apolipoprotein A-I (apoA-I), the main protein constituent of HDL. Despite many years of investigation into the structure of apoA-I, very little is known of apoA-I's HDL subclass-specific structure and the role of apoA-I structure in HDL subclass-specific properties due to the high dynamics of apoA-I in HDL. Here, we use our developed novel technique, individual particle electron tomography, to examine the structure of each individual particle of reconstituted nascent discoidal HDL subclasses via tilted viewing and three-dimensional reconstruction. Comparison of the structures from different individual particles demonstrates the dynamics of nascent discoidal HDL.

2

The Interaction of Perlecan Core Protein with LDL Is Mediated by Its LDLR-like Domain II

Yu-Xin Xu[1], David Ashline[2], Li Liu[3], Katya Ravid[4], Matthew Layne[5], Vernon Reinhold[2], Sekar Kathiresan[1]

[1]Center for Human Genetic Research/Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA; [2]Glycomics Center, Department of Chemistry, University of New Hampshire, Durham, NH; [3]Department of Molecular and Cell Biology, Boston University Goldman School of Dental Medicine, Boston, MA; [4]Department of Medicine, Boston University School of Medicine, Boston, MA; [5]Department of Biochemistry, Boston University School of Medicine, Boston, MA

Perlecan is the major proteoglycan in the arterial wall. Various studies have linked it to atherosclerosis. Perlecan is highly expressed in atherogenic lesions, and its expression correlates with the lesion progression in mice. It is widely believed that the retention of atherogenic lipoproteins by the proteoglycan is an early step in the development of atherosclerosis, and the retention is largely based on the ionic interaction between the basic amino acids of apolipoprotein B-100 (apoB-100) and the negatively charged heparan sulfate (HS). Perlecan contains a core protein and three HS side chains. Its core protein has five domains (I–V) with disparate structures, and domain II is highly homologous to the ligand-binding portion of low density lipoprotein receptor (LDLR), but its binding activity has never been investigated. Here we show that the domain is functional in LDL binding. Surprisingly, the binding is largely mediated by O-linked glycans that are only present in the secreted domain II. Among the five repeat units of domain II, most of the glycosylation comes from the second unit, which is highly deviated and rich in serine/threonine but contains no cysteine residues. Interestingly, most of the glycans contain the terminal sialic acid that is well known for its negative charge, and we have provided evidence that the sialic acid moiety is critical for the LDL binding. We also demonstrate an additive effect of HS and domain II on binding with LDL/apoB-100. Unlike LDLR, which directs the LDL uptake through endocytosis, the data reveal a distinct feature of the LDLR-like domain II, a receptor-mediated lipoprotein retention in a glycosylation-dependent manner. Our study strongly indicates that perlecan and its sialic acid-containing glycosylation are highly associated with the development of human atherosclerosis. Thus, the arterial perlecan glycosylation may provide an attractive therapeutic target for early prevention of atherosclerosis.

3

A New Lysosome-mediated Degradation Mechanism for Hepatic Lipid Droplet Turnover

Zemin Yao[1, 2], Mohsen Amir Alipour[1, 2]

[1]Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada; [2]Ottawa Institute of Systems Biology, University of Ottawa, Ottawa, ON, Canada

Dietary supplementation with fish oil, rich in n-3 fatty acids, such as EPA (20:5n-3), has been shown in many studies to exert a hypotriglyceridemic effect by reducing both plasma triglycerides (in the form of VLDL) and intrahepatic triglycerides (presented in cytoplasm as lipid droplets). To date, no cellular or molecular mechanisms have been defined to explain the hypotriglyceridemic action of n-3 fatty acids. Intrahepatocellular triglycerides are an important contributor to plasma fatty acid and lipoprotein level. We have discovered a direct interaction between lysosome and lipid droplets, in a "kiss-and-run" fashion, as the principal mechanism for the lipid droplets turnover upon EPA treatment. Inactivating lysosome function or disrupting lysosome movement resulted in accumulation of lipid droplets under EPA treatment conditions, whereas inactivating the known lipolysis enzyme ATGL or HSL was unable to attenuate droplet turnover. Lipophagy has been reported as a selective autophagic mechanism for degradation of lipid droplets under starvation conditions. However, knockdown of autophagy proteins did not prevent droplet turnover induced by EPA. On the other hand, silencing lysosome-associated GTPase Rab7 or the Rab7 effectors, such as FYCO1 and RILP, entirely blocked EPA-induced droplet turnover. The current study thus unveiled a new lysosome-mediated lipid degradation mechanism that is responsible for the hypotriglyceridemia action of n-3 fatty acids. The therapeutic potential for the treatment of non-alcoholic hepatosteatosis using n-3 fatty acids will be discussed.

4

The Interplay of Protein Kinase A and Perilipin 5 Regulates Cardiac Lipid Droplet Triacylglycerol Mobilization

Nina M. Pollak[1], Doris Jaeger[1], Stephanie Kolleritsch[1], Thomas Rülicke[2, 3], Achim Lass[1], Rudolf Zechner[1], Guenter Haemmerle[1]

[1]Institute of Molecular Biosciences, University of Graz, Graz, Austria; [2]Biomodels Austria, University of Veterinary Medicine, Vienna, Austria; [3]Institute of Laboratory Animal Science, University of Veterinary Medicine, Vienna, Austria

Cardiac triacylglycerol (TG) catabolism critically depends on the TG hydrolytic activity of adipose triglyceride lipase (ATGL). ATGL deficiency in mice provokes severe cardiac steatosis and heart dysfunction. Similarly, cardiac perilipin 5 (Plin5) overexpression leads to severe TG accumulation in the heart, involving impaired ATGL- and hormone-sensitive lipase (HSL)-mediated lipid droplet (LD) TG mobilization. Interestingly, the cardiac phenotype of Plin5 transgenic mice is very moderate and does not significantly impact life span compared with the early and lethal cardiac phenotype of ATGL-deficient mice. These findings suggested that cardiac Plin5 overexpression does not completely and persistently block cardiac TG catabolism. Notably, Plin5 protein expression levels were very similar in cardiac muscle of non-fasted and fasted mice, suggesting posttranslational mechanisms that facilitate TG breakdown of Plin5-enriched LDs. The established role of protein kinase A (PKA) in stimulating lipolysis prompted us to examine the impact of PKA on TG catabolism of Plin5-enriched LDs. Plin5-coated LDs were resistant toward ATGL- and HSL-mediated TG catabolism, whereas the presence of PKA significantly increased TG mobilization. In accordance, PKA phosphorylation was markedly increased in cardiac lysates of Plin5 transgenic mice. Additionally, fasting provoked a moderate but significant reduction in cardiac TG levels of Plin5 transgenic mice, suggesting that Plin5-enriched LDs are accessible to TG catabolism in response to increased energy demands. Nonetheless, the reduction in cardiac TG levels was accompanied by increased cardiac glucose utilization, demonstrating that cardiac lipolysis is not completely recovered upon fasting. In addition to our previous findings, these data emphasize the essential role of Plin5 in the control of cardiac lipid homeostasis.

5

Role of the Endocytic Adaptor Disabled-2 in Adipogenesis

Wensi Tao[1, 2, 3], Robert Moore[1, 3], Toni Yeasky[1, 3], Xiang-Xi Xu[1, 3]

[1]Miller School of Medicine, University of Miami, Miami, FL; [2]Cell and Developmental Biology Graduate Program, University of Miami, Miami, FL; [3]Department of Cell Biology, University of Miami, Miami, FL

Disabled-2 (Dab2) is a widely expressed endocytic adaptor protein that mediates the endocytosis of transmembrane receptors, including members of the LDL receptor family proteins and several integrin isoforms. In cell culture studies, it is thought that Dab2 plays roles in endocytic uptake and recycling and modulation of signal transduction pathways through its endocytosis function. We produced conditional Dab2 knockout mice by using Sox2-cre to delete the flox dab2 allele. Both copies of the dab2 gene were efficiently deleted in the embryo proper but not in the extraembryonic endoderm. The mice bypass the early embryonic lethal phenotype found in constitutive knockout and showed no obvious developmental phenotypes. However, we found that the Dab2-deficient mice are lean and resistant to high caloric diet-induced obesity. The impact of Dab2 was only found in young mice; in older mice; wild type and Dab2-deficient mice showed no significant difference in weight gain after feeding a high caloric diet. In Dab2-deficient mice, the cell size of adipocytes was enlarged to the same extent as in wild type; rather, the number of adipocytes was reduced. In an in vitro assay, the Dab2-deficient fibroblasts and mesenchymal stromal cells have a reduced ability to differentiate into adipocytes. The mechanism appears related to the regulation of Erk activity by Dab2 and subsequently impacts the phosphorylation and nuclear entry of PPAR-γ. The results suggest that Dab2 is required for the excessive calorie-induced differentiation of an adipocyte progenitor cell population, which is depleted in older animals.

6

Raising HDL with CETP Inhibitor Torcetrapib Improves Glucose Homeostasis in Dyslipidemic and Insulin-resistant Hamsters

François Briand[1], Bénédicte Prunet-Marcassus[1], Quentin Thieblemont[1], Clément Costard[1], Elodie Muzotte[1], Sylvie Sordello[1], Thierry Sulpice[1]

[1]Physiogenex SAS, Labège, France

Recent studies suggest that high density lipoprotein (HDL) particles have the potential to modulate glucose metabolism and inhibit adipocyte lipolysis. Here we investigated whether raising HDL cholesterol levels with cholesteryl ester transfer protein (CETP) inhibition improves insulin resistance in a hamster model. Hamsters were made dyslipidemic and insulin-resistant with a 2-week high fat/fructose-enriched diet and were then treated daily with vehicle or CETP inhibitor torcetrapib, 30 mg/kg/day (TOR) for 10 days. Compared with vehicle, TOR significantly increased HDL cholesterol levels by 38%, with higher apolipoprotein A-I and apolipoprotein E levels in HDL particles. As well, TOR significantly increased 3H-tracer appearance by ~40% in HDL after [3H]cholesterol-labeled macrophage i.p. injection.

TOR significantly reduced fasting plasma triglyceride, free glycerol, and free fatty acid levels by 65, 31, and 23%, respectively. TOR also reduced blood glucose levels and plasma insulin by 20 and 49%, respectively, which led to a 60% reduction in HOMA-IR index (all $p < 0.01$). After 2-[3H]deoxyglucose and insulin injection, TOR significantly increased glucose uptake in oxidative soleus muscle, liver, and heart by 26, 33, and 70%, respectively. We next tested whether the effects of torcetrapib were also related to higher HDL apolipoprotein A-I levels and subsequent AMP-activated protein kinase (AMPK) activation. HDL particles from both treatment groups were incubated ex vivo with soleus muscles dissected from insulin-resistant hamsters. Compared with basal conditions, HDL derived from torcetrapib-treated hamsters significantly increased AMPK phosphorylation by 1.5-fold in soleus muscle, whereas HDL derived from vehicle-treated hamsters showed no effect. In conclusion, raising HDL levels with the CETP inhibitor torcetrapib improves glucose homeostasis in dyslipidemic and insulin-resistant hamsters. These results remain to be confirmed with other CETP inhibitors.

7

Defining the Relationships between GPIHBP1, ANGPTL4, and LPL Function

Xun Chi[1], Emily Malcolm[1], Hannah Shows[1], Alex Hjelmaas[1], Matthew Potthoff[2, 3], Brandon Davies[1, 3]

[1]Department of Biochemistry, University of Iowa, Iowa City, IA; [2]Department of Pharmacology, University of Iowa, Iowa City, IA; [3]Fraternal Order of Eagles Diabetes Research Center, Iowa City, IA

Triglycerides derived from diet are packaged into lipoproteins for delivery to peripheral tissues. The hydrolysis of lipoprotein triglycerides in the capillaries of heart, skeletal muscle, and adipose tissue liberates fatty acids for tissue uptake and is critically dependent on the enzymatic activity of lipoprotein lipase (LPL). LPL is synthesized by parenchymal cells (e.g. adipocytes and cardiomyocytes) but only becomes functional in processing plasma triglycerides when it has been transported across endothelial cells into capillaries. GPIHBP1 (GPI-anchored HDL-binding protein 1) was recently identified as the protein that binds LPL and transports it across endothelial cells. Like GPIHBP1, ANGPTL4 (angiopoietin-like 4) interacts with LPL extracellularly. ANGPTL4 inhibits LPL activity, but where and when ANGPTL4 acts on LPL physiologically remains unclear. Like LPL, ANGPTL4 is secreted from parenchymal cells and thus probably interacts with LPL in the interstitial space, but how ANGPTL4 interacts with GPIHBP1-LPL complexes and its role in controlling levels of functional LPL are yet to be determined. Interestingly, although GPIHBP1 and ANGPTL4 appear to have somewhat opposing functions, under some conditions (such as fasting), expression of both genes increases. Our goal is to define the relationships between GPIHBP1, ANGPTL4, and functional LPL. We have found that LPL inactivated by ANGPTL4 loses its affinity for GPIHBP1 and that active LPL bound to GPIHBP1 remains susceptible to ANGPTL4 inactivation. We have also found that although fasting increases both Angptl4 and Gpihbp1 expression, the timings of these increases are very different.

8

Central Role for MafG in the Negative Feedback Regulation of Bile Acid Synthesis

Thomas A. Vallim[1], Hannah Ahn[1], Lee Hagey[2], Hozumi Motohashi[3], Elizabeth J. Tarling[1], Masayuko Yamamoto[3], Peter A. Edwards[1]

[1]UCLA, Los Angeles, CA; [2]University of California, San Diego, La Jolla, CA; [3]Tohoku University, Sendai, Japan

Cholesterol catabolism via bile acid synthesis represents the major route of cholesterol excretion from the body. Bile acid synthesis is tightly regulated by complex negative feedback loops that together maintain bile acid homeostasis. Here we identify *MafG* as a critical regulator of bile acid metabolism. We demonstrate that *MafG* is transcriptionally controlled by the nuclear receptor *Fxr*, a major regulator of the enterohepatic circulation of bile acids. Hepatic overexpression of *MafG* in mice or in human HepG2 cells leads to the repression of *Cyp8b1*, the enzyme required for the synthesis of the major bile acid species cholic acid. Consistent with repression of *Cyp8b1*, hepatic overexpression of *MafG* also decreased cholic acid levels, whereas *MafG*$^{-/+}$ mice have increased cholic acid levels. ChIP-Seq studies and hepatic gene expression profiling reveal that *MafG* regulates several additional genes involved in maintaining bile acid homeostasis. The identification of the *Fxr-MafG* pathway as a modulator of cholic acid levels and bile acid metabolism may have implications for the treatment of a number of metabolic diseases, including cardiovascular disease, diabetes, and obesity.

9

LXRs Regulate ER Stress and Inflammation through Dynamic Modulation of Membrane Phospholipid Composition

Xin Rong[1, 2], Carolyn J. Albert[3], Cynthia Hong[1, 2], Mark A. Duerr[3], Brian T. Chamberlain[4, 5], Elizabeth J. Tarling[6], Ayaka Ito[1, 2], Jie Gao[1, 2], Bo Wang[1, 2], Peter A. Edwards[6, 7], Michael E. Jung[4, 5], David A. Ford[3], Peter Tontonoz[1, 2]

[1]Howard Hughes Medical Institute, UCLA, Los Angeles, CA; [2]Department of Pathology and Laboratory Medicine, UCLA, Los Angeles, CA; [3]Department of Biochemistry and Molecular Biology, Saint Louis University, St. Louis, MO; [4]California NanoSystems Institute, UCLA, Los Angeles, CA; [5]Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA; [6]Division of Cardiology, Department of Medicine, UCLA, Los Angeles, CA; [7]Department of Biological Chemistry, UCLA, Los Angeles, CA

The fatty acyl composition of phospholipids determines the biophysical character of membranes and impacts the function of membrane proteins. Here, we define a nuclear receptor pathway for the dynamic modulation of membrane composition in response to changes in cellular lipid metabolism. Ligand activation of liver X receptors (LXRs) preferentially drives the incorporation of polyunsaturated fatty acids into phospholipids through induction of the remodeling enzyme Lpcat3. Promotion of Lpcat3 activity ameliorates endoplasmic reticulum (ER) stress induced by saturated free fatty acids *in vitro* or by hepatic lipid accumulation *in vivo*. Conversely, Lpcat3 knockdown in liver exacerbates ER stress and inflammation. Mechanistically, Lpcat3 modulates inflammation both by regulating inflammatory kinase activation through changes in membrane composition and by affecting substrate availability for inflammatory mediator production. These results outline an endogenous mechanism for the preservation of membrane homeostasis during lipid stress and identify Lpcat3 as an important mediator of LXR effects on metabolism.

10

Fatty Acid-inducible Myokine ANGPTL4 Governs Lipid Metabolic Response to Exercise

Milene Catoire[1], Sheril Alex[1], Eric Kalkhoven[3], Patrick Schrauwen[2], Sander Kersten[1]

[1]Wageningen University, Wageningen, The Netherlands; [2]Maastricht University, Maastricht, The Netherlands; [3]Utrecht University Medical Center, Utrecht, The Netherlands

Physical activity increases energy metabolism in exercising muscle. Whether acute exercise elicits metabolic changes in non-exercising muscles remains unclear. Here we show that one of the very few genes that during acute exercise is more highly induced in non-exercising muscle compared with exercising human muscle encodes angiopoietin-like 4 (ANGPTL4), an inhibitor of lipoprotein lipase-mediated plasma triglyceride clearance. Using a combination of human, animal, and *in vitro* data, we show that induction of ANGPTL4 in non-exercising muscle is mediated by elevated plasma free fatty acids via PPARδ, leading to reduced local uptake of plasma triglyceride-derived fatty acids and their sparing for use by exercising muscle. In contrast, in exercising muscle, induction of ANGPTL4 is counteracted probably via AMPK-mediated down-regulation of ANGPTL4, promoting use of plasma triglycerides as fuel for active muscles. Our data suggest a key role of non-exercising muscle and local regulation of ANGPTL4 via AMPK and FFA in governing lipid homeostasis during exercise.

11

A Peptide Derived from G_0/G_1 Switch Gene 2 Acts as Non-competitive Inhibitor of Adipose Triglyceride Lipase

Ines K. Cerk[1], Barbara Salzburger[1], Andras Böszörmenyi[1], Christoph J. Pillip[1], Christoph Heier[1], Matthias Romauch[1], Martina Schweiger[1], Achim Lass[1], Robert Zimmermann[1], Rudolf Zechner[1], Monika Oberer[1]

[1]Institute of Molecular Biosciences, University of Graz, Austria

Adipose triglyceride lipase (ATGL) is the rate-limiting enzyme for the release of free fatty acids (FA) from triglycerides (TG) stored in lipid droplets during lipolysis. The mobiliziation of stored energy requires regulatory and control mechanism at multiple levels (*e.g.* via hormones and post-translational modifications). On a protein level, ATGL activity is enhanced by interaction with the protein comparative gene identification 58 (CGI-58), and inhibited by interaction with G_0/G_1 switch gene 2 (G0S2). Until now, very little is known about the mode of activation and inhibition by the 351-amino acid protein CGI-58 and the small 103-amino acid protein G0S2, respectively. Both proteins interact with the N-terminal half of ATGL. G0S2 is predicted to be mostly α-helical, yet experimental structural knowledge is not available. G0S2 dose-dependently binds and inhibits ATGL even in the presence of ATGL's activator CGI-58, indicating a non-competitive mechanism. In this work, we characterize the interaction of ATGL with G0S2 and identify a short peptide sufficient for ATGL inhibition. We truncated G0S2 and tested the resulting variants for their inhibiting ATGL activity. C-terminal truncations until Met^{43} and N-terminal truncations until Tyr^{27} maintained the ability to inhibit ATGL. Interestingly, when introducing C- and N-terminal truncations, slightly longer variants were required for full ATGL inhibition. For detailed kinetic characterization of the ATGL inhibition by peptides derived from G0S2, we used a synthetic peptide identical to the human G0S2 sequence from Lys^{20} to Ala^{52}. Notably, this peptide is fully capable of inhibiting ATGL, eliminating the possibility of any post-translational modification required for the interaction. The short peptide is active in the nanomolar range and inhibits ATGL independently of the presence of CGI-58. Kinetic analysis revealed the peptide to inhibit ATGL in a non-competitive mode. Moreover, the inhibitory peptide is specific for ATGL and does not affect the activity of other intracellular lipases, including HSL and all proteins of the PNPLA family. Consequently, identification of this synthetic peptide sequence opens up new avenues for the development of lipolytic inhibitors for basic research and pharmacological applications.

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TTC39B Deficiency Stabilizes LXR and Protects from Hepatic Steatosis

Joanne Hsieh[1], Masahiro Koseki[1, 2], Matthew M. Molusky[1], Emi Yakushiji[1], Jahangir Iqbal[3], Daniel J. Rader[4], Mahmood M. Hussain[3], Alan R. Tall[1]

[1]Department of Medicine, Columbia University, New York, NY; [2]Graduate School of Medicine, Osaka University, Suita, Japan; [3]Department of Cell Biology, SUNY Downstate Medical Center, Brooklyn, NY; [4]University of Pennsylvania School of Medicine, Philadelphia, PA

Ttc39b (T39) is a novel locus identified in human GWAS to be associated with HDL and total cholesterol levels. Nothing is known about the function of T39 except that the presence of a functional tetratricopeptide repeat domain implicates it as a scaffolding protein. We aimed to understand the function of T39 by characterizing T39 KO mice. T39 KO mice had modestly higher HDL cholesterol on both chow and high cholesterol diets, associated with increased intestinal *Abca1* mRNA expression. In T39-deficient intestine, there was increased LXR protein that was not accompanied by a change in its mRNA. Livers from mice fed a chow diet did not show any differences between WT and KO. However, when fed a high fat/high cholesterol diet, T39 KO mice exhibited up-regulation of LXR targets *Abcg5*, *Scd1*, and *Srebf1* along with preservation of LXRα protein in the liver. Surprisingly, the gene expression profile of LXR activation was accompanied by decreased hepatic lipid accumulation that was not associated with changes in glucose tolerance. Despite the increase in hepatic *Srebf1* mRNA, *de novo* lipogenesis was not induced due to a remarkable inhibition of SREBP-1 processing, possibly attributable to a marked increase in *Insig2a* transcription. T39 KO mice also had reduced dietary cholesterol absorption and a delay in dietary TG uptake. In an overexpression system, T39 was shown to associate with LXRα, and in primary *T39*$^{-/-}$ hepatocytes, LXRα was less ubiquitinated. We therefore postulate that T39 is involved in mediating the polyubiquitination of LXRα that signals for its proteasomal degradation. T39 deficiency results in the posttranslational stabilization of LXRα and thus enhances the cellular response to endogenous sterol LXR ligands, which are elevated on a high cholesterol diet and not as lipogenic as synthetic agonists. Inhibiting T39 can therefore be a therapeutic approach to harness the beneficial cholesterol efflux and anti-atherogenic effects of LXR while avoiding, and possibly treating, steatohepatitis.

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Angptl4 Is a Regulator of Lipid Uptake in Brown and White Adipose Tissue during Cold Exposure

Wieneke Dijk[1], Mariette Boon[2], Laurent Vergnes[3], Jorg Heeren[4], Patrick Rensen[2], Gunilla Olivecrona[5], Sander Kersten[1]

[1]Nutrition, Metabolism and Genomics, Human Nutrition, Wageningen University, Wageningen, The Netherlands; [2]Endocrinology and Metabolic Diseases, Leiden University Medical Center, Leiden, The Netherlands; [3]Department of Human Genetics, UCLA, Los Angeles, CA; [4]Department of Biochemistry and Molecular Cell Biology, UMC Hamburg-Eppendorf, Hamburg, Germany; [5]Department of Medical Biosciences, Physiological Chemistry, Umeå University, Umeå, Sweden

Angiopoietin-like factor 4 (Angptl4) is a secreted protein that inhibits lipoprotein lipase (LPL) activity in various tissues, including white adipose tissue (WAT) and muscle. In mice, Angptl4 is highly expressed in brown adipose tissue (BAT), the tissue responsible for non-shivering thermogenesis. BAT has recently emerged as a new target in the treatment of obesity. The function of Angptl4 in BAT is, however, unknown. To examine the function of Angptl4 in BAT, Angptl4 knock-out, wild-type, and transgenic mice were placed at 4|°C for 10 days or at thermo-neutrality. Upon cold exposure, LPL activity and triglyceride (TG) uptake were dramatically increased in BAT. Increased LPL activity and TG uptake were accompanied by marked down-regulation of Angptl4 mRNA levels and protein in BAT of wild type mice. This down-regulation of Angptl4 was not mediated via activation of the β-adrenergic signaling pathway, but probably involves activation of AMPK. Opposite to the changes seen in BAT, Angptl4 mRNA and protein levels were induced in WAT during cold, suppressing the activity of LPL in WAT. Induction of Angptl4 in WAT appears to be mediated via activation of the β-adrenergic signaling pathway. Interestingly, no notable differences in body temperature, food intake, organ weights, or tissue morphology were observed between the Angptl4 genotypes upon cold exposure. Taken together, these data suggest that Angptl4 modulates the increase in TG uptake by BAT during cold exposure by directly acting upon the activity of LPL in BAT and WAT. The opposite regulation of LPL activity in these tissues by Angptl4 suggests that Angptl4 may be part of a physiological mechanism that stimulates the shuttling of fatty acids to BAT during cold.

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Cholesterol Efflux Pathways in Dendritic Cells Suppress Autoimmune Responses

Marit Westerterp[1, 2], Emmanuel L. Gautier[3], Anjali Ganda[1], Gwendalyn J. Randolph[3], Vivette D. D'Agati[1], Laurent Yvan-Charvet[1], Alan R. Tall[1]

[1]Columbia University, New York, NY; [2]Academic Medical Center, Amsterdam, The Netherlands; [3]Washington University, St. Louis, MO

Patients with autoimmune disorders have low HDL levels. Mice deficient in genes regulating cholesterol homeostasis, such as liver X receptor (LXR) or apolipoprotein A-I (apoA-I), show an autoimmune phenotype. LXR regulates the expression of ATP binding cassette A1 and G1 (ABCA1 and ABCG1), which mediate cholesterol efflux to apoA-I and HDL. ABCG1 is highly expressed in dendritic cells (DCs). We hypothesized that ABCA1 and ABCG1 regulate autoimmunity. On a chow diet, 40-week-old *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice showed enlarged lymph nodes (LNs), increased plasma autoantibodies to dsDNA, and glomerulonephritis, with characteristics typical for lupus nephritis. Using the Cre loxP system, we investigated whether these effects were due to *Abca1/g1* deficiency in T-cells, macrophages, or DCs. Only *Abca1/g1* deficiency in DCs in *CD11cCreAbca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice replicated the autoimmune phenotype found in *Abca1*$^{-/-}$*Abcg1*$^{-/-}$ mice. This suggests a major role for DC cholesterol homeostasis in autoimmunity.

DCs present antigens to T-cells, leading to their activation. *CD11cCreAbca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice showed increased T-cell activation in blood, spleen, and LNs. After immunization, DCs from *CD11cCreAbca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice showed increased antigen presentation to T-cells *in vitro* and *in vivo*. *CD11cCreAbca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice had increased CD80+ DCs. CD80 is a co-stimulatory molecule required for antigen presentation. *Abca1/g1* deficiency in DCs increased endosomal cholesterol accumulation *in vitro*. Ligands for Toll-like receptor 3 and 4 (TLR3 and -4) increased CD80 mRNA in *CD11cCreAbca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ compared with *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ DCs, where the effect of ligands for TLR3 was greater than that of ligands for TLR4. Cholesterol depletion by cyclodextrin decreased CD80 mRNA and antigen presentation in *CD11cCreAbca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ DCs *in vitro*. The increased CD80 mRNA in *CD11cCreAbca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ DCs was also reversed by a type I IFN antibody, suggesting excessive signaling by the known TLR3-IFN-CD80 axis. These studies show for the first time a role for cholesterol efflux pathways in DCs in maintaining immune tolerance.

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Hepatocyte Toll-like Receptor 4 Regulates Obesity-induced Inflammation and Insulin Resistance

Lin Jia[1], Claudia Vianna[1], Makoto Fukuda[2], Eric Berglund[3], Chen Liu[1], Kai Sun[4], Matthew Harper[1], Charlotte Lee[1], Syann Lee[1], Philipp Scherer[4], Joel Elmquist[1]

[1]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX; [2]Department of Pediatrics, Baylor College of Medicine, Houston, TX; [3]Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, TX; [4]Touchstone Diabetes Center, University of Texas Southwestern Medical Center, Dallas, TX

Obesity is characterized by chronic low grade inflammation, which has been recognized as a key contributor to the development of insulin resistance. Accumulating evidence has been shown that obese subjects and diet-induced obese animal models have increased circulating levels of lipopolysaccharide (LPS). As the receptor of LPS, Toll-like receptor 4 (Tlr4) plays a critical role in mediating LPS-induced inflammatory response. Indeed, mice with global Tlr4 deletions are protected from diet-induced inflammation and insulin resistance. However, Tlr4 is widely expressed in various cell types. Thus, it is still unclear which cell type expressing Tlr4 links obesity-induced low grade inflammation and other metabolic abnormalities. The liver is a key insulin target tissue and is actively involved in glucose homeostasis and lipid metabolism. Importantly, growing lines of evidence suggest the role of hepatocyte-initiated inflammation in the development of insulin resistance. Notably, the expression of Tlr4 in primary hepatocytes and hepatoma cell lines has been reported. However, the role of hepatocyte Tlr4 in diet-induced inflammation and insulin resistance remains to be determined. In the present study, we generated hepatocyte-specific Tlr4 knockout ($Tlr4^{LKO}$) mice and found that $Tlr4^{LKO}$ mice had dramatically reduced circulating concentrations of tumor necrosis factor α (Tnfα) and interleukin 6 (IL-6) despite the development of obesity after a high fat diet (HFD) challenge. In addition, we observed that obese $Tlr4^{LKO}$ mice exhibited significantly down-regulated mRNA expressions of several inflammatory genes in the liver (IL-6 and IL-1β) and adipose tissue (IL-6, IL-1β, and Tnfα). Furthermore, HFD-induced glucose intolerance and systemic insulin resistance were greatly attenuated in $Tlr4^{LKO}$ mice. Strikingly, euglycemic-hyperinsulinemic clamp studies showed that hepatic glucose production was dramatically suppressed in $Tlr4^{LKO}$ mice, indicating enhanced insulin sensitivity in the liver. Finally, hepatic triglyceride contents were significantly decreased in $Tlr4^{LKO}$ mice after HFD feeding, which was associated with down-regulated mRNA expressions of genes involved in de novo lipogenesis, such as acetyl-CoA carboxylase 1, fatty acid synthase, and stearoyl-CoA desaturase 1. Collectively, our findings suggest that activation of hepatocyte Tlr4 contributes to obesity-related inflammation and insulin resistance and that targeting hepatocyte Tlr4 might be a useful therapeutic strategy for the treatment of type 2 diabetes.

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The Influence of Ginkgo Extract on the Microcirculation of the Eye in Patients with Type 2 Diabetes

Nijole Savickiene[1], Asta Spadiene[2], Nijole Jurgeviciene[3], Andrejs Skesters[4], Alisa Silova[4], Migle Francaite-Daugeliene[5], Rytas Ostrauskas[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Lithuanian University of Health Sciences, Kaunas, Lithuania; [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Lithuanian University of Health Sciences; Kaunas, Lithuania; [3]Endocrinology Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania; [4]Laboratory of Biochemistry, Riga Stradins University, Riga, Latvia; [5]Endocrinological Clinic, Hospital of Lithuanian University of Health Sciences Kaunas Clinics, Kaunas, Lithuania

Diabetic retinopathy is the most common diabetic eye complication and the leading cause of visual impairment and acquired blindness. This randomized double blind placebo-controlled study was conducted in the Endocrinological Clinic, Hospital of Lithuanian University of Health Sciences Kaunas Clinics, Lithuania. 44 patients with type 2 diabetes mellitus were randomized to Ex.Gb 160 mg/day or placebo and were followed up for 9 months. The dose of Ex.Gb was increased to 240 mg in the next 9 months. The baseline ophthalmological parameters and biochemical measurements were repeated after 9 and 18 months of receiving preparations. Vascular, intravascular, and perivascular alterations were evaluated, and total conjunctival index (TCI) was calculated. Total antioxidant status (TAS) of plasma was measured using the Trolox equivalent antioxidant capacity assay. This was performed in the Laboratory of Biochemistry, Riga Stradins University, Latvia. Data were analyzed using the computer software packages SPSS for Windows version 20.0 and GraphPad version 3.00. Significant differences were observed between three different measurements in the Ex.Gb group: The index of the vascular changes (VC) decreased by 1.2 point after 9 months ($p = 0.04$) and by 0.94 point after 18 months ($p = 0.14$) as compared with baseline. The index of the perivascular changes (PVC) decreased by 0.45 point after 9 months ($p = 0.11$) and by 0.7 point after 18 months ($p = 0.02$) as compared with baseline. The values of TAS were normal in both groups as compared with the reference values (between 1.30 and 1.77 mmol/liter) given by RANDOX. Evaluation of plasma TAS did not show statistically significant differences between Ex.Gb and placebo groups. The results of this study show that ingestion of Ginkgo biloba L. leaf extract determines significant changes in microcirculation in the conjunctiva blood vessels and might enhance the TAS, but further exploration needs to be done to confirm the results in different conditions.

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A Synthetic POVPC Analogue Binds to CD36 and Induces Expression of Inflammatory Genes in Macrophages via Toll-like Receptor 2 and 1

Philipp Wiesner[1], Erica N. Montano[1], Ishita Shah[1], Oswald Quehenberger[1], Edward A. Dennis[1], Sangderk Lee[2], Casey E. Romanoski[1], Aldons J. Lusis[2], Judith A. Berliner[2], William W. Turner[3], Michael S. VanNieuwenhze[3], Christopher K. Glass[1], Peter Tobias[4], Joseph L. Witztum[1]

[1]University of California, San Diego, La Jolla, CA; [2]UCLA, Los Angeles, CA; [3]Indiana University, Bloomington, IN; [4]The Scripps Research Institute, La Jolla, CA

1-Palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphorylcholine (POVPC) is an oxidized phospholipid (OxPL) found in oxidized LDL (OxLDL) and atherosclerotic lesions. We recently reported the development of a synthetic analogue that consists of a POVPC-peptide adduct. It is stable and water-soluble and could facilitate the study of one class of OxPLs. Here we show that the POVPC-peptide is highly bioactive and able to induce the expression of a variety of pro-inflammatory cytokines, including IL-1β, Ccl2, Ccl3, Ccl4, Cxl2, and Tnf-α, in RAW 264.7, murine bone marrow-derived macrophages, human-derived macrophages, and endothelial cells. It also increased the expression of Cox-2 in these cells and stimulated eicosanoid production. Using CD36 transfected CHO cells, we showed that the POVPC-peptide specifically binds to CD36. Because oxidized PAPC (OxPAPC) signals in macrophages through TLR2, we also examined the role of TLR2, TLR1, and TLR6 to mediate POVPC-peptide signaling. Utilizing thioglycolate-elicited macrophages from respective TLR KO mice, we found that inflammatory gene expression was dependent on TLR2 and TLR1, but not TLR6. We are currently investigating whether CD36 and TLR4 also participate in this signaling pathway. Compared with OxPAPC-stimulated gene expression in human macrophages and endothelial cells, the POVPC-peptide stimulated ~25-50% of the same genes. This work shows that the POVPC-peptide adduct reproduces the inflammatory activity of a subset of OxPLs and should be a valuable reagent in elucidating the biological importance of OxPL.

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Human Obesity and Insulin Resistance: Lessons from Genetics

Stephen O'Rahilly[1]

[1]University of Cambridge, Cambridge, UK

The genetic component of quantitative metabolic traits is complex, with a mixture of common alleles of small effect and rarer alleles of larger effect. We have principally focused on finding the latter through the study of extreme human phenotypes of obesity and insulin resistance, including lipodystrophy. By applying both candidate and hypothesis-free genetic approaches, we have identified multiple different genetic variants that cause highly penetrant forms of these diseases. Through detailed phenotypic studies in humans and relevant murine and cellular models, these disorders continue to provide new insights into the physiology and pathophysiology of energy balance and metabolism.

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Systematic and Non-biased Approach for Identifying a Compound(s) That Encourages the "Browning" of Subcutaneous Human Adipose Stem Cells (hASCs)

Björn Magnusson[1], Stefan Hallén[2], Eva K. Carlsson[2], Maria Andersson[2], Simonetta Wallin[2], Stefano Bartesaghi[2], Ola Engkvist[3], Gavin O'Mahony[4], Anna Forslöw[1], Xiao-Rong Peng[2]

[1]Reagents and Assay Development, Discovery Sciences, AstraZeneca RD, Mölndal, Sweden; [2]Bioscience, CVMD, AstraZeneca RD, Mölndal, Sweden; [3]Computational Chemistry, Discovery Sciences, AstraZeneca, RD, Mölndal, Sweden; [4]Medicinal Chemistry, CVMD, AstraZeneca RD, Mölndal, Sweden

Classic brown adipocytes are characterized by the presence of multilocular lipid droplets, high mitochondria content, and the mitochondrial protein uncoupling protein 1 (UCP-1). When activated by increased sympathetic tone, UCP-1 uncouples the mitochondrial proton gradient to generate heat for maintenance of core body temperature, a process termed non-shivering thermogensis. BAT is abundant in rodents but has also been visualized in the neck, clavicle, and spinal cord areas of adult humans, using ^{18}F-FGD uptake by PET-CT. The amount of human BAT varies greatly between individuals and is partly dependent on BMI and age.

In rodents, brown adipocytes are very metabolically active and utilize both glucose and lipids for non-shivering thermogenesis. During cold exposure or stimulation with β3-adrenergic agonists, BAT contributes significantly to whole body glucose and lipid metabolism. However, the relative contribution of BAT to human whole body metabolism remains to be explored.

In addition, "brown-like" adipocytes ("beige" or "brite" adipocytes) have recently been identified in rodent white adipose tissue (WAT). Morphologically, these cells resemble brown adipocytes and express UCP-1 upon cold exposure or thiazolidinedione (TDZ) or β3-adrenergic agonist treatment. Emerging evidence suggests that mouse beige adipocytes could take up energy to a similar extent as seen in classic brown adipocytes *in vivo*, and an increase in the number of beige adipocytes is metabolically beneficial. These observations have sparked great interest in finding ways to utilize the special properties of the beige adipocytes for treatment of type 2 diabetes. We will present a screen strategy to identify compounds/pathways that enhance the "browning" of hASCs isolated from human subcutaneous fat biopsies. Our strategy combines measurement of brown adipocyte-specific marker UCP1 mRNA and protein with functional readout of basal and stimulated proton leak, using the Seahorse Extracellular Flux (XF) analyzer. By applying this screening strategy, we will select compounds encouraging a more metabolically active human adipocyte phenotype. Indeed, we have identified compounds that increase UCP1 mRNA, protein, and proton leakage in differentiated adipocytes *in vitro*.

We believe this work will lead to a deepened understanding of the pathways involved in brown adipogenesis and ultimately identification of new chemical entities for the management of diabetes.

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Liver X Receptors Balance Lipid Stores in Hepatic Stellate Cells via Rab18, a Retinoid-responsive Lipid Droplet Protein

Fiona O'Mahony[1], Kevin Wroblewski[1], Sheila M. O'Byrne[2], Hongfeng Jiang[2], Jihane Benhammou[1], William S. Blaner[2], Simon W. Beaven[1]

[1]Division of Digestive Diseases, UCLA School of Medicine, Los Angeles, CA; [2]Department of Medicine, Columbia University, New York, NY

Liver X receptors (LXRs) are determinants of hepatic stellate cell activation and liver fibrosis. Freshly isolated stellate cells from *Lxra*β-/- mice have larger lipid droplets (LD), but the functional consequences of this are unknown. The aim of this study was to determine whether LXRs link cholesterol to retinoid storage in stellate cells and how this impacts stellate cell activation. Primary stellate cells from *Lxra*β-/- and wild-type (WT) mice were profiled by gene array during *in vitro* activation. Retinoid and oxysterol content was quantified by HPLC and mass spectroscopy. Primary stellate cells were transfected with siRNA constructs, analyzed by immunocytochemistry, and treated with specific nuclear receptor ligands. *Lxra*β-/- stellate cells have increased cholesterol and retinyl esters. Retinoid increase leads to intrinsic retinoic acid signaling receptor (RAR) signaling, such that stellate cell activation occurs more rapidly in *Lxra*β-/- stellate cells. We identify a novel all-*trans*-retinoic acid (ATRA)-responsive gene, *Rab18*, which is reciprocally down-regulated by LXRs. *Rab18* mRNA and protein are increased during stellate cell activation and correlate with loss of stellate cell LDs. Knockdown of *Rab18* by siRNA retards the loss of the lipid droplets in culture and blocks the induction of the mature, activated phenotype in stellate cells. We demonstrate a novel connection between retinoid and cholesterol metabolism within hepatic stellate cells that is mediated by the lipid droplet-associated protein, *Rab18*. Retinoid overload helps explain the profibrotic phenotype of *Lxra*β-/- mice. Consequently, interference with *Rab18* may have significant therapeutic benefit in ameliorating liver fibrosis.

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Antisense Inhibition of Angiopoietin-like Protein 3 Reduces Atherosclerosis and Hepatic Steatosis in Dyslipidemic Mouse Models

Richard G. Lee[1], Wuxia Fu[1], Mark Graham[1], Thomas A. Bell[1], Rosanne Crooke[1]

[1]Isis Pharmaceuticals, Carlsbad, CA

Patients with loss of function mutations in angiopoietin-like protein 3 (angptl3) have reduced plasma apoB-containing lipoproteins similar to that seen with null mutations in apolipoprotein B (*APOB*) and microsomal triglyceride transfer protein (*MTP*). However, in contrast, *ANGPTL3* mutants do not develop the hepatic steatosis observed in those other two genetic deficiency states. To recapitulate the human phenotype, antisense oligonucleotides (ASOs) targeting murine angptl3 were characterized in both LDLr$^{-/-}$ and diet-induced obesity (DIO) mice. Administration of angptl3 ASOs (50 mg/kg/week) to Western diet-fed LDLr$^{-/-}$ mice for 16 weeks led to significant reductions in angptl3 hepatic mRNA (28 ± 2% of control ASO) and plasma protein (117 ± 6 ng/ml with angptl3 ASO *versus* 484 ± 36 ng/ml with control ASO). Angptl3 inhibition resulted in reductions in total cholesterol (TPC) (416 ± 30 mg/dl with angptl3 ASO *versus* 1208 ± 135 mg/dl with control ASO) and plasma triglyceride (TG) (129 ± 10 mg/dl with angptl3 ASO *versus* 661 ± 69 mg/dl with control ASO), leading to significant reductions in *en face* atherosclerosis (5 ± 1% lesion area with angptl3 ASO *versus* 11 ± 1% lesion area with control ASO). Furthermore, administration of angptl3 ASOs (50 mg/kg/week) for 4 weeks to LDLr $^{-/-}$ mice led to significant reductions in TPC and plasma TG but tended to reduce hepatic TG (9 ± 1 mg/g liver with angptl3 ASO *versus* 15 ± 2 mg/g liver with control ASO). To determine the effects on angptl3 in the face of impaired VLDL secretion, DIO mice were administered an angptl3 ASO, MTP ASO, or a combination for 6 weeks. Angptl3/MTP ASO co-administration led to further significant reductions in TPC and hepatic TG accumulation when compared with MTP ASO monotherapy. These data suggest that angptl3 inhibition may have the therapeutic advantage of reducing both atherogenic lipids and cardiovascular disease without elevating the risk for developing hepatic steatosis.

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Association between APOE, SCARB1, and PPARα Polymorphisms and Serum Lipids in a Population of Lithuanian Adults

Alina Smalinskiene[1], Janina Petkeviciene[1], Kristina Jureniene[1], Jurate Klumbiene[1], Vaiva Lesauskaite[1]

[1]Lithuanian University of Health Sciences, Kaunas, Lithuania

Dyslipidemia is one of several known risk factors for coronary heart disease, a leading cause of death in Lithuania. Epidemiological studies have demonstrated the impact of nutrition on lipid levels within the Lithuanian population, although the role of genetic factors for dyslipidemias has not yet been studied. The objective of this study was to assess the distribution of the *APOE*, *SCARB1*, and *PPARα* genotypes in the Lithuanian adult population and to determine the relationship of these genotypes with dyslipidemia. A cross-sectional health survey was carried out in a representative random sample of the Lithuanian population aged 25–64 (n = 1030). A variety of single-nucleotide polymorphisms (SNPs) of the *APOE* (rs429358 and rs7412), *SCARB1* (rs5888), and *PPARα* (rs1800206) genes were assessed using real-time polymerase chain reaction. Serum lipids were determined using enzymatic methods. Men and women with the *APOE2* genotype had the lowest level of total and low density lipoprotein cholesterol (LDL-C). Men with the *APOE2* genotype had significantly higher levels of triglycerides (TG) than those with the *APOE3* genotype. In men, the carriers of the *APOE4* genotype had higher odds ratios (OR) of reduced (<1.0 mmol/liter) high density lipoprotein cholesterol (HDL-C) levels versus *APOE3* carriers (OR = 1.98; 95% CI = 1.05-3.74). The odds of having elevated (>1.7 mmol/liter) TG levels were significantly lower in *SCARB1* genotype CT carriers compared with men with the *SCARB1* genotype CC (OR = 0.50; 95% CI = 0.31-0.79). In men, carriers of the *PPARα* genotype CG had higher OR of elevated TG levels versus carriers of *PPARα* genotype CC (OR = 2.67; 95% CI = 1.15-6.16). The odds of having high LDL-C levels were lower in women with the *APOE2* genotype as compared with *APOE3* genotype carriers (OR = 0.35; 95% CI = 0.22-0.57). Our data suggest a gender difference in the associations between *APOE*, *SCARB1*, and *PPARα* genotypes and lipid levels. In men, the *APOE4* genotype and PPARα genotype CG were correlated with an atherogenic lipid profile, whereas the *SCARB1* genotype CT had an atheroprotective effect. In women, *APOE2* carriers had the lowest odds of high LDL-C.

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Transgenic Expression of Dominant-Active IDOL in Liver Causes Diet-induced Hypercholesterolemia and Atherosclerosis in Mice

Anna C. Calkin[1, 2], Stephen D. Lee[1, 2], Jason Kim[3], Caroline Van Stijn[3], Cynthia Hong[1, 2], Rajendra Tangirala[3], Peter Tontonoz[1, 2]

[1]Howard Hughes Medical Institute, UCLA, Los Angeles, CA; [2]Department of Pathology and Laboratory Medicine, UCLA, Los Angeles, CA; [3]Department of Medicine, Division of Endocrinology, UCLA, Los Angeles, CA

The E3 ubiquitin ligase IDOL triggers lysosomal degradation of the LDL receptor. The tissue-specific effects of the IDOL pathway on plasma cholesterol and atherosclerosis have not been examined. Given that the liver is the primary determinant of plasma cholesterol levels, we sought to examine the effect of chronic liver-specific expression of a dominant active form of IDOL in mice. We expressed a degradation-resistant, dominant-active form of IDOL (sIDOL) in C57Bl/6J mice from the liver-specific albumin promoter (L-sIDOL transgenics). L-sIDOL mice were fed a Western diet for 20 or 30 weeks and then analyzed for plasma lipid levels and atherosclerotic lesion formation. L-sIDOL mice showed dramatic reductions in hepatic LDLR protein and increased plasma LDL cholesterol levels on both chow and Western diets. Moreover, L-sIDOL mice developed marked atherosclerotic lesions when fed a Western diet. Lesion formation in L-sIDOL mice was more robust than has been reported for other transgenic models in the absence of concurrent LDLR or apoE mutation and did not require the addition of cholate to the diet. Western diet-fed L-sIDOL mice had elevated expression of LXR target genes and pro-inflammatory genes in their aortas. Liver-specific expression of dominant-active IDOL is associated with hypercholesterolemia and a marked elevation in atherosclerotic lesions. Our results show that increased activity of the IDOL pathway in the liver can override other LDLR regulatory pathways, leading to cardiovascular disease. L-sIDOL mice are a robust, dominantly inherited, diet-inducible model for the study of atherosclerosis.

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Apolipoprotein A-I-binding Protein Deficiency Exacerbates Inflammation and High Fat Diet-induced Metabolic Syndrome

Dina A. Schneider[1], Longhou Fang[1], Dorothy D. Sears[1], Yury I. Miller[1]

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

Apolipoprotein A-I-binding protein (AIBP) is a conserved protein that is found in the mitochondria, cytoplasm, and nucleus in addition to being secreted. Our laboratory recently demonstrated that extracellular AIBP-mediated cholesterol efflux controls angiogenesis in zebrafish. Although secreted AIBP has been shown to regulate cholesterol efflux, little is known about its intracellular functions. To investigate these, our laboratory has generated *Aibp*$^{-/-}$ mice, which are viable and fertile. A recent paper suggests that AIBP is an NAD(P)H-hydrate epimerase, giving it a presumptive role in mammalian NAD$^+$ homeostasis. We hypothesized that by maintaining an appropriate cellular NAD$^+$ level, AIBP indirectly regulates SIRT1, an NAD+-dependent histone deacetylase with targets including inflammatory and metabolic regulators, such as NF-κB, AP-1, and Akt. *Aibp*$^{-/-}$ resident macrophages exhibit increased expression of pro-inflammatory cytokines that fit an NF-κB activation pattern. SIRT1 expression was decreased in *Aibp*$^{-/-}$ mice, as determined by *Sirt1* mRNA transcript levels and acetylation status of SIRT1 targets. The presumptive role of AIBP in NAD$^+$ homeostasis indicates a link between energetic status and AIBP activity, suggesting that metabolic disturbances should exacerbate the effects of *Aibp* knockout. Indeed, *Aibp*$^{-/-}$ mice fed a 45% high fat diet (HFD) gained significantly more weight than wild type mice, despite consuming a similar quantity of food. In contrast, chow-fed knockout mice did not weigh more than their wild type counterparts. HFD-fed *Aibp*$^{-/-}$ mice were also glucose-intolerant and had increased M1 pro-inflammatory macrophage infiltration in their white adipose tissue, indicating the development of metabolic syndrome. A better understanding of AIBP's regulation of inflammation and metabolism will provide new mechanistic insights and therapeutic targets for metabolic disorders.

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Hepatic S1P Deficiency Lowers Plasma Cholesterol Levels in ApoB-containing Lipoproteins When LDLR Function Is Compromised

Debapriya Basu[1], Afroza Huq[1], Jahangir Iqbal[1, 2], M. Mahmood Hussain[1, 2], Xian-Cheng Jiang[1], Weijun Jin[1]

[1]Department of Cell Biology, SUNY Downstate Medical Center, Brooklyn, NY; [2]Department of Pediatrics, SUNY Downstate Medical Center, Brooklyn, NY

Site-1 protease (S1P) is the key enzyme required for activation of the sterol regulatory element-binding proteins (SREBPs) that govern lipid synthesis. S1P inhibition has been shown to lower plasma total cholesterol (TC) and triglyceride (TG) levels, but whether it has an effect on plasma apoB-containing lipoprotein (Blp) metabolism is unknown. A hepatic specific knockdown (KD) of S1P using floxed S1P mouse models ($S1P^{f/f}$) and hepatic expression of Cre recombinase resulted in a 45 and 38% reduction in plasma TC and TG levels, respectively. With regard to Blp-c, hepatic S1P KD had a minimal effect in $S1P^{f/f}$ mice but significantly reduced Blp-c levels in LDLR+/-$S1P^{f/f}$ and LDLR-/-$S1P^{f/f}$ mice. This suggested that in $S1P^{f/f}$ mice, hepatic S1P KD impaired LDLR function, reducing degradation of Blp-c. Despite decreasing LDLR mRNA expression by 50%, hepatic S1P KD resulted in reduced liver LDLR protein expression only under fasting, not fed, conditions. Further assessment of hepatic S1P deficiency revealed that it increased LDLR protein stability in vivo. In addition, hepatic S1P KD was shown to decrease the liver and plasma levels of proprotein convertase subtilisin/kexin type 9 (PCSK9) that degrades LDLR protein. This effect was more prominent in the fed condition, which could explain the discordance between LDLR mRNA and protein expression after hepatic S1P KD. Mechanistically, hepatic S1P was shown to regulate PCSK9 expression through activation of the SREBPs. In conclusion, hepatic S1P is a physiological modulator of the liver LDLR and PCSK9 mRNA expression, both of which are mediated by activation of SREBPs. This regulation is critical for fine-tuning both LDLR protein at the post-translational level and plasma Blp-c levels. The study shows that putative S1P inhibitors could constitute a novel class of Blp-c-lowering drugs, of particular use when LDLR function is compromised.

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Stimulation of Lipoprotein Internalization into Brown and White Adipose Tissues by β3-Adrenergic Receptor Signaling

Markus Heine[1], Alexander Fischer[1], Kristina Gottschling[1], Alexander Bartelt[1, 2], Ludger Scheja[1], Joerg Heeren[1]

[1]Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA

The activation of brown adipose tissue (BAT) has been shown to confer beneficial effects on adiposity, insulin resistance, and hyperlipidemia. In response to activation by cold, BAT accelerates the clearance of triglyceride-rich lipoprotein (TRL) in a process that includes lipoprotein lipase-mediated TRL processing as well as lipoprotein particle internalization. Cold can be pharmacologically mimicked using the β3-adrenergic receptor agonist CL316,243 (CL). Similar to sustained cold exposure, repeated CL treatment results in a marked remodeling of white adipose tissue (WAT), which is characterized by the appearance of multilocular beige adipocytes ("browning"). In the current study, we investigated the role of acute and sustained β3-adrenergic receptor activation for TRL catabolism mediated by brown, beige, and white adipocytes. In order to induce browning, C57BL/6J mice were injected daily with CL for 7 days. For acute activation, CL was injected 4 h prior to the experiment. Then we exteriorized BAT or epididymal WAT and placed the anesthetized animal directly on a confocal microscope equipped with a resonant scanner. Recombinant TRL, labeled with hydrophobic fluorescent nanocrystals, was injected via a tail vein catheter, and its *in vivo* processing within BAT and browned WAT was recorded in real time. CL injections markedly increase lipoprotein and lipid uptake into BAT. As expected, accelerated processing and transport of TRL is stimulated either by short term or sustained activation. This process comprised a two-step mechanism: initial binding followed by endocytosis into endothelial cells and subsequent lipid transport into brown adipocytes. Notably, TRL processing was accelerated in epididymal WAT by both short term and repeated CL treatment, suggesting that beige as well as white adipocytes promote lipoprotein catabolism in response to β3-adrenergic receptor signaling. Our data suggest that brown and beige but also white adipocytes are able to manipulate their endothelial microenvironment, resulting in increased local influx of lipoproteins and thus nutrient for combustion or storage.

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CD36 and Caveolin-1 Mediate Lipoprotein Uptake into Brown Adipose Tissue across the Endothelial Barrier

Kristina Gottschling[1], Alexander Bartelt[1, 2], Kerstin Röser[1], Markus Heine[1], Ludger Scheja[1], Joerg Heeren[1]

[1]Department of Biochemistry and Molecular Cell Biology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [2]Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA

Recently, we showed that brown adipose tissue (BAT) activity influences lipoprotein metabolism by controlling plasma triglyceride clearance. This process includes whole lipoprotein particle internalization into BAT. However, the molecular mechanisms underlying lipoprotein trafficking into BAT are unclear. Here, we elucidate the contribution of endothelial cells, macrophages, and brown adipocytes to BAT-mediated lipoprotein processing and delineate key molecules involved in the internalization. Wild-type and caveolin-1- and CD36-deficient mice were injected with fluorescence-labeled triglyceride-rich lipoproteins (TRL) after exposure to 22|°C (control) or 4|°C (cold). BAT tissues were harvested, and immunofluorescence studies were performed to visualize caveolin-1, CD36, and TRL in different BAT cell types. Brown adipocytes and endothelial cells from control or cold-activated BAT were separated by MACS® technology, and expression was quantified by Taqman®. TRL radiolabeled with ^{14}C-cholesteryl oleyl ether and 3H-triolein were used as a tool to follow the fate of the lipoprotein core and liberated fatty acids into the respective cell types. CD36 and caveolin-1 are present at the plasma membrane of brown adipocytes and endothelial cells. Caveolin-1 deficiency impaired plasma membrane localization of CD36. Interestingly, the expression of proteins known to regulate lipoprotein clearance, such as CD36, lipoprotein lipase, and fatty acid transport protein 4, was induced primarily in endothelial cells of cold-activated BAT. Mechanistically, TRL particles are first taken up by BAT endothelial cells, a process dependent on CD36 and caveolin-1. Subsequent processing of radiolabeled lipoproteins indicates efficient lipid transfer to brown adipocytes. Our data indicate that caveolin-1 is an essential scaffold protein at the plasma membrane of BAT endothelial cells necessary for proper CD36 localization and function. Our data demonstrate that endothelial cells play a key role in triglyceride-rich particle internalization into BAT, providing fuels for brown adipocyte thermogenesis.

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Regulation of Metabolic Flux by FoxOs during the Fasting-Refeeding Transition

Rebecca A. Haeusler[1], Kirsten Hartil[2], Bhavapriya Vaitheesvaran[2], Isabel Arrieta-Cruz[2], Colette M. Knight[2], Helene L. Kammoun[3], Mark A. Febbraio[3], Roger Gutierrez-Juarez[2], Irwin J. Kurland[2], Domenico Accili[1]

[1]Columbia University, New York, NY; [2]Albert Einstein College of Medicine, Bronx, NY; [3]Cellular and Molecular Metabolism Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia

Insulin integrates hepatic glucose and lipid metabolism, directing nutrients to glycogen storage and lipogenesis. In type 2 diabetes, the former process is impaired, and the latter is exaggerated, posing a pathophysiologic and therapeutic conundrum. A branching model of insulin signaling, with FoxO1 presiding over glucose production and Srebp1c regulating lipogenesis, provides a potential explanation. Here we illustrate an alternative mechanism that integrates glucose production and lipogenesis under the unifying control of FoxO. Liver-specific ablation of three FoxOs results in constitutive derepression of glucokinase and activation of lipogenesis at the expense of glucose production. We document a similar pattern in the early phases of diet-induced insulin resistance and propose that FoxOs are required to enable the liver to direct nutritionally derived carbons to glucose *versus* lipid metabolism. Our data underscore the heterogeneity of hepatic insulin resistance during progression from the metabolic syndrome to overt diabetes and the conceptual challenge of designing therapies that curtail glucose production without promoting hepatic lipid accumulation.

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A Central Role for Brown and Beige Adipocytes in Dyslipidemia and Atherosclerosis

Alexander Bartelt[1, 2], Clara John[1], Jimmy F. Berbée[3], Mariëtte R. Boon[3], Padmini P. Khedoe[3], Anna Worthmann[1], M. Lisa Cherradi[1], Franz Rinninger[4], Markus Heine[1], Ludger Scheja[1], Patrick C. Rensen[3], Joerg Heeren[1]

[1]Department of Biochemistry and Molecular Cell Biology, UKE, Hamburg, Germany; [2]Department of Genetics and Complex Disease, Harvard School of Public Health, Boston, MA; [3]Department of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands; [4]Department of Internal Medicine, UKE, Hamburg, Germany

Obesity and diabetes are major risk factors for atherosclerotic heart disease. An excess of white adipose tissue (WAT) on the one hand and a decline in brown adipose tissue (BAT) activity on the other hand might be causal for high triglycerides, low HDL cholesterol, and atherosclerosis in obese individuals. However, the underlying mechanism for the relation between obesity, dyslipidemia, and atherosclerosis still remains ambiguous. Here we investigate the systemic effects of BAT activation and browning on circulating triglycerides, HDL, and atherosclerosis in mice. Wild-type, adipocyte-specific lipoprotein lipase (LPL) knock-out and Western diet-fed APOE3*Leiden.CETP mice, a humanized mouse model of atherosclerosis (all C57Bl/6 background), were treated with vehicle or a ß3-adrenergic receptor agonist CL316,243 (CL) to induce browning. Lipoprotein profiling was performed using FPLC; in vivo HDL function was assessed using a reverse cholesterol transport macrophage assay and HDL turnover studies; high resolution lipidomics was performed on lipoproteins as well as BAT and WAT; atherosclerosis was assessed in the aortic root. CL injection for 7 days lowered plasma glucose and triglyceride levels strongly, whereas HDL cholesterol was increased. We observed that browning leads to rapid remodeling of the HDL lipidome, a process crucially dependent on adipocyte LPL. The lipid fingerprint of HDL resembled increased de novo lipogenesis in BAT and WAT. HDL remodeling was associated with decreased HDL half-life and increased HDL-mediated cholesterol excretion into feces. Treatment of APOE3*Leiden.CETP mice with CL for 10 weeks reduced dyslipidemia, increased cholesterol excretion via HDL, and resulted in strongly reduced development of atherosclerotic lesions. BAT activation and browning protect from dyslipidemia and atherosclerosis by both lowering deleterious blood lipids and increasing HDL-mediated reverse cholesterol transport. Because adipocyte-LPL is a prerequisite for HDL remodeling, our results provide a molecular mechanism linking triglyceride breakdown to HDL formation. Taking these results together, we uncover a novel and powerful role for brown and beige adipocytes in atheroprotection.

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Natural Antibodies Recognize Shared Mimitopes on MDA-LDL and Streptococcus Group A

Ayelet Gonen[1], Jason Cole[1], Romulo Oliveira[2], Cody J. Diehl[1], Jeffrey Copps[1], Victor Nizet[1], Partho Ghosh[1], Joseph Witztum[1]

[1]University of California, San Diego, La Jolla, CA; [2]Department of Pathology, State University of Campinas, Campinas, Brazil

When LDL undergoes lipid peroxidation, a wide variety of modifications occur, generating oxidation-specific epitopes (OSE), which are both pro-inflammatory and immunogenic.

We have shown that OSE are "danger-associated molecular patterns" (DAMPs), to which has evolved a concerted innate immune response, which are mediated by "pattern recognition receptors" (PRRs). In addition, it has been suggested that all natural antibodies (NAbs) have dual specificities and bind to *both* endogenous antigens (DAMPs) and "pathogen-associated molecular patterns" (PAMPs) on infectious pathogens. Work from our group and others showed that MDA is the target of three different PRRs: the macrophage scavenger receptor SR-A, the NAb E014 (hapten-specific mAb), and the innate plasma protein, CFH. We hypothesized that EO14 will be able to recognize an immunologically similar epitope on pathogens as well. We screened a pathogen library with E014 and discovered that it bound to group A streptococcus (GAS) with high affinity and that this could be inhibited dose-dependently by MDA-LDL or MDA-BSA. Reciprocally, E014 binding to MDA-LDL was inhibited by GAS, demonstrating molecular mimicry between GAS and MDA. We further showed that E014 only bound to GAS strains containing protein M, a major virulence factor, and also bound to recombinant protein M. Using an array of recombinant protein M fragments and a synthetic peptide array, we identified a 24-amino acid peptide on the M protein to which E014 bound. Thus, the M protein harbors a linear peptide that is a mimotope for MDA-lysine. We are currently studying the immunological consequences of molecular mimicry between the M protein of GAS and the ubiquitous MDA epitope found in atherosclerotic lesions and inflammatory tissue. We previously showed a similar molecular identity between the PC epitope of oxidized phospholipids and the cell wall of *Streptococcus pneumoniae*.

Together, these data support the hypothesis that OSE are an important target of innate immunity, and both oxidative events and infectious pathogens have led to the natural selection of shared and potent innate immune responses.

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Spleen Tyrosine Kinase Regulates Macrophage MHC II Expression via Activation of Autophagy in Response to Oxidized Lipoprotein

Soo-Ho Choi[1], Ayelet Gonen[1], Jungsu Kim[1], Felicidad Almazan[1], Joseph L. Witztum[1], Yury I. Miller[1]

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

Adaptive immunity, which plays an important role in the development of atherosclerosis, is mediated by major histocompatibility complex (MHC)-dependent antigen presentation. In atherosclerotic lesions, macrophages constitute an important class of antigen-presenting cells (APCs) that activate adaptive immune responses to oxidized low density lipoprotein (OxLDL). Autophagy is a process of degradation of dysfunctional cytoplasmic compartments for recycling. It has been reported that autophagy regulates adaptive immune responses by enhancing antigen presentation to MHC class II (MHC-II). In a previous study, we demonstrated that spleen tyrosine kinase (SYK) regulates reactive oxygen species (ROS) generation and c-Jun N-terminal kinase (JNK) activation in macrophages. Here we show that OxLDL induced autophagosome formation, MHC-II expression, and phosphorylation of SYK in macrophages. Pharmacological inhibitors of SYK, ROS, and JNK reduced autophagosome formation in macrophages. Using bone marrow-derived macrophages (BMDM) isolated from wild type and myeloid cell-specific SYK knockout mice, we demonstrated that OxLDL-induced ROS generation and JNK activation were decreased in SYK-deficient macrophages. Intraperitoneal injections of OxLDL resulted in macrophage autophagy and MHC-II expression in vivo, and these effects were significantly diminished in SYK knockout mice. Further, Ldlr-/-/Syk-/- mice fed a high fat diet (HFD) produced lower levels of IgG2c to malondialdehyde (MDA)-LDL, malondialdehyde acetaldehyde (MAA)-LDL, and OxLDL compared with Ldlr-/- mice. The results of this study provide new insights into the mechanisms by which SYK regulates MHC-II expression via autophagy in macrophages and thereby contributes to the regulation of adaptive immune responses in atherosclerosis.

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Reducing Macrophage Proteoglycan Sulfation Exacerbates Diet-induced Obesity via Type-I Interferon Signaling

Philip L. Gordts[1], Roger Lawrence[1], Risha Sinha[1], Chris K. Glass[1], Aldons J. Lusis[2], Jeffrey D. Esko[1]

[1]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA; [2]Department of Cardiology, UCLA, Los Angeles, CA

Obesity is a major contributor to cardiovascular disease-related mortality and is setting back progress made by risk factor prevention. Diet-induced obesity has become a worldwide epidemic and is associated with a chronic low grade inflammation, called metabolic inflammation, which is considered to be the driving force for obesity-related metabolic consequences, such as Type-2 diabetes and atherosclerosis. A key mechanism underlying obesity-induced inflammation is accumulation of increased numbers of pro-inflammatory macrophages in obese adipose tissue. In order to examine the role of macrophage heparan sulfate proteoglycans in diet-induced obesity, we inactivated the biosynthetic gene GlcNAc *N*-deacetylase/*N*-sulfotransferase 1 (*Ndst1*) selectively in macrophages by crossing *Ndst1*$^{f/f}$ mice with *LysMCre*$^{+}$ mice. *Ndst1* inactivation reduced the overall sulfation of HSPG in macrophages by 30%. Despite this modest change in heparan sulfate, *Ndst1f/fLysMCre*$^{+}$ mice on a high fat diet had excessive body weight gain compared with control mice. Age-matched mice on a chow diet did not show any differences in body weight gain. The increased fat content in liver, white adipose tissue, and brown adipose tissue seen in *Ndst1*$^{f/f}$*LysMCre*$^{+}$ mice on the high fat diet was associated with increased macrophage infiltration and CCL2 expression, hallmarks for advanced metabolic inflammation. Glucose and insulin tolerance tests confirmed that *Ndst1*$^{f/f}$*LysMCre*$^{+}$ mice had reduced insulin sensitivity. Microarray analysis of bone marrow-derived macrophages from *Ndst1*$^{f/f}$*LysMCre*$^{+}$ mice showed significantly increased expression of inflammatory genes, such as CCL5, CCL7, CCL8, and TNF-α. Motif analysis of promoters of up-regulated genes revealed increased Type-I interferon (IFN) signaling in mutant macrophages. Also, IFN-β-induced STAT1 phosphorylation was elevated in *Ndst1*$^{f/f}$*LysMCre*$^{+}$ macrophages. We show that IFN-β interacts with macrophage heparan sulfate, suggesting that macrophage proteoglycans control inflammation by maintaining Type-I interferon reception in a quiescent state through sequestration of IFN-β. Altogether, our data imply that differences in macrophage heparan sulfation can possibly predict and determine the outcome of metabolic inflammation in diet-induced obesity.

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The Antioxidant Enzyme Prdx1 Alters Nuclear Receptor DNA Binding during Conditions of Cellular Stress

Nina V. Titova[1], Jonathan Deans[1], John Prins[1], Yinsheng Wang[1], Kenneth Huffman[2], Frances M. Sladek[1]

[1]University of California, Riverside, CA; [2]University of Texas Southwestern Medical Center, Dallas, TX

Prdx1 is an abundant antioxidative enzyme that is nitrated on tyrosine and has been shown to reduce nitration of plant and bacterial proteins. Protein nitration is significantly increased in mammals during metabolic stress, diabetes, aging, and cancer, and mice lacking Prdx1 exhibit accelerated aging and develop cancer in multiple tissues. Prdx1 has been shown to act as a co-activator for the androgen receptor, but little else is known about transcriptional effects of this enzyme, which is abundant in the nucleus as well as the cytoplasm. Two other nuclear receptors (NRs), glucocorticoid receptor (GR) and HNF4α, regulate metabolism in the liver under various stress conditions. We used co-immunoprecipitation, mass spectrometry, and protein binding microarray (PBM) technology to determine whether Prdx1 could act as a co-activator for GR and HNF4α. We found that GR and HNF4α were immunoprecipitated by anti-nitrotyrosine antibodies and that the signal correlated with the degree of cellular stress. Co-immunoprecipitations also showed that Prdx1 interacts with GR and HNF4α in mouse liver in the absence of DNA. PBMs using mouse liver nuclear extracts showed that Prdx1 co-localizes to specific NR response elements, which were also bound by GR and HNF4α. Prdx1 seemed to enhance the ability of HNF4α to bind certain DNA sequences during stress.

In cancer cells, where metabolic stress is high, the levels of Prdx1 protein are often increased dramatically. We used human lung cancer cell lines from a primary and metastatic tumor with differential sensitivity to GR ligands from the same patient to assess the role of human Prdx1 in GR DNA binding. We found comparable high levels of nuclear GR and Prdx1 proteins after dexamethasone treatment in both cell lines, although the metastatic line was more sensitive to GR ligands than the primary line. We observed specific interactions between GR and Prdx1 in the GR ligand-sensitive line. This work shows for the first time that Prdx1 interacts with NRs HNF4α and GR and that it may affect their ability to bind DNA and hence regulate transcription. Ongoing experiments will determine whether Prdx1 alters NR nitration and whether that nitration affects DNA binding.

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Non-classical Secretion of Adipokine aP2 Is Regulated by Lipolysis in Adipocytes

Meric Erikci Ertunc[1], Jorgen Sikkeland[2], Federico Fenaroli[2], Gareth Wyn Griffiths[2], Mathew P. Daniels[3], Haiming Cao[1, 3], Fahri Saatcioglu[2], Gokhan S. Hotamisligil[1]

[1]Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA; [2]Department of Biosciences, University of Oslo, Oslo, Norway; [3]Center for Molecular Medicine, National Institutes of Health, NHLBI, Bethesda, MD

Adipocyte/macrophage lipid chaperone aP2 is a critical immunometabolic regulator involved in the pathogenesis of several diseases, such as type 2 diabetes, atherosclerosis, fatty liver disease, asthma, and cancer. The physiological and pathological functions of aP2 have long been attributed to its intracellular actions. However, we and others have detected aP2 in conditioned media of adipocytes and in serum, and recent work in our laboratory demonstrated a role for aP2 secreted from adipocytes in the regulation of liver glucose output. Whereas recombinant protein infusion to lean mice increased gluconeogenesis, treatment with an aP2-neutralizing antibody resulted in significant improvement in glucose metabolism in obese diabetic mice. Taken together, these data demonstrate that aP2 is a potent adipokine integrating adipose tissue with critical systemic metabolic pathways. Because this novel adipokine does not resemble any classically secreted proteins, we sought to understand the mechanisms and route that lead to its secretion. We have previously shown that the serum level of aP2 in mice is regulated by feeding/fasting cycles and that secretion is induced by lipolytic stimuli. Here we show that aP2 secretion is dependent on lipolysis *in vitro* and *ex vivo*. aP2 lacks a canonical signal peptide, and inhibitors of classical secretion are not effective in blocking its secretion from adipocytes. We found that aP2 secretion occurs via a vesicular pathway, and extracellular aP2 is found in both free and extracellular vesicle-bound forms. Taken together, our data demonstrate that aP2 is secreted from adipocytes in a highly regulated manner and via a non-classical pathway in response to lipolysis. Considering the significant metabolic effects of secreted aP2, characterization of the mechanisms by which it is secreted from adipocytes has the potential to yield new insights for treatment of immunometabolic diseases, such as type 2 diabetes, and related complications.

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The Effect of High Carbohydrate and High Fat Diets on ChREBP and Its Target Genes in C57BL/6 Mice

Tara N. Jois[1], Victor Howard[1], Kristina Youngs[1], Claudia Thalmann[1], Mark W. Sleeman[1]

[1]Monash University, Clayton, Victoria, Australia

The transcription factor carbohydrate response element-binding protein (ChREBP) is a key mediator of intracellular glucose sensing and regulates energy metabolism through the modulation of key glycolytic and lipogenic genes. Recently, a new ChREBP isoform has been identified, ChREBP-β. Increased expression of this isoform in adipose tissue, but not liver, is correlated with improved health in obesity, suggesting tissue-specific regulation of ChREBP may be important in human metabolic disease. We sought to investigate the effect of diet composition on ChREBP isoform expression and correlate any changes with metabolic function.

In order to accomplish this, we fed mice one of three diets for a period of 12 weeks: low fat diet (LFD; energy from fat: 12%, carbohydrate: 65%; 14 MJ/kg), high carbohydrate diet (HCD; energy from fat: 9%, carbohydrate: 70%; 18 MJ/kg), or high fat diet (HFD; energy from fat: 43%, carbohydrate: 36%; 19 MJ/kg). A HFD is known to lead to significant increases in body weight with accompanying impairments in glucose and insulin tolerance. In our experiments, these changes in metabolic parameters can be correlated with an increase in hepatic but decrease in adipose expression of ChREBP-α and ChREBP-β isoforms ($p < 0.05$). There were also similar changes in expression of SREBP1c and ACC1, so it is difficult to dissect out an effect mediated by ChREBP from SREBP1c. In contrast, animals on a HCD surprisingly exhibited a mild but significant impairment in glucose tolerance ($p < 0.05$) but a significant improvement in insulin sensitivity ($p < 0.05$), with no changes in body weight. Assessment of adipose ChREBP-α or ChREBP-β expression revealed no changes; however, a HCD did result in a preferential increase in ChREBP-β in liver. This was accompanied by increased liver ACC1 expression ($p < 0.05$), despite no changes in SREBP1c levels. These results show that not only are ChREBP isoforms differentially expressed between tissues, but also composition of diet can modulate their expression. They also support the current idea that ChREBP-β is more transcriptionally active than ChRBEP-α, because changes in ChREBP target genes followed ChREBP-β expression. However, understanding the metabolic effects of HCD, in particular the differential effects on glucose and insulin tolerance, will require further investigation.

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Localized Immune Response to Oxidized Lipids in *Abcg1*$^{-/-}$ Mice: A New Model for Lipid-driven Autoimmunity

Angel Baldan[1, 3], Ayelet Gonen[2], Christina Choung[1], Tyler Marquart[3], Xuchu Que[2], Cody Diehl[2], David A. Ford[3], Joseph L. Witztum[2], Elizabeth J. Tarling[1]

[1]UCLA, Los Angeles, CA; [2]University of California, San Diego, La Jolla, CA; [3]Saint Louis University, St. Louis, MO

Many metabolic diseases, including atherosclerosis, type 2 diabetes, pulmonary alveolar proteinosis (PAP), and obesity, have a chronic inflammatory component involving both innate and adaptive immunity. Mice lacking the ATP binding cassette (ABC) transporter ABCG1 develop chronic inflammation in the lungs, associated with lipid accumulation (cholesterol, cholesterol ester, phospholipid, and oxidized lipids) and cholesterol crystal deposition, characteristic of atherosclerotic lesions and PAP. Here we demonstrate that specific lipids, probably oxidized phospholipids and/or sterols, elicit a lung-specific immune response in *Abcg1*$^{-/-}$ mice. Loss of ABCG1 results in increased levels of specific oxysterols, phosphatidylcholines, and oxidized phospholipids, including 1-palmitoyl-2-(5′-oxovaleroyl)-sn-glycero-3-phosphocholine (POVPC), in the lungs. Further, we identify a niche-specific increase in natural antibody (NAb)-secreting B-1 B cells in response to this lipid accumulation that is paralleled by increased titers of IgM, IgA, and IgG against oxidation-specific epitopes, such as those on oxidized LDL and malondialdehyde-modified LDL (MDA-LDL). Finally, we identify a cytokine/chemokine signature in the lungs of *Abcg1*$^{-/-}$ mice reflective of increased B cell activation, antibody secretion, and homing. Collectively, these data demonstrate that the accumulation of lipids in *Abcg1*$^{-/-}$ mice induces the specific expansion and localization of B-1 B cells, which secrete NAbs that may help protect against the development of atherosclerosis. Indeed, despite chronic lipid accumulation and inflammation, hyperlipidemic mice lacking ABCG1 develop smaller atherosclerotic lesions compared with controls. These data also suggest that *Abcg1*$^{-/-}$ mice may represent a new model in which to study the protective functions of B-1 B cells/NAbs and may provide novel targets for pharmacologic intervention and treatment of disease.

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Identification of Novel Regulators of Endosomal Cholesterol Transport

Ximing Du[1], Hongyuan Yang[1]

[1]University of New South Wales, Sydney, New South Wales, Australia

The delivery of low density lipoprotein (LDL)-derived cholesterol from endosomal compartments to the plasma membrane and the endoplasmic reticulum (ER) is an important yet poorly understood cellular process. Niemann-Pick C1 (NPC1), a multipass integral membrane protein on the limiting membranes of late endosomes and lysosomes, is known to insert lumenal LDL-derived cholesterol into the limiting membrane. We have identified novel cytoplasmic proteins that regulate the exit of LDL-derived cholesterol from late endosomes and lysosomes, such as ORP5, a member of the oxysterol-binding protein (OSBP)-related protein (ORP) family; Hrs/VPS27, a well established regulator of the ESCRT (endosomal sorting complex required for transport) pathway; and VPS4, an AAA ATPase that has a defined role in disassembling the ESCRT-III polymer. Depletion of ORP5, Hrs, or VPS4 causes cholesterol accumulation in late endosomes and lysosomes, which is reminiscent of the cholesterol trafficking defect in Niemann-Pick C (NPC) fibroblasts. We propose that these proteins may work together with NPC1 at the membrane junction between late endosomes/lysosomes and the ER to facilitate removal of endosomal cholesterol. The newly identified regulators of endosomal cholesterol transport also provide important insights into the budding of intralumenal vesicles and the functional separation of Hrs and VPS4 from other ESCRT proteins.

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AIBP Inhibits Inflammation and Reduces Foam Cell Formation

Longhou Fang[1], Ayelet Gonen[1], Soo-Ho Choi[1], Felicidad Almazan[1], Yury Miller[1]

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

ApoA-I-binding protein (AIBP) is a conserved, ubiquitously expressed and secreted protein with largely unknown functions. In a recent study, we demonstrated that AIBP accelerates cholesterol efflux from endothelial cells (EC), decreases lipid rafts, and inhibits VEGFR2 signaling, which in turn impairs angiogenesis in vitro and in zebrafish. The role of AIBP in atherosclerosis is unknown. Here we show that AIBP promoted cholesterol efflux from macrophages. This result led us to hypothesize that AIBP has an atheroprotective function. We generated transgenic zebrafish with conditional expression of Aibp and fed them a high cholesterol diet. In the hypercholesterolemic transgenic zebrafish, induced expression of Aibp diminished vascular lipid accumulation. We also created an AIBP knockout mouse. Naïve AIBP$^{-/-}$ mice manifested increased expression of pro-inflammatory cytokines in the peritoneum compared with control mice. Peritoneal macrophages isolated from wild type and AIBP$^{-/-}$ mice were treated with mmLDL or OxLDL, and increased accumulation of free cholesterol and cholesterol esters was found in AIBP$^{-/-}$ macrophages. We further documented that AIBP was expressed in murine atherosclerotic lesions, probably in macrophages and vascular smooth muscle cells but not in EC. We performed a bone marrow transplantation experiment, transferring wild type or AIBP$^{-/-}$ bone marrow (BM) into irradiated LDLR$^{-/-}$ mice and feeding recipient mice a high fat diet. Mice that received AIBP$^{-/-}$ BM had increased plasma IL-6 protein and peritoneal MCP-1 mRNA levels compared with control recipients. Furthermore, IgM Ab titers to MDA-LDL were higher, and IgM Ab titers to Cu-OxLDL and mmLDL trended higher in mice that received AIBP$^{-/-}$ BM. Collectively, the data demonstrate that AIBP regulates macrophage cholesterol efflux, vascular lipid accumulation, and inflammatory and immune responses. Further studies in systemic AIBP null mice on an atheroprone background are warranted to determine the role of AIBP in atherosclerosis. Our studies of the roles of AIBP in atherosclerosis may lead to the development of therapeutic approaches for prevention and treatment of atherosclerotic cardiovascular diseases.

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Apoc2 Knock-out Zebrafish Model of Hypertriglyceridemia

Chao Liu[1], Longhou Fang[1], Yury I. Miller[1]

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

APOC2 is a protein found on VLDL and chylomicrons and is obligatory for lipoprotein lipase activity to hydrolyze plasma triglycerides. APOC2 is one of the most frequently mutated genes in patients with familial hypertriglyceridemia. Hypertriglyceridemia is associated with acute pancreatitis and is an independent risk factor for atherosclerosis. However, there is no genetic mouse model for APOC2 deficiency and hypertriglyceridemia. Here, we describe an *apoc2* knock-out (KO) zebrafish model of hypertriglyceridemia, created with the transcription activator-like effector nuclease (TALEN) technique. The *apoc2* mutants survive to adulthood and are fertile. Homozygous *apoc2* embryos have retarded development and show increased triglyceride and cholesterol levels in blood, as detected by Oil Red O and BODIPY staining and colorimetric assays. Interestingly, the number of blood cells in *apoc2* mutants is decreased starting from the 3rd day post-fertilization, and this phenomenon is persistent over time. Our findings suggest that *apoc2* and/or lipoprotein lipase may play an important role in hematopoietic stem cell (HSC) specification and/or maintenance. The *apoc2* KO zebrafish can be a useful animal model to study mechanisms involved in the development of atherosclerosis, acute pancreatitis, and, possibly, defects of hematopoiesis.

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Inhibition of Secretory Phospholipase A2 (sPLA2) Caused Rapid Changes in Plasma HDL Composition and Function in ApoE* Leiden x hCETP Mice and Reduced the *ex Vivo* Enzyme Effects

Eva Hurt-Camejo[1], German Camejo[1], Birgitta Rosengren[1], Maria Wågberg[1], Daniel Karlsson[1], Mikael Brusberg[1]

[1]AstraZeneca, CVMD iMed, Mölndal, Sweden

The aim of this work was to investigate effect of sPLA2 inhibition in lipoproteins. The sPLA2 pan-pro-drug AZ12755733 was administered orally (150 μM/kg) to apoE*3Leiden x CETP transgenic mice (n = 5-7). Plasma was collected after 2 and 6 h of compound/vehicle administration. Plasma active drug (AZ10897140) exposure, lysophosphatidylcholine (lyso-PC) level, sPLA2 enzymatic activity, and CETP activity were measured. Lipoproteins were isolated by ultracentrifugation in D_2O/sucrose solutions and characterized by electrophoretic techniques and composition. HDL cholesterol efflux capacity was measured in human macrophages preloaded with radiolabeled cholesterol. For the *ex vivo* studies, exogenous mouse or human recombinant sPLA-X was added to mouse and/or human plasma in the presence and absence of active drug (IC_{50} = 19 and 38 μM for mouse and human sPLA2-X, respectively; IC_{20} = 15 μM in mouse whole plasma). Active compound AZ10897140 reached plasma exposures of 20 and 5 μM after 2 and 6 h, respectively. Treatment decreased plasma sPLA2 enzymatic activity and lyso-PC levels and induced changes in HDL cholesterol/protein, phospholipid/protein ratios, and particle size. In treated animals, HDL was a better inducer of cholesterol efflux than in non-treated animals. Exogenous recombinant sPLA2-X added to mouse and human plasma rapidly induced (4 h) size reduction of HDL and LDL and changes in electrophoretic properties. The enzyme was found associated with VLDL/LDL and HDL. The co-addition of inhibitor blocked most of these changes. *In vivo* inhibition of sPLA2 plasma activity increased potentially atherosclerosis-protective properties of HDL. The addition of sPLA2-X to plasma caused rapid alterations in HDL that were reduced by inhibition of the enzyme. These results show for the first time the involvement *in vivo* of plasma sPLA2 activity in HDL composition, structure, and possible function.

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Defective ATGL-mediated Lipolysis Protects Mice from Diet-induced Obesity

Renate Schreiber[1], Peter J. Voshol[2], Peter Hofer[1], Petra Kotzbeck[1], Ulrike Taschler[1], Robert Zimmermann[1], Guenter Haemmerle[1], Antonio Vidal-Puig[2], Rudolf Zechner[1]

[1]Institute of Molecular Biosciences, University of Graz, Heinrichstrasse 31, 8010 Graz, Austria; [2]Institute of Metabolic Science, Metabolic Research Laboratories, University of Cambridge, UK

Obesity results from an imbalance between energy intake, expenditure, or storage. Excessive energy is stored as intracellular triacylglycerol (TG) in adipose but also non-adipose tissues. Defects in lipid homeostasis may cause metabolic disorders, such as dyslipidemia, insulin resistance, and type 2 diabetes. The mobilization of intracellular TGs is initiated by adipose triglyceride lipase (ATGL). Mice globally lacking ATGL (AKO) are obese and insulin-sensitive despite ectopic lipid accumulation. We hypothesized that mice lacking ATGL will become fatter upon HFD intervention but remain metabolically healthy. To address this question, we used a mouse model expressing ATGL exclusively in the heart (AKO/cTg) to circumvent cardiomyopathy and early lethality normally observed in AKO mice. Importantly, AKO/cTg mice exhibited a normal life span and showed increased adiposity similar to AKO mice.

Unexpectedly, we found that AKO/cTg mice were protected from HFD-induced obesity. Hyperinsulinemic-euglycemic clamps demonstrated that AKO/cTg animals maintained insulin sensitivity in particular also in adipose tissues. Thus, the obesity-resistant phenotype of AKO/cTg mice persisted despite normal/increased response to the adipogenic hormone insulin. Using metabolic analyses, we showed that energy expenditure was not increased in AKO/cTg mice. However, food intake was moderately decreased in AKO/cTg mice upon HFD intervention. Moreover, mRNA levels of the major adipogenic transcription factors C/EBP-α and PPAR-γ2, and its target genes were decreased in AKO/cTg mice compared with controls. These data indicate that defective ATGL-mediated lipolysis affects PPAR-γ signaling. Furthermore, ex vivo fat pad analyses showed that fatty acid and glucose incorporation into the TG moiety was impaired in AKO/cTg mice. Supplemention of HFD with the PPAR-γ agonist rosiglitazone restored the obesity phenotype of AKO/cTg mice. Together, our data provide evidence that a moderate hypophagia and reduced adipogenesis/lipid synthesis protects mice lacking ATGL from diet-induced obesity.

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Mutations in ABCA8 Result in HDL Deficiency and Cholesterol Efflux Defects

Julian C. vanCapelleveen[1], Kees Hovingh[1], Linhua Zhang[2], Geesje Dallinga-Thie[1], Ian Tietjen[3], Kenny Wong[4], John J. Kastelein[1], Michael R. Hayden[2], Roshni R. Singaraja[5]

[1]Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands; [2]Centre for Molecular Medicine and Therapeutics and Child and Family Research Institute, Vancouver, BC, Canada; [3]Xenon Pharmaceuticals, Burnaby, BC, Canada; [4]Merck Research Laboratories, Rahway, NJ; [5]Translational Laboratory in Genetic Medicine (TLGM), Agency for Science, Technology and Research, Singapore

The molecular basis of extreme HDL cholesterol (HDL-C) levels is incompletely understood. To identify mutations in novel putative HDL genes, we sequenced select genes in 80 unrelated individuals with extremely low HDL-C (HDL percentile ≤ 10th) and, as controls, 120 individuals with high HDL (HDL percentile ≥ 90th). We identified two variants in *ABCA8* in subjects with low HDL-C levels. *ABCA8* belongs to the family of ATP binding cassette transporters. The E17-2 A>G mutation is located in a highly conserved region and gives rise to a disruption of a splice site. The A>G variant occurs at a site conserved in vertebrates, at position 64425884 (build HG18), and results in a Pro609→Arg substitution. No *ABCA8* mutations were identified in HHDL individuals. Family expansion followed by genotyping identified additional mutation carriers and first degree relative controls in whom segregation analyses were performed. Compared with controls, mutation carriers showed a significant 32.6% decrease in plasma HDL-C levels and decreased HDL percentiles (HDL-C: controls = 1.23 ± 0.26 mmol/liter, mutation carriers = 0.83 ± 0.37 mmol/liter, p=0.0007; HDL percentile: controls = 41.8±21.2, mutation carriers = 17.3 ± 19.2, $\underline{p}$ = 0.004, n = 23 controls and 12 carriers). No changes in LDL cholesterol, triglycerides, or BMI were observed. Because mutations in *ABCA8* reduce plasma HDL-C levels, we assessed the role of ABCA8 in lipid efflux. Wild type and P609R *ABCA8* cDNA clones were generated from human liver RNA. A significant ~200% increase in cholesterol efflux to lipid-free APOA-I was observed with wild type ABCA8, suggesting that ABCA8 is a lipid transporter. A significant 75% decrease in the efflux of cholesterol to lipid-free APOA-I (p = 0.02) was observed with the mutant P609R ABCA8 protein compared with wild type. We show here that ABCA8 is a cholesterol transporter and that mutations in *ABCA8* are a novel cause for reduced plasma HDL-C levels in humans.

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

Kirstin Albers[1], Britta Hoffzimmer[1], Philip Gordts[2], Hartwig Schmale[1], Jörg Heeren[1]

[1]Department of Biochemistry and Molecular Cell Biology, University Medical Center, Hamburg, Germany;
[2]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA

The LDL receptor-related protein 1 (LRP1) is important for the rapid clearance of pro-atherogenic lipoprotein remnants into the liver. Furthermore, in adipose tissues, LRP1 is present in intracellular vesicles positive for the glucose transporter 4 (Glut4). The insulin-dependent fusion of these vesicles with the plasma membrane enables efficient glucose uptake via Glut4 in the postprandial phase. The phosphotyrosine interaction domain-containing protein 1 (PID1) has been identified as an adaptor protein for LRP1. In the current study, we investigated the functional consequences of PID1 loss with regard to LRP1 localization and its role in systemic lipoprotein and glucose metabolism. GST pull-down experiments were performed to investigate interaction between LRP1 and PID1. Silencing of PID1 was achieved in hepatoma cells and primary hepatocytes using lentiviral shRNA- and siRNA-based transfection systems. Radioactively labeled triglyceride-rich lipoproteins were used to study lipoprotein uptake in primary hepatocytes. After feeding chow and diabetogenic high fat diets, metabolic studies were conducted in wild type and PID1-deficient mice. The adaptor protein PID1 bound exclusively to the distal, dephosphorylated NPXY motif of the intracellular domain of LRP1. Although LRP1 is known to act as an endocytotic cell surface receptor, it is primarily located within perinuclear, endosomal compartments. This intracellular localization seems to be dependent on PID1 expression, because LRP1 was found primarily located at the plasma membrane in hepatic PID1 knock-out cells. Subsequently, LRP1 missorting resulted in altered lipoprotein remnant uptake in primary hepatocytes. Furthermore, loss of PID1 expression leads to an increased uptake of glucose and lipids into metabolically active organs. Our data support the model that phosphorylation of LRP1 induced by exogenous stimuli interrupts the LRP1-PID1 interaction, resulting in LRP1 translocation to the cell surface. We propose that PID1 acts as a molecular switch controlling the function of LRP1 as an endocytic receptor for remnant proteins *in vivo* in analogy to the well known function of the adaptor protein ARH for the LDL receptor. Next to an altered lipoprotein uptake into the liver, PID1-dependent sorting of LRP1 influences glucose uptake via Glut4 in adipose tissue.

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FGF21 Acts on the Nervous System to Cause Weight Loss and Improve Other Metabolic Parameters in Obese Mice

Bryn Owen[1], Xunshan Ding[1], Katie Colbert[1], Donald Morgan[2], Kamal Rahmouni[2], David Mangelsdorf[1], Steven Kliewer[1]

[1]University of Texas Southwestern Medical Center, Dallas, TX; [2]University of Iowa, Iowa City, IA

The liver-derived hormone FGF21 causes weight loss and improves insulin sensitivity and lipid profiles when administered to obese animals and humans. FGF21 acts through a cell surface receptor complex that includes the obligate co-receptor, βKlotho. In the nervous system, βKlotho is selectively expressed in the suprachiasmatic nucleus and the dorsal-vagal complex. Using tissue-specific knock-out mice, we show that βKlotho in the nervous system is required for FGF21 to exert its effects on energy expenditure, body weight, and other metabolic parameters in obese mice. FGF21 acts centrally to stimulate sympathetic nerve activity and to induce uncoupling protein 1 and other thermogenic genes in adipose tissues. Our findings provide an unexpected mechanistic explanation for the strong pharmacologic effects of FGF21 on energy expenditure and body weight in obese animals.

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A Role for Macrophage Lipopolysaccharide-binding Protein in Atherosclerosis

Tamer Sallam[1, 2], Thomas Gilliland[1, 3], Ayaka Ito[1, 3], Xin Rong[1, 3], Jason Kim[4], Caroline Van Stijn[4], Brian T. Chamberlain[5, 6], Michael Jung[5, 6], Lily C. Chao[7], Marius Jones[1, 3], XiaoHui Wu[2], Grace Su[8, 9], Rajendra K. Tangirala[3], Peter Tontonoz[1, 2], Cynthia Hong[1, 2]

[1]Howard Hughes Medical Institute, UCLA, Los Angeles, CA; [2]Department of Medicine, Division of Cardiology, UCLA, Los Angeles, CA; [3]Department of Pathology and Laboratory Medicine, UCLA, Los Angeles, CA; [4]Department of Medicine, Division of Endocrinology, UCLA, Los Angeles, CA; [5]California NanoSystems Institute, UCLA, Los Angeles, CA; [6]Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA; [7]Saban Research Institute, Children's Hospital Los Angeles, Los Angeles, CA; [8]Medical Service, Department of Veterans Affairs Medical Center, Ann Arbor, MI; [9]Department of Medicine, University of Michigan Medical School, Ann Arbor, MI

The liver X receptors (LXRs) are a family of nuclear receptors that regulate cellular sterol homeostasis and inflammation. The loss of LXR in macrophages greatly accelerates the development of atherosclerosis, whereas pharmacological LXR activation has potent athero-protective effects. Targeted deletion of LXR target genes in macrophages can have opposing effects on atherosclerosis development. For example, deletion of ABCA1 using bone marrow transfer in atherogenic mouse models showed increased atherosclerotic plaque burden due to impairment of reverse cholesterol transport. Conversely, knockout of ABCG1 or Aim1 decreased lesion formation in bone marrow transplantation experiments due to an increase in macrophage apoptosis. We identify the lipopolysaccharide-binding protein (LBP), a secreted glycoprotein, as an LXR target in macrophages. Treatment with modified LDL or oxysterols induces LBP expression in an LXR-dependent manner, suggesting a potential role for LBP in the cellular response to cholesterol overload. To investigate this further, we performed bone marrow transplant studies using WT or $LBP^{-/-}$ donors and $LDLR^{-/-}$ recipients. After 18 weeks of Western diet, atherosclerotic lesion burden was assessed by *en face* and aortic root section analysis. $LDLR^{-/-}$ mice receiving $LBP^{-/-}$ bone marrow had markedly smaller lesions compared with those receiving WT bone marrow ($p < 0.0001$ in *en face* analysis). Furthermore, loss of bone marrow LBP expression lead to a significant increase in apoptosis in atherosclerotic lesions as determined by TUNEL staining. *In vitro* studies with isolated macrophages showed that LBP expression does not affect cholesterol efflux but promotes the survival of macrophages in the setting of cholesterol loading. Our study outlines an unexpected role for bone marrow-derived LBP in macrophage survival and atherosclerosis that may be exploited from a diagnostic or therapeutic standpoint.

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Exploring the Binding Partner of TTC39B by Using LC/MS Analysis in HeLa Cells

Masahiro Koseki[1, 2], Ayami Saga[1], Joanne Hsieh[2], Alan R. Tall[2], Yasushi Sakata[1], Shizuya Yamashita[1]

[1]Cardiovasucular Medicine, Osaka University Graduate School, Osaka, Japan; [2]Molecular Medicine, Columbia University Medical Center, New York, NY

TTC39B (T39) was identified in a genome-wide association study as a novel gene influencing HDL cholesterol (HDL-C) levels. We have shown that HDL-C levels were significantly increased in *T39*$^{-/-}$ mice on a chow diet. Moreover, *T39*$^{-/-}$ mice were protected from fatty liver when mice were challenged with a high fat/high cholesterol/bile salt diet and *Ldlr*$^{-/-}$,*T39*$^{-/-}$mice on the Western diet had shown increased HDL-C, decreased V/LDL cholesterol, and decreased atherosclerosis. However, molecular mechanism has not been fully understood. To know intracellular localization of *T39*, we generated the HeLa cells stably expressing *T39* with an AcGFP tag. By using confocal laser-scanning microscopy, most AcGFP-tagged *T39* genes were homogeneously distributed in cytoplasm but some were localized in the nucleus. Overexpressed *T39* did not have any impact on expression levels of LXR target genes. Second, we established other HeLa cells that expressed drug-inducible *T39* with a FLAG tag. Under the relatively low level of expression of *T39*, which was controlled by doxycycline concentration, we performed co-immunoprecipitation experiments using monoclonal anti-FLAG antibody-conjugated magnetic beads. We identified PRMT5 as one of the binding partners with *T39* by LC/MS analysis.

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Neuronal Signals via the Hepatic Vagus Nerve Contribute to the Development of Obesity and Steatohepatitis in *Pemt*$^{-/-}$ Mice Fed a High Fat Diet

Xia Gao[1], Jelske N. van der Veen[1], Linfu Zhu[2], Todd Chaba[3], Marta Ordoñez[4], Antonio Gomez-Muñoz[4], Dennis E. Vance[1], René L. Jacobs[5]

[1]Department of Biochemistry, University of Alberta, Edmonton, AB, Canada; [2]Department of Surgery, University of Alberta, Edmonton, AB, Canada; [3]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, AB, Canada; [4]Department of Biochemistry and Molecular Biology, University of the Basque Country (UPV/EHU), Bilbao, Spain; [5]Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB, Canada

Phosphatidylethanolamine *N*-methyltransferase (PEMT) is an enzyme highly expressed in the liver, responsible for ~30% of hepatic phosphatidylcholine biosynthesis through the sequential methylation of phosphatidylethanolamine. When fed a high fat (HF) diet, *Pemt*$^{-/-}$ mice are protected from adiposity and insulin resistance; however, these mice develop steatohepatitis. The vagus nerve relays signals between the liver and brain, regulating peripheral adiposity and pancreas function. To explore a possible role of the hepatic branch of the vagus nerve in HF-induced steatohepatitis and resistance to the HF-induced obesity in *Pemt*$^{-/-}$ mice, 8-week-old*Pemt*$^{-/-}$ and *Pemt*$^{+/+}$ mice were subjected to hepatic vagotomy (HV) compared with sham-operated mice, and then fed the HF diet for 10 weeks. Dramatically, HV abolished the protection against HF-induced obesity and glucose intolerance in *Pemt*$^{-/-}$ mice. HV also reduced oxygen consumption in *Pemt*$^{-/-}$ mice. Moreover, HV prevented hepatomegaly and hepatic triacylglycerol accumulation in *Pemt*$^{-/-}$ mice. HV increased the hepatic anti-inflammatory cytokine interleukin-10, reduced chemokine monocyte chemotactic protein-1, and protected *Pemt*$^{-/-}$ mice from the HF-induced steatohepatitis, showing less cellular inflammation and ballooning in the liver and normal plasma alanine aminotransferase. Furthermore, HV reversed the lower expression of mitochondrial electron transport chain proteins and the higher expression of C/EBP homologous protein, an ER stress marker, in the liver of *Pemt*$^{-/-}$ mice. HV reversed the lower expression of proteins involved in fatty acid synthesis, acetyl-CoA carboxylase and fatty acid synthase. Our data suggest that neuronal signals via the hepatic vagus nerve contribute to the protection against diet-induced obesity and the development of steatohepatitis in HF-fed *Pemt*$^{-/-}$ mice.

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Acute Knockdown of Hepatic Apoc3 Induces Rapid Regression of Pre-established Hypertriglyceridemia

Lazar A. Bojic[1], Sheng-Ping Wang[1], Sajesh Parathath[1], Ray Rosa[1], Walter Strapps[2], Laura Sepp-Lorenzino[2], Marija Tadin-Strapps[2], Douglas G. Johns[1]

[1]Merck & Co. Inc., Merck Research Laboratories, Kenilworth, NJ; [2]Merck & Co. Inc., RNA Discovery Biology, West Point, PA

A common characteristic of diabetic-dyslipidemia is increased plasma concentrations of triglyceride (TG)-rich very low density lipoprotein (VLDL), an independent risk factor for the development of coronary heart disease. Given the well established relationship between negative regulators of lipoprotein lipase activity and plasma VLDL, the antagonism of apoC-III is a potential therapeutic strategy for the treatment of hypertriglyceridemia. However, the effect of inhibiting *Apoc3* in a setting of pre-established hypertriglyceridemia has not been reported. We used siRNA to silence *Apoc3* in male *Ldlr*$^{-/-}$ mice that had been fed a high fat diet for the preceding 4 weeks. Ten days after injection with siRNA, liver *Apoc3* mRNA was markedly reduced (87%), whereas intestinal *Apoc3* mRNA was unchanged. The hepatic knockdown of *Apoc3* was accompanied with decreased plasma TG levels (~30%); total plasma cholesterol was unaffected. Reduced plasma apoC-III is known to enhance fatty acid (FA) delivery to peripheral tissues, potentially stimulating a local inflammatory response. However, si-*Apoc3* animals exhibited reduced pro-inflammatory cytokine expression (15-40%) and increased anti-inflammatory cytokine expression (~3-fold) in adipose tissue and muscle. Additional analyses revealed that hepatic knockdown of *Apoc3* enhanced the expression of genes involved in FA oxidation in muscle. These studies demonstrate that liver-specific silencing of *Apoc3* reduces pre-established hypertriglyceridemia, with dampened peripheral inflammatory tone.

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Gender-specific Differences in Sterol Metabolism and Diabetes Risk in the Insulin Resistance Atherosclerosis Study

Tracy Shafizadeh[1], Lynn Wagenknecht[2], Steven Watkins[1], Waqas Qureshi[2]

[1]Metabolon, Inc., Durham, NC; [2]Wake Forest School of Medicine, Winston-Salem, NC

Dysregulation in sterol metabolism is central to cardiovascular diseases, but a link between total blood cholesterol and type 2 diabetes risk has not been established. Although total cholesterol levels do not predict incident diabetes, several reports link T2D, IR, and other dysregulations of glucose metabolism to changes in the increased levels of cholesterol synthesis intermediates and decreased levels of cholesterol absorption. It is not clear whether sterol intermediate concentrations are predictive of conversion to T2D; nor has the effect of gender on these associations been established. To address this, we quantitatively measured 22 metabolites, covering sterol absorption, synthesis, and excretion, in baseline serums from 690 subjects in

the Insulin Resistance Atherosclerosis Study (IRAS). IRAS is a 5-year prospective multiethnic cohort with frequently-sampled IVGTT measures taken at baseline. We identified associations between each sterol and insulin sensitivity (SI) and the 5-year risk of incident diabetes. As expected, serum cholesterol was not associated with diabetes risk in this study. Baseline serum sterol concentrations were significantly different in this population when stratified by gender. Endogenously produced sterols, such as lanosterol and lathosterol, however, were significantly associated with diabetes risk in females but not males. Alternatively, markers of sterol absorption, including phytosterols, were protective and remained significant after controlling for clinical variables, such as fasting glucose, insulin, and BMI. No gender-specific differences were found in the association of sterol absorption intermediates and diabetes risk. Surprisingly, the associations of sterol absorption and cholesterol synthesis markers with incident diabetes were independent phenomena.

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A Unique Class of Surfactant Concentrated in Mammalian Bile

Jacob M. McGlashon[1, 2], Matthew P. Gillum[1, 2]

[1]University of Iowa, Iowa City, IA; [2]University of Copenhagen, Copenhagen, Denmark

N-acyl taurines (NATs) are polar endogenous lipids of unknown function. Because genetic deletion of the enzyme (which is principally present in the liver and gastrointestinal tract) that degrades them causes hepatic insulin resistance, we hypothesized that they might be metabolic regulators. Here we show that they are nearly identical in structure to *N*-oleoyl *N*-methyl taurine, a compound from cattle bile used as a soap substitute during World War I. We further demonstrate that the most abundant NAT in humans, *N*-arachidonyl taurine, exhibits surfactant properties that rival those of the commercial detergent sodium dodecyl sulfate. Finally, we show that NATs are concentrated in bile in both humans and rodents. These findings suggest that NATs comprise a distinctive class of natural detergent that may regulate nutrient emulsification and metabolic homeostasis in a capacity that complements canonical bile acids.

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Cavin-3 Protects against Cachexia

Victor J. Hernandez[1], Hongyun Dong[1], Jian Weng[1], Peter Michaely[1]

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

Cachexia is a wasting syndrome that affects both adipose tissue and skeletal muscle. *In vivo* knockout of cavin-3 causes cachexia. The goal of this project is to determine why loss of cavin-3 does so. Cavin-3 is a cytoskeletal linker protein that anchors caveolae to the peripheral actin cytoskeleton. This linkage facilitates signal transduction between surface receptors and ERK, resulting in increased levels of active ERK (pERK) and reduced levels of active Akt (pAkt). Cavin-3 reduces pAkt levels because pERK drives expression of the transcription factor, early growth response protein 1 (EGR1). EGR1 is necessary for normal expression of the phosphatase and tensin homolog protein (PTEN). Activation of Akt requires phosphatidylinositol 3,4,5-trisphosphate (PIP3), and PTEN dephosphorylates PIP3 back to phosphatidylinositol 4,5-bisphosphate (PIP2). Loss of cavin-3 greatly reduces pERK levels and largely eliminates expression of EGR1 and PTEN with a concomitant increase in the level of pAkt. Our data now show that the changes to pERK and pAkt caused by loss of cavin-3 drive production of multiple cachectic factors, including tumor necrosis factor α (TNF), interleukin 6 (IL-6), and zinc α2-glycoprotein (ZAG). This combination of factors is necessary and sufficient to drive triglyceride lipolysis in adipocytes and myofilament proteolysis in skeletal muscle fibers. Our data also show that cavin-3 normally functions as part of a pathway that determines whether cells undergo apoptosis or proliferate in response to stress. We propose that cavin-3 function is down-regulated during tissue repair and that the consequent production of cachectic factors facilitates proliferation of the cells that regenerate damaged tissues by mobilizing fatty acids and amino acids from adipose and skeletal muscle stores. Cachexia is the principal cause of death for many individuals with chronic diseases, such as chronic obstructive pulmonary disease (COPD), chronic heart disease (CHD), cancer, and chronic kidney disease (CKD). These diseases all involve tissue damage, and this damage may drive cachexia via suppression of cavin-3 function.

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Los Angeles, CA 90095-1662
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Cardiovascular Research Institute
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555 Mission Bay Blvd South
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Department of Nutrition
665 Huntington Avenue
Building 1 Room 207
Boston, Massachusetts 02115
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University of California, Los Angeles
Department of Medicine
Division of Cardiology
650 Charles E. Young Drive, South
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Los Angeles, CA 90095
Phone: (310) 825-4934
FAX: (310) 206-0865

LOCAL ARRANGEMENTS

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
bgordon@asbmb.org

Conference Attendees

Domenico Accili
Columbia University
New York, NY, USA
da230@columbia.edu

Ahmad Al-Sarraf
UBC
KUWAIT, Kuwait
hoora2000@gmail.com

Kirstin Albers
University Medical Center Hamburg-Eppendorf
Hamburg, Germany
k.albers@uke.de

Mohmed Ashmaig
Health Diagnostics Laboratory
Richmond, VA, USA
mashmaig@hdlabinc.com

Debapriya Basu
SUNY Downstate Medical Center
Brooklyn, NY, USA
debapriya.basu@downstate.edu

Simon Beaven
UCLA
Los Angeles, CA, USA
sbeaven@mednet.ucla.edu

Anne Beigneux
UCLA
Los Angeles, CA, USA
abeigneux@mednet.ucla.edu

Daniel Bloomfield
Merck & Co
Rahway, NJ, USA
daniel_bloomfield@merck.com

Lazar Bojic
Merck Research Laboratories
Kenilworth, NJ, USA
lazar.a.bojic@gmail.com

Karin Bornfeldt
University of Washington
Seattle, WA, USA
bornf@uw.edu

Martin Brenner
Merck
Kenilworth, NJ, USA
martin.brenner@merck.com

Francois Briand
Physiogenex
Labege, France
f.briand@physiogenex.com

John Brunzell
University of Washington
Seattle, WA, USA
brunzell@u.washington.edu

Laura Calabresi
University of Milano
Milano, Italy
laura.calabresi@unimi.it

Qiong June Cao
Amgen
South San Francisco, CA, USA
qcao@amgen.com

Arthur Charles
UCSF
Suasalito, CA, USA
macharle@uci.edu

Ajay Chawla
UCSF
San Francisco, CA, USA
ajay.chawla@ucsf.edu

Soo-Ho Choi
UCSD
La Jolla, CA, USA
soc002@ucsd.edu

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

Jane C. De Souza
Columbia University
New York, NY, USA
janenutri@gmail.com

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

Wieneke Dijk
Wageningen University
Wageningen, Netherlands
wieneke.dijk@wur.nl

Ximing Du
The University of New South Wales
Sydney, Australia
x.r.du@unsw.edu.au

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

Peter Edwards
University of California Los Angeles
Los Angeles, CA, USA
pedwards@mednet.ucla.edu

Meric Erikci Ertunc
Harvard School of Public Health
Boston, MA, USA
merikci@fas.harvard.edu

Derek Erion
Pfizer Inc.
Cambridge, MA, USA
derek.erion@pfizer.com

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

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

Longhou Fang
UC San Diego
La Jolla, CA, USA
lofang@ucsd.edu

Robert Farese
J. David Gladstone Institute
San Francisco, CA, USA
bfarese@gladstone.ucsf.edu

Loren Fong
UCLA
Los Angeles, CA, USA
lfong@mednet.ucla.edu

Guido Franceschini
University of Milano
Milano, Italy
guido.franceschini@unimi.it

Omar Francone
Shire Pharmaceuticals
Lexington, MA, USA
ofrancone@shire.com

Jacob Friedman
University of Colorado Denver
Aurora, CO, USA
jed.friedman@ucdenver.edu

Philip Frost
UCSF
San Francisco, CA, USA
philip.frost@ucsf.edu

Xia Gao
University of Alberta
Edmonton, AB, Canada
xgao5@ualberta.ca

Ricardo Garcia
Bristol Myers Squibb
Pennington, NJ, USA
ricardo.garcia@bms.com

Thomas Gilliland
University of California, Los Angeles
Los Angeles, CA, USA
thomas.gilliland@gmail.com

Matthew Gillum
University of Copenhagen
Copenhagen, Denmark
zlv613@ku.dk

Ayelet Gonen
UCSD
La Jolla, CA, USA
agonen@ucsd.edu

Barbara Gordon
American Society for Biochemistry & Molecular Biology
Rockville, MD, USA
bgordon@asbmb.org

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

Kristina Gottschling
University Medical Center Hamburg-Eppendorf
Hamburg, Germany
k.gottschling@uke.de

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

Rebecca Haeusler
Columbia University
New York, NY, USA
rah2130@cumc.columbia.edu

Joerg Heeren
University Medical Center Hamburg Eppendorf
Hamburg, Germany
heeren@uke.de

Markus Heine
University Medical Center Hamburg Eppendorf
Hamburg, Germany
ma.heine@uke.de

Marten Hofker
University Medical Center Groningen
Groningen, Netherlands
m.h.hofker@umcg.nl

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

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

Eva Hurt-Camejo
AstraZeneca
Mölndal, Sweden
Eva.Hurt-Camejo@astrazeneca.com

Lin Jia
UT Southwestern Medical Center
Dallas, TX, USA
lin.jia@utsouthwestern.edu

Tara Jois
Monash University
Melbourne, Australia
tnjoi1@student.monash.edu

Marius Jones
UCLA
Los Angeles, CA, USA
mcjones@mednet.ucla.edu

John Kane
UCSF
San Francisco, CA, USA
john.kane@ucsf.edu

Sekar Kathiresan
MGH
Boston, MA, USA
skathiresan@partners.org

Sander Kersten
Wageningen University
Wageningen, Netherlands
sander.kersten@wur.nl

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

Masahiro Koseki
Osaka University Graduate School
Suita, Japan
kosekey@yahoo.co.jp

Mark Kowala
Eli Lilly and Company
Indianapolis, IN, USA
kowala_mark@lilly.com

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

Richard Lee
Isis Pharmaceuticals
Carlsbad, CA, USA
rlee@isisph.com

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

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

Chen Li
University of Alberta
Edmonton, AB, Canada
cli10@ualberta.ca

Jihong Lian
University of Alberta
Edmonton, AB, Canada
jlian1@ualberta.ca

Chao Liu
UCSD
San Diego, CA, USA
chl029@ucsd.edu

David Mangelsdorf
UT Southwestern Medical Center
Dallas, TX, USA
davo.mango@utsouthwestern.edu

Brendan Manning
Harvard School of Public Health
Boston, MA, USA
bmanning@hsph.harvard.edu

Laura Michael
Eli Lilly and Company
Indianapolis, IN, USA
michael_laura@lilly.com

Peter Michaely
University of Texas Southwestern Medical Center
Dallas, TX, USA
peter.michaely@utsouthwestern.edu

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

Matthew Molusky
Columbia University Medical Center
New York, NY, USA
mmm2338@columbia.edu

Sarah Nordstrom
UCSF
San Francisco, CA, USA
sarah.nordstrom@ucsf.edu

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

Monika Oberer
University of Graz
Graz, Austria
m.oberer@uni-graz.at

Eveline Oestreicher Stock
UCSF
San Francisco, CA, USA
eveline.sf@gmail.com

Bryn Owen
UT Southwestern Medical Center
Dallas, TX, USA
bryn.owen@utsouthwestern.edu

Lynne Parker
JT Pharma
New York, NY, USA
parker@trilliummed.com

John Parks
Wake Forest School of Medicine
Winston-Salem, NC, USA
jparks@wakehealth.edu

Sampath Parthasarathy
University of Central Florida
Orlando, FL, USA
spartha@ucf.edu

Xiao-Rong Peng
Astrazeneca RD Sweden
Mölndal, Sweden
xiao-rong.peng@astrazeneca.com

Robert Phair
IBI
Mountain View, CA, USA
rdphair@gmail.com

Stephen Pinkosky
Esperion
Plymouth, MI, USA
spinkosky@esperion.com

Michael Ploug
State University Hospital
Copenhagen, Denmark
m-ploug@finsenlab.dk

Nina Pollak
University of Graz
Graz, Austria
nina.pollak@uni-graz.at

Matthew Potthoff
University of Iowa
Iowa City, IA, USA
matthew-potthoff@uiowa.edu

Christina Priest
UCLA
Los Angeles, CA, USA
CPriest@mednet.ucla.edu

Clive Pullinger
UCSF
San Francisco, CA, USA
clive.pullinger@ucsf.edu

Daniel Rader
Univ of Pennsylvania
Philadelphia, PA, USA
rader@mail.med.upenn.edu

Karishma Rahman
NYU Langone Medical Center
New York, NY, USA
karishma.rahman24@gmail.com

Prashant Rajbhandari
University of California-Los Angles
Los Angles, CA, USA
prajbhandari@ucla.edu

Karen Reue
UCLA
Los Angeles, CA, USA
reuek@ucla.edu

Shunxing Rong
UT Southwestern Medical Center
Dallas, TX, USA
shunxing.rong@utsouthwestern.edu

Xin Rong
University of California, Los Angeles
Los Angeles, CA, USA
xrong@ucla.edu

Kerry-Anne Rye
Centre for Vascular Research, University of New South Wales
Sydney, Australia
karye@ozemail.com.au

Juro Sakai
The University of Tokyo, RCAST
Tokyo, Japan
jmsakai-tky@umin.ac.jp

Tamer Sallam
UCLA
Los Angeles, CA, USA
sallamt@gmail.com

Jaspreet Sandhu
UCLA
Los Angeles, CA, USA
jsandhu1@ucla.edu

Nijole Savickiene
Lithuanian University of Health Sciences
Kaunas, Lithuania
savickienenijole@takas.lt

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

Marcus Schindler
AstraZeneca
Mölndal, Sweden
Marcus.Schindler@astrazeneca.com

Dina Schneider
UC San Diego
La Jolla, CA, USA
dschneider@ucsd.edu

Renate Schreiber
University of Graz
Graz, Austria
renate.schreiber@uni-graz.at

Tracy Shafizadeh
Metabolon, Inc.
West Sacramento, CA, USA
tshafizadeh@metabolon.com

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

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

Carol Shoulders
William Harvey Research Institute
London, United Kingdom
c.shoulders@qmul.ac.uk

Alan Shuldiner
Univ of Md Baltimore
Baltimore, MD, USA
ashuldin@medicine.umaryland.edu

Debra Simmons
University of Utah
Salt Lake City, UT, USA
Debra.Simmons@hsc.utah.edu

Roshni Singaraja
National University of Singapore
Singapore
roshni@cmmt.ubc.ca

Mark Sleeman
Monash University
Melbourne, Australia
mark.sleeman@monash.edu

Aline Smalinskiene
Lithuanian University of Health Sciences
Kaunas, Lithuania
smalinskienealina@takas.lt

Jun Sonoda
Genentech, Inc.
South San Francisco, CA, USA
junichis@gene.com

Ira Tabas
Columbia University
New York, NY, USA
iat1@columbia.edu

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

Wensi Tao
University of Miami
Miami, FL, USA
wtao@med.miami.edu

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

Ryan Temel
University of Kentucky
Lexington, KY, USA
ryan.temel@uky.edu

Nina Titova
University of California Riverside
Riverside, CA, USA
ntitova@ucr.edu

Peter Tobias
Scripps Research Institute
La Jolla, CA, USA
tobias@scripps.edu

Peter Tontonoz
University of California Los Angeles
Los Angeles, CA, USA
ptontonoz@mednet.ucla.edu

Thomas Vallim
UCLA
Los Angeles, CA, USA
tvallim@mednet.ucla.edu

Murielle Véniant
Amgen
Thousand Oaks, CA, USA
mveniant@amgen.com

Laurent Vergnes
UCLA
Los Angeles, CA, USA
lvergnes@ucla.edu

Claudio Villanueva
University of Utah School of Medicine
Salt Lake City, CA, USA
villanueva@biochem.utah.edu

Tobias Walther
Yale School of Medicine
New Haven, CT, USA
tobias.walther@yale.edu

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

Steven Watkins
Metabolon, Inc.
West Sacramento, CA, USA
swatkins@metabolon.com

Ethan Weiss
UCSF
San Francisco, CA, USA
ethan.weiss@ucsf.edu

Marit Westerterp
Columbia University
New York, NY, USA
mw2537@cumc.columbia.edu

Philipp Wiesner
University of California, San Diego
La Jolla, CA, USA
pwiesner@ucsd.edu

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

Samuel Wright
CSL Limited
Westfield, NJ, USA
samuel.wright@cslbehring.com

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

Yu-Xin Xu
Massachusetts General Hospital
Boston, MA, USA
yxu17@partners.org

Zemin Yao
University of Ottawa
Ottawa, ON, Canada
zyao@uottawa.ca

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

Liqing Yu
University of Maryland
College Park, MD, USA
lyu123@umd.edu

Rudolf Zechner
University of Graz
Graz, Austria
rudolf.zechner@uni-graz.at

Notes

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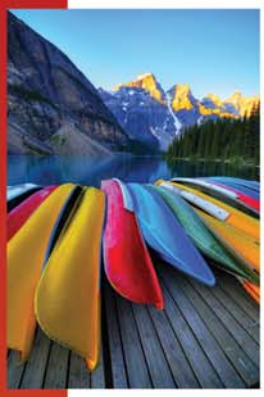

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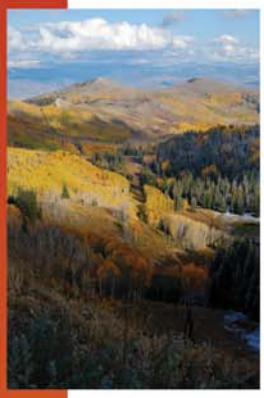

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