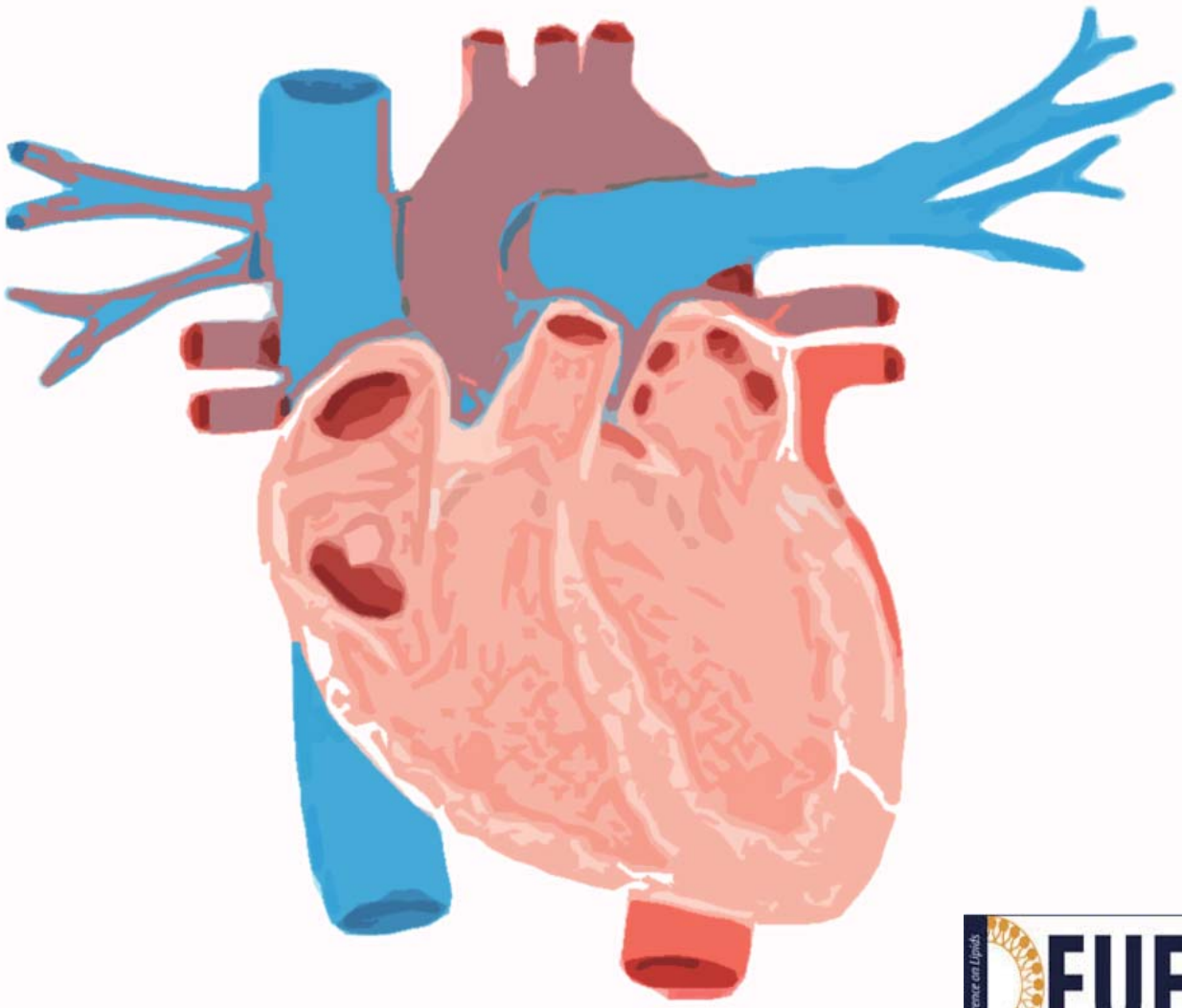
March 5–8, 2013

Napa Valley, CA

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

DEUEL CONFERENCE ON LIPIDS



DEUEL Conference on Lipids

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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 five scientific sessions, with an eminent lipid scientist chairing each session Each session includes three to four original scientific presentations followed by in-depth discussions of the topic.

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

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ASBMB Lipid Research Division

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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; 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.

2013 - Rick Lifton, Yale University

"From human genetics to validated therapeutic targets"

2012 Gokhan Hotamisligil, Harvard University

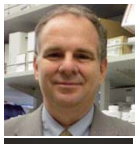
"Inflammation, Endoplasmic Reticulum Stress and Lipids:
Emerging Networks Regulating Metabolism"

2011 Christopher K. Glass, University of California San Diego

"Oxysterol regulation of macrophage gene expression"

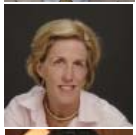
2010 David J. Mangelsdorf, University of Texas Southwestern

"Nuclear receptor control of lipid metabolism"



2009 Stephen G. Young, UCLA School of Medicine

"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 Medical Center, Dallas, TX

"SREBPs: Master Regulators of Lipid Metabolism" 2002 - Joseph L. Goldstein, UT Southwestern

Schedule of Events

	Tuesday, March 5	Wednesday, March 6	Thursday, March 7		Friday, March 8
7 AM		Breakfast 7-8:30	Breakfast 7-8:30	Board Mtg. 7:00- 8:30	Breakfast 7-8:30
8 AM					
9 AM		Session I 8:30-10:00	Session 2 8:30-10:15		Session 4 8:30-10:15
10 AM		Coffee Break 10:00	Coffee Break 10:15		Coffee Break 10:15
11 AM		Session I, Continued 10:15-11:45	Session 2, Continued 10:30-12:00		Session 4, Continued 10:30-12:00
12 PM		Free Time			
1 PM			Free Time		
2 PM					
3 PM	Registration 3-6:30				
4 PM					
5 PM			Sponsor Reception 5:00-6:00		
6 PM	Opening Reception & Dinner 6:30	Dinner 6:00	Dinner 6:00		
7 PM	Welcome Reception and Dinner	HAVEL LECTURE 7:30-8:30	Session 3 7:30-9:30		
8 PM					
9 PM					
10 PM		Poster Session 8:30			

Meeting Program

The Deuel Conference on Lipids, March 5–8, 2013
Silverado Resort, Napa Valley, California

"New biology and therapeutic targets revealed through human genetics"

Tuesday, March 5

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

Wednesday, March 6

Wednesday, March 6, 8:30 – 11:45 AM
Session Chair: Richard Lehner

Session 1 **Novel pathways regulating lipoprotein metabolism**

8:30 – 9:05 "MicroRNAs in the regulation of lipoprotein metabolism"
Kathryn Moore, New York University, New York, NY

9:05 -9:40 "Role of ABC transporters in atherosclerosis and autoimmunity"
Alan Tall, Columbia University, New York, NY

9:40 -10:00 "TDAG51 deficiency protects against atherosclerosis by modulating cholesterol efflux, apoptosis, and peroxiredoxin-1 expression"
Edward Lynn, McMaster University, Hamilton, Ontario, Canada

10:00 - 10:15 Coffee Break

10:15-10:50 "Novel regulators of VLDL assembly"
Edward A. Fisher, New York University, New York, NY

10:50 -11:05 "Diet1 Functions in the FGF15/19 Enterohepatic Signaling Axis to Modulate Bile Acid and Lipid Levels"
Jessica Lee, UCLA, Los Angeles

11:05-11:40 "The role of Tribbles in lipid metabolism and vascular biology"
Endre Kiss-Toth, University of Sheffield, Sheffield,UK

11:40 – 12:00 "Tribbles-1 regulates hepatic lipogenesis in the mouse"
Robert Bauer, University of Pennsylvania, Philadelphia, PA

Wednesday, March 6, 7:30 – 10:00 PM
Session Chair: Daniel Rader

7:30-8:30 The Havel Lecture: "From human genetics to validated therapeutic targets"
Richard P. Lifton, Yale University, New Haven, CT

8:30-10 Wine reception and Poster Session

Thursday, March 7

Thursday, March 7, 8:30 AM to 12:00 Noon
Session Chair: Ajay Chawla

Session 2 **Approaches to new pathway discovery**

8:30 -9:05 "New genes associated with plasma lipid traits"
Cristin Willer, University of Michigan, Ann Arbor, MI

9:05 -9:40 "Exome and whole genome sequencing as a discovery tool"
Deborah Nickerson, University of Washington, Seattle, WA

9:40-10:15 "Exome sequencing for common complex diseases: myocardial infarction"
Sekar Kathiresan, Massachusetts General Hospital, Boston, MA

10:15- 10:30 Coffee Break

10:30 –11:05 "Cell-based screening for cholesterol traits"
Heiko Runz, University of Heidelberg, Heidelberg, Germany

11:05-11:40 "Cellular response to statins reveals unexpected new pathways"
Ron Krauss, Children's Hospital of Oakland Research Institute, Oakland, CA

11:40-12:00 "HNRNPA1 regulates HMGCR alternative splicing and modulates cholesterol metabolism"
Chi-Yi Yu, Children's Hospital of Oakland Research Institute, Oakland, CA

Thursday, March 7, 7:30-10:15 PM

Session Chair: Karin Bornfeldt

Session 3 New pathways in atherosclerosis and coronary artery disease

7:30 – 8:05 **The *Journal of Lipid Research* Lecture**
"Sphingosine-1-phosphate signaling and cardiovascular disease"
Introduction: Stephen G. Young, University of California, Los Angeles, CA
Shaun Coughlin, University of California, San Francisco, San Francisco, CA

8:05-8:25 "Liver-specific overexpression of apo-M stimulates production of larger, apo-M/S1P-enriched plasma HDL"
Mingxia Liu, Wake Forest School of Medicine, Winston-Salem, NC

8:25- 9:00 "The biology of TCF21 and its relationship to atherosclerosis"
Tom Quertermous, Stanford University, Stanford, CA

9:00-9:35 "The role of the metalloproteinase ADAMTS7 in vascular injury and atherosclerosis"
Muredach Reilly, University of Pennsylvania, Philadelphia, PA

9:35-9:55 "HDAC9 deficiency in myeloid cells protects against atherosclerosis in mice"
Nilamadhab Mishra, Wake Forest School of Medicine, Winston-Salem, NC

9:55 -10:15 "Competitive binding of CXCL12 to CXCR7 reduces atherosclerosis in apoE-deficient mice"
Miao Wang, Pfizer, Cambridge, MA

Friday, March 8

Friday, March 8, 8:30 AM to 12:00 PM

Session Chair: Murielle Véniant

Session 4 Translating biology into therapeutics

8:30 -9:05 "DGAT1 inhibition"
Deborah Keefe, Novartis, East Hanover, NJ

9:05 -9:40 "Apo-CIII inhibition"
Rosanne Crooke, Isis, Carlsbad, CA

9:40-10:15 "Gene therapy for the treatment of LPL deficiency: Journey from the laboratory to the clinic"
Colin Ross, University of British Columbia, Vancouver, BC, Canada

10:15- 10:30 Coffee Break

10:30 – 10:45 "Lipoprotein Lipase Activator LP071 Improves the Plasma Lipid Profile in ApoE3L:CETP Mice"
Stefan K. Nilsson, Umeå University, Umeå, Sweden

10:45 – 11:20 "Update on clinical development of CSL II2, a recombinant HDL infusion therapy""
Sam Wright, CSL, Ltd., Westfield, NJ

11:20-11:40 "Response of subjects with familial hypercholesterolemia to treatment with RN316, a PCSK9-binding antibody"
Bart Duell, Oregon Health and Science University, Portland, OR

11:40-12:00 "An oxidation-specific antibody reduces atherosclerosis and hepatosteatosis in LDLR-/- mice"
Xuchu Que, University of California, San Diego, La Jolla, CA

Poster Presentations

1
Rapid Lipoprotein Density Profiling using Microliter Quantities of Serum or Plasma

Rapid Lipoprotein Density Profiling using Microliter Quantities of Serum or Plasma

Rosemary L. Walzem1, Xiuzhi Wu1, Tomomi Minamoto2, Jan Suchodolski2, and Jorg Steiner2

1Poultry Science Department, 2Small Animal Clinical Sciences, School of Veterinary Medicine, Texas A&M University, College Station, TX

Continuous density profiling resolves key physical property of lipoproteins. The approach is underutilized compared with size exclusion chromatography due to perceived technical complexity and analytical time requirements. Improved methods in ultracentrifugation, lipoprotein staining and imaging, and image informatics now allow for rapid density profiling using as little as 6 µl of plasma or serum. Samples from a wide variety of species and clinical conditions were profiled after NBD-C6-ceramide staining using self-forming gradients of differing steepness within a MLA-130 rotor and a Beckman Optima MAX-LP ultracentrifuge. 0.18 M NaBi-EDTA gradient (1.0063–1.2200 g/ml) and 0.24 M CsBi-EDTA gradient (1.000–1.261 g/ml) allowed for improved imaging of lower and higher density regions, respectively. Paired human plasma and serum samples from the same blood draw showed no difference in density distributions. Reproducibility trials using human plasma of low (55.6 mg/100 ml), medium (102.2 mg/100 ml), and high (199.3 mg/100 ml) triglyceride content showed that variability within 10 defined density intervals averaged 5% within day (n = 3) and 3.5% between days (n = 5). Variability in TGRL and HDL3c regions were greatest but averaged <10%. Bovine serum albumin (Sigma A7638) was imaged and used to identify the serum albumin region within intact serum lipoprotein density profiles and was determined to begin at 1.116 g/ml, indicating that fatty acids were complexed to albumin. A stability study, comparing freshly prepared plasma (n = 3) with samples stored at –80°C for 1, 2, 3, or 4 months and thawed once, showed similar patterns. Density distributions of samples frozen and thawed two or three times were also remarkably similar; perhaps due to the small sample volume used. Density profiles from >15 species were distinct, showing variation in LDL or HDL dominance in accord with being an herbivore, omnivore, or carnivore and species characteristics. The method demonstrates potential as a rapid screening method.

ABSTRACTS

2

Reducing Macrophage Proteoglycan Sulfation Increases Atherosclerosis via Type I Interferon Signaling

Philip L. Gordts1, Erin M. Foley1, 2, Roger Lawrence1, Chris K. Glass1, Aldons J. Lusis3, Joseph Witztum4, and Jeffrey D. Esko1, 2

1Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA; 3Department of Cardiology, UCLA, Los Angeles, CA; 4Department of Medicine, University of California San Diego, La Jolla, CA; 2Biomedical Sciences Graduate Program, University of California San Diego, La Jolla, CA

Atherogenesis initiates by retention of atherogenic lipoproteins by proteoglycans within the vessel wall. Macrophage uptake of these atherogenic lipoproteins triggers formation of foam cells and plaque deposition. To examine the role of macrophage heparan sulfate proteoglycans (HSPGs) in atherogenesis, we inactivated the biosynthetic gene GlcNAc N-deacetylase/N-sulfotransferase 1 (Ndst1) selectively in macrophages by crossing Ndst1f/f mice with LysMCre+ mice. When bred onto an Ldlr–/– background and placed on an atherogenic diet, Ndst1f/fLysMCre+Ldlr–/– mice demonstrated increased atherosclerosis compared with Ldlr–/– mice. Plaque analysis also revealed significantly increased macrophage content in lesions from Ndst1f/fLysMCre+Ldlr–/– mice. Diminished HSPG sulfation in macrophages from Ndst1f/fLysMCre+ mice resulted in significantly increased expression of inflammatory genes such as CCL5, CCL7, CCL8, and ACAT2. Increased ACAT2 expression correlated with more ACAT enzyme activity and increased foam cell formation compared with wild-type macrophages. Motif analysis of promoters of up-regulated genes revealed increased type I interferon signaling in macrophages with reduced HSPG sulfation. Also, IFN-α- and IFN-β-induced STAT1 phosphorylation was elevated in Ndst1f/fLysMCre+ macrophages. In conclusion, our data suggest that macrophage HSPGs are atheroprotective and act by maintaining type I interferon reception in a quiescent state either through sequestration of type I interferons or by forming complexes with type I interferon receptors.

3

Visualizing Intestinal Caveolin-1 Localization and Caveolar Endocytosis in the Context of Lipid Metabolism: Using the Zebrafish to Image Cell Biological Processes in Vivo

Jessica P. Otis and Steven A. Farber

Carnegie Institute, Baltimore, MD

We use larval zebrafish to study the roles of caveolae in lipid metabolism and transport in intestinal enterocytes. The larval intestine is optically clear, facilitating confocal microscopy studies of subcellular biology in the presence of bile, mucus, and symbiotic organisms, which are factors absent from cultured cells. Caveolae are pit-shaped plasma membrane (PM) endocytotic structures of caveolin-1 (Cav1). Cav1 intestinal subcellular localization and its functional significance have not been characterized. Because Cav1 binds to cholesterol and caveolae form in cholesterol rich PM regions, we hypothesized that Cav1 plays a key role in intestinal lipid metabolism. Our Cav1-GFP zebrafish exhibit Cav1 localization to the basolateral and lateral PM of enterocytes, but not to the luminal brush border, as verified by WT Cav1 IHC. A lipid-rich feed did not alter Cav1-GFP localization, suggesting that Cav1 functions on the basolateral enterocyte PM, but not in dietary lipid absorption. To visualize endocytosis in live larvae, three cell culture reagents were optimized in vivo: D-lactosylceramide (LacCer) and albumin (caveolar transport), and L-LacCer (noncaveolar transport). By basolateral injection of the fluorescent-labeled cargos, we observed D-LacCer and albumin internalization, and localization on the basolateral and lateral PM, but not on the brush border; no internalization occurred following luminal injection. L-LacCer was endocytosed by all PM regions. Ongoing research focuses on elucidating the function of Cav1 on the basolateral enterocyte PM by developing methods to disrupt caveolae in vivo. Three methods of caveolar disruption are in development: L-LacCer disruption of endocytosis, a dominant negative Cav1-GFP construct, and shRNA-mediated knockdown of Cav1. In sum, the unique properties of zebrafish facilitated the first ever in vivo imaging of intestinal caveolae and development of tools to investigate the roles of intestinal Cav1 in lipid metabolism, transport, and disease.

4

Trimethylamine-N-oxide, a Metabolite Associated with Atherosclerosis, Exhibits Complex Genetic and Dietary Regulation

Thomas A. Vallim1, Bennett J. Brian1, Zeneng Wang2, Diana Shih1, Yonghong Meng1, Jill Gregory2, Hooman Allayee3, Richard Lee4, Mark Graham4, Rosanna Crooke4, Stanley Hazen2, Jake Lusis1, and Peter A. Edwards1

1Department of Medicine, UCLA, Los Angeles, CA; 3University of Southern California Keck School of Medicine, Los Angeles, CA; 2Department of Cardiovascular Medicine, Cleveland Clinic, Cleveland, OH; 4ISIS Pharmaceuticals, Carlsbad, CA

Circulating trimethylamine-N-oxide (TMAO) levels are strongly associated with atherosclerosis. We now examine genetic, dietary, and hormonal factors regulating TMAO levels. We demonstrate that two flavin mono-oxygenase family members, FMO1 and FMO3, oxidize trimethylamine (TMA), derived from gut flora metabolism of choline, to TMAO. Further, we show that FMO3 exhibits 10-fold higher specific activity than FMO1. FMO3 overexpression in mice significantly increases plasma TMAO levels whereas silencing FMO3 decreases TMAO levels. In both humans and mice, hepatic FMO3 expression is reduced in males compared with females. In mice, this reduction in FMO3 expression is due primarily to down-regulation by androgens. FMO3 expression is induced by dietary bile acids by a mechanism that involves the farnesoid X receptor, a bile acid-activated nuclear receptor. Analysis of natural genetic variation among inbred strains of mice indicates that FMO3 and TMAO are significantly correlated, and TMAO levels explain 11% of the variation in atherosclerosis.

5

TDAG51 Deficiency Protects against Atherosclerosis by Modulating Cholesterol Efflux, Apoptosis, and Peroxiredoxin-1 Expression

Edward G. Lynn1, 2, Gazi S. Hossain1, 2, Kenneth N. Maclean3, Ji Zhou1, 2, Jeffrey G. Dickhout1, 2, Šárka Lhoták1, 2, Bernardo Trigatti1, Damu Tang1, 2, Dov Shiffman4, and Richard C. Austin1, 2

1McMaster University, Hamilton, ON, Canada; 3University of Colorado Health Sciences Center, Aurora, CO; 2St. Joseph's Healthcare, Hamilton, ON, Canada; 4Celera, Alameda, CA

Cardiovascular disease (CVD) and atherothrombosis account for the majority of deaths in North America. Acute clinical manifestations of atherothrombosis result from plaque rupture, thrombus formation, and vessel occlusion. Macrophage infiltration, lipid accumulation, and foam cell formation are key events in atherogenesis. Apoptosis caused by endoplasmic reticulum (ER) stress in lesion-resident macrophages, smooth muscle cells, and endothelial cells also contributes to atherosclerotic lesion growth and plaque necrosis. T cell death-associated gene 51 (TDAG51), a member of the pleckstrin homology-like domain gene family, is induced by ER stress, causes apoptosis when overexpressed, and is present in lesion-resident macrophages as well as vascular endothelial and smooth muscle cells. To study the role of TDAG51 in atherosclerosis, male mice deficient in TDAG51 and apolipoprotein E (TDAG51–/–/apoE–/–) were generated. TDAG51–/–/apoE–/– mice fed a normal chow diet showed reduced atherosclerotic lesion growth and necrosis without changes in plasma levels of lipids, glucose, and inflammatory cytokines. TDAG51 deficiency was associated with decreased lipid accumulation in lesions and caused several phenotypic changes in macrophages that enhanced PPARγ-dependent reverse cholesterol transport and cytoprotection against oxidative and ER stress. Cytoprotection versus ER and oxidative stress in TDAG51–/– cells was associated with increased expression of peroxiredoxin-1, an antioxidant enzyme with antiatherogenic properties. Two independent, case control studies found that genetic variants in the human TDAG51 gene region were associated with CVD. These findings provide evidence that TDAG51 impacts specific cellular pathways known to reduce atherogenesis, suggesting that modulation of TDAG51 expression or its activity may have therapeutic benefit for the treatment of CVD.

6

Growth Hormone Signaling via JAK2 in Adipocytes Regulates Insulin Sensitivity in Mice Independent of Body Composition and Hepatic Lipid Content through Alteration of Hepatic Insulin Sensitivity

Sarah M. Nordstrom, Jennifer L. Tran, Dongmei Wu, and Ethan J. Weiss

University of California San Francisco, Cardiovascular Research Institute, San Francisco, CA

Growth hormone (GH) is a known regulator of metabolism; levels of circulating GH are inversely correlated with body fat, ostensibly through its promotion of lipolysis. GH excess also leads to insulin resistance (IR), whereas disruption of GH signaling improves insulin sensitivity (IS). The mechanisms by which GH regulates carbohydrate metabolism are unclear. We disrupted GH signaling in hepatocytes by deleting the signaling mediator, JAK2 (JAK2L). JAK2L mice have elevated circulating GH and thus, greater lipolysis and reduced body fat. JAK2L have severe fatty liver (FL), hepatic IR, and whole body IR. To determine whether the IR of JAK2L mice can be attributed to the accumulation of hepatic lipid, we additionally disrupted JAK2 in adipocytes to reduce lipolysis (JAK2L/A). JAK2L/A had hepatic lipid content and IS comparable with controls. However, following high fat diet (HFD), JAK2L and JAK2L/A had similar hepatic lipid accumulation, but JAK2L/A maintained improved IS versus JAK2L, indicating that the IS is not necessarily related to hepatic lipid. Furthermore, JAK2L/A had higher body fat on both normal chow and HFD relative to matched JAK2L, indicating that the improved IS in JAK2L/A was not due a reduction in fat. To determine whether the singular deletion of JAK2 in adipocytes (JAK2A) influences IS, we compared JAK2A and controls. Like JAK2L/A, JAK2A were obese versus controls; yet, JAK2A had improved IS. Following HFD, both controls and JAK2A developed obesity, but JAK2A remained relatively IS. Improved whole body IS was not explained by changes in hepatic lipid content or IS in muscle or fat. Overall, our findings indicate that GH signaling via JAK2 in adipocytes regulates hepatic IS and whole body IS independent of changes in body fat and hepatic lipid content. We speculate that the effects of GH on IS are mediated by a JAK2-regulated adipokine that alters IS in liver. Further defining the effects of GH on metabolism could lead to novel treatments for IR that are independent of weight loss and FL

7

Novel Mouse Models to Study LDLR mRNA Stability and Regulation by the Natural Hypocholesterolemic Compound Berberine through Bioluminescence Imaging in Living Mice

Amar B. Singh, Kelvin Kan, Bin Dong, and Jingwen Liu

Veterans Affairs Palo Alto Health Care System, Palo Alto, CA

The 3′-untranslated region (UTR) of LDL receptor (LDLR) mRNA contains three AU-rich elements (AREs) responsible for rapid mRNA turnover in liver tissue and mediates the stabilization induced by the natural cholesterol-lowering compound berberine (BBR). Currently it is unclear whether 3′-UTR mediates LDLR mRNA decay in a tissue-specific fashion, and the ARE-binding proteins responsible for LDLR mRNA degradation in liver tissue remain unknown. In this study, we engineered two transgenic mouse models for studying the regulatory role of 3′-UTR in LDLR mRNA stability under in vivo conditions. First, we generated transgenic mice expressing luciferase-LDLR3′-UTR reporter gene (Luc-UTR) driven by the CMV promoter. The tissue expression pattern of Luc-UTR transcript was visualized noninvasively in living mice using in vivo bioluminescent imaging and was further analyzed by measuring luciferase activity in dissected tissues. We demonstrate that Luc-UTR transgene is expressed differentially, with highest levels in heart, intestine, muscle, skin, and testis and lowest levels in spleen and liver. These results provided the first in vivo evidence of tissue-specific regulation of LDLR mRNA stability through 3′-UTR. In the second model referred as Alb-Luc-UTR, the expression of Luc-UTR transgene is under the control of mouse albumin promoter which directs the transgene expression in liver tissue specifically. BBR treatment of Alb-Luc-UTR mice led to a 2-fold increase in bioluminescense signals, a 2-fold increase in Luc-UTR mRNA and a 30% elevation of mouse LDLR mRNA levels over control mice in liver. These changes were accompanied by a significant reduction of mRNA and protein levels of the mRNA decay-promoting protein hnRNP D in liver of BBR-treated mice. The inverse relationship of hnRNP D protein abundance and LDLR mRNA levels suggests a functional role of hnRNP D in mediating LDLR mRNA degradation in liver tissue.

8

Transgenic Ldlr–/– Mice Expressing an Oxidation-specific Antibody Have Less Atherosclerosis and Decreased Hepatosteatosis

Xuchu Que1, Antti Maatta2, Jennifer Pattison1, Karen Bowden1, Seppo Ylä-Herttuala2, Sotirio Tsimikas1, and Joseph L. Witztum1

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Innate natural antibodies (NAbs) provide the first line of host defense against common oxidation-specific epitopes (OSEs) on endogenous neo-epitopes (OxLDL and apoptotic cells) and exogenous epitopes of pathogens. OSEs are ubiquitous, formed in many inflammatory tissues, including atherosclerotic lesions, and are a major target of NAbs. We previously showed that the prototypic NAb E06, which binds the phosphocholine head group in oxidized phospholipids (OxPLs), blocks uptake of OxLDL by macrophages. To determine the impact in vivo of sustained titers of E06, we generated transgenic mice (Tg) expressing a single-chain antibody fragment (scFv) of E06 in Ldlr–/– background. The E06-scFv was secreted into the plasma and inhibited binding of OxLDL to macrophages in culture at high plasma dilution. We compared the extent of atherosclerosis in male Ldlr–/– and Tg Ldlr–/– mice fed 1% cholesterol diet for 16 weeks (n = 10-12). In the Tg mice, en face lesion area was decreased 57% (8.28 versus 3.54%) and the lesion area at the aortic root decreased 55%. Peritoneal macrophages from Tg mice had 49% less cholesterol, consistent with decreased OxLDL uptake, and also had decreased inflammatory gene expression. Plasma SAA was also reduced 32%, consistent with generalized decreased inflammation. Because macrophages also secreted E06scFv, we performed bone marrow transplantation (BMT) from Tg into irradiated Ldlr–/– recipients; this also decreased aortic root lesions by 31% compared with BMT from WT mice donors. Hepatic steatosis was also dramatically decreased in Tg mice as was hepatic inflammatory gene expression. The E06scFv lacks functional effects of an intact antibody other than the ability to bind OxPLs and inhibit OxLDL uptake in macrophages. Thus, these data demonstrate that OxPLs are profoundly proinflammatory and proatherogenic, which E06 counteracts in vivo.

9

Hepatic Clearance of Triglyceride-rich Lipoproteins by Heparan Sulfate Proteoglycan Receptors Is Mediated by Apolipoproteins E and A-V and Is Atheroprotective

Jon C. Gonzales, Philip L. Gordts, and Jeffrey D. Esko

Department of Cellular and Molecular Medicine, Glycobiology Research and Training Center, University of California, San Diego, La Jolla, CA

The heparan sulfate proteoglycan, syndecan-1, acts as a major receptor for triglyceride-rich lipoprotein (TRL) clearance in the liver. Here, we sought to identify the relevant apolipoproteins on TRLs that mediate binding to syndecan-1 and determine their clinical relevance. Evidence supporting apoE as a major determinant of binding arose from enrichment of apoE in TRLs from mice defective in hepatic heparan sulfate (Ndst1f/fAlbCre+), decreased binding of apoE–/– TRLs to heparan sulfate proteoglycans on human hepatoma cells, and decreased clearance of apoE-deficient TRLs in vivo. Evidence for a second ligand was suggested by the faster clearance of apoE-deficient TRLs after injection into wild-type mice versus Ndst1f/fAlbCre+ mice and elevated fasting and postprandial plasma triglycerides in Ndst1f/fAlbCre+; apoE–/– mice compared with either single mutant. We excluded apoB as a candidate based on lack of synthetic phenotypes in apoB mutants lacking Ndst1, normal binding of apoB-restricted particles to heparan sulfate-deficient hepatocytes, and failure of mAbs to block binding of human TRLs to isolated proteoglycan ectodomains. ApoA-V emerged as a candidate based on a 6-fold enrichment of apoA-V in TRLs accumulating in Ndst1f/fAlbCre+ mice, decreased binding of TRLs to proteoglycans after depletion of apoA-V or addition of anti-apoA-V monoclonal antibody, and decreased binding of apoA-V-depleted TRLs to hepatocytes. Importantly, disruption of hepatic heparan sulfate-mediated clearance increased atherosclerosis by 2.5-fold. We conclude that clearance of TRLs by hepatic heparan sulfate proteoglycans is atheroprotective and mediated by multivalent binding to apoE and apoA-V.

10

Familial Hypercholesterolemia: Molecular Characterization and Response to Treatment with RN316, a PCSK9-binding Antibody

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Autosomal dominant familial hypercholesterolemia (ADH or FH) is commonly caused by mutations in the low density lipoprotein receptor (LDLR) and less often by mutations in apolipoprotein B (APOB) or proprotein convertase subtilisin/kexin type 9 (PCSK9). Patients with FH require aggressive lowering of LDL cholesterol (LDL-C) because of a very high risk for coronary artery disease, stroke, and early death. Many FH patients fail to achieve desirable levels of LDL-C even with maximum doses of lipid-lowering drugs, highlighting the need for additional targeted therapies. We assessed the prevalence of mutations in these three genes in FH patients in a nontreatment study and explored the relationship between genotypes and efficacy of RN316, a humanized monoclonal antibody binding to PCSK9, in two clinical trials. We hypothesized that RN316 treatment would lead to LDL-C lowering in subjects with genotypic FH. A total of 93 men and 107 women, age 19-80 years who met Simon Broome criteria for heterozygous FH and were on concurrent LDL-C-lowering therapy with LDL-C >100 mg/dl, were recruited from academic lipid disorders clinics. The mean on-treatment LDL-C was 167 ± 64 mg/dl; 33% patients had levels of LDL-C <130 mg/dl. Targeted sequencing analysis of LDLR and PCSK9 coding regions and exon 26 of APOB was performed, followed by detection of LDLR deletions and duplications. LDLR mutations (total 60, 13 novel) were found in 132 patients and APOB mutations in 6. No known PCSK9 causative mutations were seen. Two Phase 2a studies were conducted to assess the efficacy, safety, and tolerability of RN316 in hypercholesterolemic subjects taking high to maximal doses of statins. Exploratory pharmacogenomic analysis showed RN316 had maximal lowering of LDL-C of 73% in the mutation carriers (n=26) and similar maximal lowering of LDL-C in 72% in the noncarriers (n = 69). Response to RN316 in specific mutation carriers will be presented.

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A Novel Mathematical Modeling Approach to Analyze Complex Metabolic Pathway Dynamics: Application to LXR-activated Lipoprotein Metabolism

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Lipoprotein metabolism is governed by a highly complex network of metabolic pathways involved in lipid and protein synthesis and degradation. Understanding the dynamics of network regulation is impossible without the help of computational modeling. We present a new modeling approach to analyze the long term effects of a dietary or pharmacological intervention in lipoprotein metabolism. A concept of time-dependent evolution of model parameters is introduced to study the dynamics of metabolic network adaptations. The progression of these adaptations is predicted by identifying necessary dynamic changes in the model parameters to describe the transition of steady states during different stages of the dietary or pharmacological treatment. The trajectories provide insight into the affected underlying biological systems and provide targeted direction to the molecular events that should be studied in more detail to unravel the mechanistic basis of treatment outcome. Modulating effects on pathways caused by interactions with the proteome and transcriptome levels can be captured by the time-dependent descriptions of the parameters. The approach was employed to identify metabolic adaptations induced upon a 3-week activation of the liver X receptor (LXR) in C57BL/6J wild-type mice. The metabolic trajectories were modeled to analyze the metabolic adaptations in time. This provided a number of sometimes counterintuitive insights into the underlying mechanisms inducing hepatic steatosis, dynamic changes in plasma triglycerides, and plasma HDL content. The model predicted for instance decreased activity of the scavenger receptor class B1 (SR-B1) despite an increased flux mediated via the receptor. This prediction was validated experimentally by immunoblotting measurements of SR-B1 in hepatic membranes. We also show that this procedure can be used quite simply to select optimal therapeutic targets successfully.

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Perturbation of Serum Cholesterol in Mice Deficient in Disabled-2 (Dab2), a LDL Receptor Endocytosis

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High serum cholesterol levels (hypercholesterolemia) are strongly associated with cardiovascular disease because the atherogenic low density lipoproteins (LDL) promote atheroma development in arteries (atherosclerosis). A cause of hypercholesterolemia is the perturbation of LDL clearance from the circulation by the LDL receptor (LDLR)-mediated endocytosis pathway. Disabled-2 (Dab2) is a LDLR endocytosis adaptor protein with structural similarity to the ARH (autosomal recessive hypercholesterolemia gene) (or LDLRAP1, LDL receptor-associated protein 1), another LDLR endocytosis adaptor. We used a conditional knockout mouse model to determine whether Dab2 also contributes to clearance of serum cholesterol. Dab2 is widely expressed in various tissues in adults and is first expressed in extraembryonic endoderm in development. Constitutive knockout in mice is early embryonic lethal due to the failure in the formation of the extraembryonic endoderm layer. We constructed a floxed Dab2 conditional mutant line and generated Dab2 mosaic deletion using SOX2-Cre to restrict gene deletion within embryonic proper. By 3 week of age, Dab2 gene deletion was found in the majority (99%) of the cells of the conditional mutant mice. The mutant mice appear normal, fertile, and have a normal life span. Serum cholesterol level was elevated slightly and was further increased when the mice were fed a high fat diet. We were also able to produce Dab2 and ARH double-knock-out mice. The serum cholesterol of the double-mutant mice was found elevated in an additive fashion, but still lower than that of LDLR-null mice. The results suggest that both Dab2 and ARH contribute to serum LDL clearance in a non-redundant mechanism, and additional mechanism contributes to LDLR function.

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Lipoprotein Lipase Activator LP071 Improves the Plasma Lipid Profile in ApoE3L:CETP Mice

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Elevated plasma triglyceride (TG) and low HDL-cholesterol (HDL-c) are risk factors for cardiovascular disease (CVD) and are often seen as contributors to residual risk. Lipoprotein lipase (LPL) is a central enzyme in lipoprotein metabolism controlling plasma TG hydrolysis and contributing to de novo HDL formation. Thus, LPL is an attractive target for correcting dyslipidemia and reducing CVD residual risk. Through high throughput screening we have identified novel, first-in-class, small molecule LPL activators with in vivo efficacy. The objective with this study was to characterize plasma lipids in mice treated with LP071. Mice were treated for 4 days with 10 mg/kg LP071 through intraperitoneal injections. Plasma lipid handling capacity was investigated after oral lipid gavage. Plasma lipid profiles were analyzed for TGs, free glycerol and cholesterol using SEC-HPLC. NEFA was measured with commercial kits. LPL activity was investigated in different tissues. Using Triton WR1339 blockade, chylomicron and VLDL secretion rates were assessed. Fasting plasma TG levels were decreased 97% in apoE3L:CETP mice treated with LP071; HDL-c levels were increased by 116% and (V)LDL-C were decreased by 91%. Plasma-free glycerol and NEFA were significantly lowered, 26 and 22%, respectively. After an oral lipid load the plasma lipid response was significantly blunted in LP071-treated mice compared with controls. Compared with controls, LPL activity in subcutaneous adipose tissue was increased 1500% and in BAT LPL was increased by 100% at 3 h after a lipid gavage in WT mice treated with LP071. We conclude that LP071 is a potent activator of LPL, improving plasma lipid levels toward a less atherogenic profile. This novel drug class can potentially help battle CVD residual risk in the future.

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HNRNPA1 Regulates HMGCR Alternative Splicing and Modulates Cholesterol Metabolism

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3-Hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) encodes the rate-limiting enzyme in the cholesterol biosynthesis pathway and is inhibited by statins, a class of cholesterol-lowering drugs. Expression of an alternatively spliced HMGCR transcript lacking exon 13, HMGCR13(–), has been implicated in variation in plasma LDL-cholesterol (LDL-C) and is also the single most informative molecular marker of variation in LDL-C response to statins. Given the physiological importance of this transcript, our goal was to identify molecules that regulate HMGCR alternative splicing. Using expression arrays, we quantified gene expression changes in 480 lymphoblastoid cell lines after in vitro incubation with simvastatin or sham buffer and identified 95 statin-responsive genes (FDR<0.0001) involved in mRNA splicing. Heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1) was chosen for follow-up because rs3846662, a single-nucleotide polymorphism (SNP) within HMGCR shown to regulate exon 13 skipping, was predicted to alter an HNRNPA1 binding motif. Sterol depletion of human hepatoma cell lines reduced HNRNPA1 mRNA levels, an effect that was reversed with sterol add-back. When determining HNRNPA1 half-life using actinomycin D, we observed that HNRNPA1 transcript stability was dramatically reduced in sterol-depleted cells.

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Adipose-specific Deletion of ARV1 Results in a Lean Phenotype with Markedly Reduced White Adipose Tissue Mass and Improved Glucose Tolerance

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ACAT-related enzyme required for viability 1 (ARV1) was identified as a gene required for viability in yeast in the absence of the ability to esterify cholesterol. ARV1 encodes a transmembrane protein of the endoplasmic reticulum (ER) believed to participate in transport of lipids from the ER to the Golgi. We hypothesized that efficient intracellular cholesterol transport may be an important factor affecting lipid droplet formation and stability in mammalian cells. To investigate the role of ARV1 in adipose lipid metabolism, we have generated mice with an adipose-specific deletion of ARV1 using Cre/loxP technology with the Ap2:Cre transgene. ARV1 adipose-specific knockout (ASKO) mice exhibited significant reductions in plasma total cholesterol (⊠21%, $p < 0.05$), HDL cholesterol (⊠25%, $p < 0.01$), and phospholipid (⊠17.6%, $p < 0.05$) levels, whereas fasting triglyceride levels were unaffected. Most strikingly, the ARV1 ASKO mice had very little white adipose tissue mass (WAT) on a chow diet including substantial reductions in the perigonadal (WT 0.41 ± 0.07 g versus KO 0.10 ± 0.07 g, $p = 0.0002$) and subcutaneous depots (WT 0.32 ± 0.03 g versus KO: 0.11 ± 0.08 g; $p = 0.0002$). In contrast to other lipodystrophic mouse models, the lean phenotype of these animals was accompanied by higher adiponectin levels and improved glucose tolerance (WT AUC 32,055 versus KO AUC 21,470 mg/dl*min; $p < 0.05$), without an increase in hepatic triglyceride content. Primary adipocytes isolated from ARV1 KO mice exhibited increased rates of lipoplysis, under both basal and stimulated conditions (increased 2.2-fold, 3.1-fold versus WT, respectively; $p < 0.05$). These studies suggest a key role for adipose ARV1 in adipocyte lipid storage and lipolysis.

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Bile Acid and Sterol Metabolism and Gene Expression in Statin-treated PCSK9 Knockout Compared with Wild-type Mice

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PCSK9 inhibitor and statin combination treatment augments plasma LDL lowering and increases liver cholesterol (C) uptake, raising questions about hepatic C and bile acid (BA) metabolism and colonic BA exposure. To address this, we treated PCSK9-KO (PCSK9-Y119X mutant) and wild-type (WT) C57BL/6J mice with atorvastatin for 12 weeks and assayed 14 individual BAs and C by LC-MS in 48-h fecal extracts. These data were compared with plasma lipid profiles and liver C & BA pathway gene expression assayed by RT-PCR. Compared with no-statin controls, atorvastatin reduced plasma LDL- and total C in both WT and PCSK9-KO mice, but plasma total BAs and liver free and esterified C content were the same in all four groups. Statin treatment increased fecal C ~3-fold in both genotypes (base lines not different) and increased fecal total BA levels ~2-fold in both genotypes (base line ~2-fold higher in PCSK9-KO). Females showed greater BA increases than males, and differences in individual BAs were seen, but secondary/primary BA ratios remained constant among groups. Atorvastatin increased liver sterol pathway mRNAs severalfold in both genotypes (base lines similar) and increased liver BA synthesis mRNA ~2-fold in both genotypes (base lines lower in PCSK9-KO). Liver mRNA levels for several BA-conjugating enzymes and BA transporters were similar in all groups. We conclude that statin treatment promoted elimination of excess liver C through induction of key liver sterol and BA synthesis genes, increasing fecal BAs but not free C in PCSK9-KO mice, and increasing both BAs and C in WT mice. Liver BA transporter and conjugating enzyme mRNAs were not affected, suggesting that reuptake and recirculation of BAs remained within normal ranges. This study supports the interpretation that normal regulation of liver gene expression and sterol/BA metabolism can accommodate the increased flux of liver C following stimulated clearance of LDL with combined statin/PCSK9 suppression.

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Adipose Subtype-selective Recruitment of TLE3 or Prdm16 by PPARγ Specifies Lipid Storage versus Thermogenic Gene Expression

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White and brown adipose have specialized physiological functions that are enabled by shared but distinct transcriptional programs. Prdm16 was identified as a critical PPARγ cofactor that drives brown adipose-selective gene expression, but very little is known about factors that specify the white adipose-selective transcriptional program. We demonstrate that TLE3 is a white adipose-selective cofactor of PPARγ, executing an opposing transcriptional program to Prdm16. Elevated expression of TLE3 in vitro reprograms brown preadipocytes to have increased levels of white fat markers and decreased levels of brown fat markers. Co-expression of TLE3 with Prdm16 antagonizes the brown adipose program induced by Prdm16. Adipose-specific TLE3 transgenic mice exhibit impaired thermogenic capacity and enhanced lipid storage in brown adipose tissue, both on the basal level and with the treatment of β-adrenergic agonist to induce browning. Contrarily, conditional deletion of TLE3 in adipose tissue improves adaptive thermogenesis and promotes depot-specific browning of inguinal white adipose tissue. Mechanistic studies reveal mutually exclusive interactions of Prdm16 with TLE3 and PPARγ on target gene promoters. Our study suggests that the differential recruitment of cofactors TLE3 and Prdm16 to PPARγ target genes dictates distinct adipose cell type-specific gene expression, thereby helping to specify white versus brown adipose phenotypes.

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Liver-specific Overexpression of Apolipoprotein M Stimulates Production of Larger, ApoM/S1P-enriched Plasma HDL

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ApoM, a member of the lipocalin family, is expressed in liver and kidney and after secretion associates with plasma HDL. ApoM is also a carrier of plasma sphingosine 1-phosphate (S1P), a signaling molecule involved in inflammation and atherogenesis. Overexpression of apoM in nonhepatic, ATP-binding cassette transporter A1 (ABCA1)-expressing HEK293 cells increases the size of nascent HDL particles. To determine whether overexpression of apoM in hepatocytes would lead to larger sized HDL particles in vivo, we generated liver-specific apoM transgenic (apoM Tg) mice, which had larger plasma HDL particles enriched in apoM, lecithin:cholesterol acyltransferase, cholesteryl ester, and S1P compared with wild-type (WT) mice. In both WT and apoM Tg mice, apoM preferentially associates with larger sized plasma HDL, suggesting that these HDLs are optimal for apoM binding. Primary hepatocytes from apoM Tg mice contained increased S1P and assembled/secreted larger nascent HDL particles compared with WT hepatocytes, suggesting that apoM catalyzes this assembly in the absence of plasma. Liver mRNA and protein expression of sphingosine kinase 2, one of the rate-limiting enzymes in S1P biosynthesis, was significantly increased in apoM Tg mice and hepatocytes from apoM Tg mice synthesized and secreted more [3H]S1P from [3H]sphingosine compared with WT mice. Our results suggest that hepatocytes are an important source of plasma S1P and that increased expression of liver apoM stimulates the synthesis and secretion of S1P and the assembly of larger HDL particles, which appear to be the preferential carrier of plasma apoM and S1P.

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Structural and Calorimetric Studies on a Homologous Series of N-Acylglycines, Antinociceptive Molecules of Mammalian Membranes

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Membrane lipids not only serve as structural components and energy storage molecules, but they also play important roles in communication and signaling within and between cells. Because aberrations in lipid metabolism are associated with neurodegenerative diseases and neurological disorders, it is essential to characterize brain lipids to develop a thorough understanding of the biochemical mechanisms underlying brain physiology and pathology. Recent research shows that various N-acylamino acids (NAAs) such as N-acylglycines and N-acylserines, are present in mammalian brain and other tissues. Therefore, characterization of various NAAs with respect to structure and membrane interactions can provide clues to understand their endogenous roles. In view of this, a homologous series of N-acylglycines (NAGs) have been synthesized and characterized by differential scanning calorimetry and single crystal x-ray diffraction. Transition enthalpies and entropies of dry and hydrated N-acylglycines exhibit linear dependence on their chain lengths. Linear least squares analyses yielded incremental values contributed by each methylene group to the transition enthalpy and entropy and the corresponding end contributions. Structures of N-myristoylglycine and N-palmitoylglycine were solved in a monoclinic system with C2/c and P21 space groups, respectively. Analysis of the crystal structures shows that N-acylglycines are organized in a bilayer fashion, which is stabilized by strong hydrogen-bonding interactions between the molecules of opposite leaflets as well as in the same leaflet and dispersion interactions among the acyl chains. Further studies on the interaction of NAGs with major membrane lipids (e.g. phospholipids and cholesterol) can provide clues to understand their role in biological membranes.

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Methylation Capacity Links Hyperlipidemia and Innate Immune Responses in Caenorhabditis elegans

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Nutritional and metabolic pathways feed into diverse cellular processes. A growing body of evidence suggests that transcriptional responses are affected by metabolic cues, coordinating gene expression, and physiological changes with nutrient availability. Knockdown of sams-1 (S-adenosylmethionine (SAMe) synthase) results in decreases in available methyl donors and phosphatidylcholine and increased lipogenesis in Caenorhabditis elegans. However, these animals also have an extended life span. Dietary addition of choline, which corrects the imbalance in PC, rescues the lipogenic defects and returns life span to wild-type levels. To determine gene regulatory circuits that might also be responding to the low SAMe levels as well as identify additional mechanisms potentially affecting lipogenesis or potentiating survival in low SAMe/low PC metabolic conditions, we performed whole genome mRNA expression analysis. To distinguish between effects of low SAMe and low SAMe/PC, we compared gene expression in sams-1(RNAi) and sams-1(RNAi) choline-rescued animals. We found several functional classes of genes with altered regulatory profiles, including up-regulation of genes in the p38 MAP kinase-based innate immune response. However, increased expression of this gene class did not confer protection against pathogenic bacteria, as sams-1(loss-of-function) animals died faster on virulent strains of Pseudomonas aeruginosa. Interestingly, the sams-1(lof) lifespan extension was evident on non-virulent P. aeruginosa isolates, suggesting that this defect is linked to immune system function rather than food source. These data suggest that metabolic changes driving hyperlipidemia can also alter immune responses, which may be important for our understanding of links among host microbiota, immune responses, and hyperlipidemia in human metabolic disease.

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HDAC9 Deficiency in Myeloid Cells Protects against Atherosclerosis in Mice

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The epigenetic role of specific histone deacetylase (HDAC) isoforms in atherosclerosis development is unknown. In this study, we show that systemic and bone marrow cell deletion of HDAC9 decreased atherosclerosis in LDLr−/− mice with minimal effect on plasma lipid concentrations. HDAC9 deletion increased accumulation of acetylated H3 and H4 at the promoters of ABCA1, ABCG1, LXR-, LXR-, and PPAR1 in macrophages, up-regulation of lipid homeostatic genes and cholesterol efflux, down-regulation of inflammatory genes, and polarization toward an M2 phenotype and ultimately leading to decreased foam cell formation. We conclude that macrophage HDAC9 up-regulation is atherogenic, and macrophage-specific HDAC9 inhibition may provide therapeutic benefit in atherosclerosis.

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Cytochrome P4502S1: A Novel Monocyte/Macrophage Fatty Acid Epoxygenase in Human Atherosclerotic Plaques

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Cytochrome P450 (CYP) epoxygenases metabolize endogenous polyunsaturated fatty acids to their corresponding epoxides, generating bioactive lipid mediators. The latter play an important role in vascular homeostasis, angiogenesis, and inflammation. Because little is known about the functional importance of extravascular sources of lipid epoxides we focused on determining whether lipid epoxide-generating CYP isoforms are expressed in human monocytes/macrophages. Epoxides were generated by freshly isolated human monocytes, and production increased markedly during differentiation to macrophages. Mass spectrometric analysis identified CYP2S1 as a novel macrophage CYP, and CYP2S1-containing microsomes generated epoxides of arachidonic, linoleic, and eicosapentaenoic acid. Macrophage CYP2S1 expression was increased by LPS and IFN-☒ (classically activated) and oxidized LDL but not IL-4 and IL-13 (alternatively activated) and was co-localized with CD68 in inflamed human tonsils but not in breast cancer metastases. Prostaglandin (PG) E2 is an immune modulator factor that promotes phagocytosis, and CYP2S1 can metabolize its immediate precursors PGG2 and PGH2 to 12(S)-hydroxyheptadeca-5Z,8E,10E-trienoic acid (12-HHT). We found that CYP inhibition and siRNA-mediated down-regulation of CYP2S1 increased macrophage phagocytosis and that the latter effect correlated with decreased 12-HHT formation. Although no Cyp2s1 protein was detected in aortae from wild-type mice it was expressed in aortae and macrophage foam cells from apoE–/– mice. Consistent with these observations CYP2S1 was co-localized with the monocyte marker CD68 in human atherosclerotic lesions. Thus, CYP2S1 generates 12-HHT and is a novel regulator of macrophage function that is expressed in classical inflammatory macrophages and can be found in murine and human atherosclerotic plaques.

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Müller Cells Regulate Notch Signaling and Retinal Angiogenesis via the Soluble Epoxide Hydrolase-dependent Generation of 19,20-Dihydroxydocosapentaenoic Acid

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Soluble epoxide hydrolase (sEH) is a promising pharmacological target based on its enzymatic function of metabolizing potent bioactive substrates, epoxyeicosatrienoic acids (EETs), epoxydocosapentaenoic acids (EDPs), and other lipid epoxides to their corresponding diols. Despite many studies characterized the proangiogenic and anti-inflammatory effect of EETs, the biological function of sEH in angiogenesis in vivo remains unclear. In this study, we found that the retinal angiogenesis was significantly delayed in sEH–/– mice and was associated with reduced tip cell numbers and filopodia. This phenotype was associated with the induction of the Notch-dependent transcription factors Hes1 and Hey1 and attenuated endothelial cell proliferation. sEH was mainly expressed in Müller glia cells, and Müller glia cell-specific sEH knockout mice displayed delayed retinal angiogenesis similar to sEH–/– mice. Lipid profile analyses revealed a significant decrease of the sEH metabolite 19,20-dihydroxydocosapentaenoic acid (DHDP) in sEH–/– retinas compared with WT littermates. 19,20-DHDP suppressed Notch signaling via inhibition of γ-secretase in vitro, as well as rescued retinal vasculature defects in a Notch overactivating mutant (Fbxw7iEC) in vivo. Moreover, intravitreal injection of 19,20-DHDP significant increased tip cell and filopodia number as well as primary vessel network density in sEH–/– mice retina. Our data demonstrated that Müller glia cells are involved in the development of the superficial retinal vasculature by secreting 19,20-DHDP which inhibits γ-secretase-mediated Notch signaling.

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A Common Polymorphism in the LDL Receptor Gene Has Multiple Effects on LDL Receptor Function

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A common synonymous single-nucleotide polymorphism in exon 12 of the LDL receptor (LDLR) gene, rs688, has been associated with increased plasma total and LDL-cholesterol in several populations. Using immortalized lymphoblastoid cell lines from a healthy study population, we confirmed an earlier report that the minor allele of rs688 is associated with increased exon 12 alternative splicing ($p < 0.05$) and showed that this triggered nonsense-mediated decay (NMD) of the alternatively spliced LDLR mRNA. However, because synonymous SNPs may influence structure and function of the encoded proteins by co-translational effects, we sought to test whether rs688 was also functional in the full-length mRNA. In HepG2 cells expressing LDLR cDNA constructs engineered to contain the major or minor allele of rs688, the latter was associated with a smaller amount of LDLR protein at the cell surface ($-21.8 \pm 0.6\%$, $p = 0.012$), a higher amount in the lysosome fraction ($+25.7 \pm 0.3\%$, $p = 0.037$) and reduced uptake of fluorescently labeled LDL (–-$24.3 \pm 0.7\%$, $p < 0.01$). Moreover, in the presence of exogenous PCSK9, a protein that reduces cellular LDL uptake by promoting lysosomal degradation of LDLR, the minor allele resulted in reduced capacity of a PCSK9 monoclonal antibody to increase LDL uptake. These findings are consistent with the hypothesis that rs688, which is located in the β-propeller region of LDLR, has effects on LDLR activity beyond its role in alternative splicing due to impairment of LDLR endosomal recycling and/or PCSK9 binding, processes in which the β-propeller is critically involved.

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Liver LXRα Plays a Critical Role in Regulating LXR-dependent Reverse Cholesterol Transport

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The liver X receptors LXRα and LXRβ are ligand-dependent nuclear hormone receptors responsible for regulating cholesterol homeostasis. LXR activation in macrophages promotes the efflux of internal cholesterol to high density lipoprotein particles (HDL) initiating the process of reverse cholesterol transport (RCT). Additionally, LXRα in the liver regulates the catabolism and excretion of cholesterol, classically considered the penultimate step in the RCT process. In animal models of cardiovascular disease treatment with LXR agonist reduces atherosclerosis, and this antiatherogenic activity is believed to be due, at least in part, to stimulating RCT. We have shown previously that liver-specific deletion of LXRα in LDLR knock-out mice significantly increases atherosclerosis and decreases RCT, identifying the liver as an important site of LXR-dependent antiatherogenic activity. Treatment of LXRα liver-specific knock-out mice with an LXR agonist, however, reduces atherosclerosis, suggesting that the ability to stimulate hepatic cholesterol catabolism and excretion is not required for the antiatherogenic activity of LXR agonists. We now show that deletion of LXRα in the liver decreases LXR agonist-dependent RCT in vivo whereas deletion of both LXR subtypes in macrophages has little or no effect. Together, these experiments suggest that the liver and not the macrophage is the critical site of LXR agonist-stimulated RCT. Nevertheless, we have shown previously that LXR expression in hematopoietic cells is required for the antiatherogenic activity of LXR agonists. Our finding that macrophage LXRs are not required for LXR agonist-stimulated RCT suggests that the site of agonist-dependent antiatherogenic activity, the LXR-dependent pathways that influence atherosclerosis, or both remain to be determined.

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Autoantigenic Protein-DNA Complexes Stimulate Plasmacytoid Dendritic Cells to Promote Atherosclerosis

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Inflammation has been closely linked to autoimmunogenic processes in atherosclerosis. Plasmacytoid dendritic cells (pDCs) produce type I interferons in response to pathogenic single-stranded nucleic acids, but also sense self-DNA released from dying cells or in neutrophil extracellular traps (NETs) complexed to the antimicrobial peptide Cramp/LL37 in autoimmune disease. However, the exact role of pDCs in atherosclerosis remains elusive. Here, we demonstrate that pDCs can be detected in murine and human atherosclerotic lesions. Exposure to modified LDL enhanced the capacity of pDCs to phagocytose and prime antigen-specific T cell responses. pDCs can be stimulated to produce interferon-α by Cramp-DNA complexes, and we further identified increased expression of Cramp and formation of NETs in atherosclerotic arteries. Whereas Cramp-DNA complexes aggravated atherosclerotic lesion formation in apolipoprotein E-deficient mice, pDC depletion and Cramp deficiency in bone marrow reduced atherosclerosis and anti-double-stranded DNA antibody titers. Moreover, activation of pDCs and interferon-α treatment promoted plaque growth, associated with enhanced anti-double-stranded DNA antibody titers. Self-DNA and an increased expression of the Cramp/LL37 in atherosclerotic lesions may stimulate a pDC-driven pathway of autoimmune activation and the generation of anti-double-stranded DNA antibodies, critically aggravating atherosclerosis lesion formation. These key factors may thus represent novel therapeutic targets.

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Structural Basis of Transfer between Lipoproteins by Cholesteryl Ester Transfer Protein

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Human cholesteryl ester transfer protein (CETP) mediates the net transfer of cholesteryl ester mass from atheroprotective high density lipoproteins to atherogenic low density lipoproteins by an unknown mechanism. Delineating this mechanism would be an important step toward the rational design of new CETP inhibitors for treating cardiovascular diseases. Using high resolution transmission electron microscope and structure analyses techniques, we discovered that CETP bridges a ternary complex with its N-terminal ⍰-barrel domain penetrating into high density lipoproteins and its C-terminal domain interacting with low density lipoprotein or very low density lipoprotein. In our mechanistic model, the CETP interacting regions, which are highly mobile, form pores that connect to a hydrophobic central cavity, thereby forming a tunnel for transfer of neutral lipids from donor to acceptor lipoproteins. These new insights into CETP transfer provide a molecular basis for analyzing mechanisms for CETP inhibition

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Endoplasmic Reticulum-targeted ACSL1 Directed Fatty Acid into Complex Lipid Synthesis but Not to Oxidation

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Long chain acyl-CoA synthetases (ACSLs) catalyze the addition of coenzyme A (CoA) to fatty acids, thereby activating them. Acyl-CoAs are then metabolized, primarily by oxidation or incorporation into complex lipids. ACSL1 contributes 50-90% of total ACSL activity in heart, adipose, and liver. Heart and adipose lacking ACSL1 are virtually unable to oxidize fatty acids, whereas liver that lacks ACSL1 has decreases in both fatty acid oxidation and complex lipid biosynthesis. Differential centrifugation of liver showed that ACSL1 accounts for 75% of total ACSL activity in mitochondria and 50% in the endoplasmic reticulum (ER). In heart and adipose, ACSL1 is responsible for >90% of mitochondrial ACSL activity. To determine whether the subcellular location of ACSL1 directs fatty acids into specific pathways, we inserted an ER-targeted ACSL1 construct into an adenovirus vector. The construct contained the transmembrane domain of FATP4, an ER-localized protein (PMID:23024797), and the active site region of ACSL1. Cultured brown adipocytes from adipose-specific ACSL1 knockout (Acsl1A–/–) mice oxidized 38% less [14C]16:0 than wild-type cells and incorporated 48% less [14C]16:0 into complex lipids. Cultured control and Acsl1 A–/– brown adipocytes were infected with adenoviruses containing GFP, wild-type Acsl1, or the ER-targeted ACSL1 (F4-Acsl1). Infection with Ad-Acsl1 or Ad-F4-Acsl1 increased ACSL activity 3- to 6-fold over Ad-GFP-infected Acsl1A–/– cells. Infecting the knock-out cells with Ad-Acsl1 increased oxidation by 33%, whereas infection with Ad-F4-Acsl1 did not rescue oxidation. Infection of Acsl1A–/– brown adipocytes with either wild-type Ad-Acsl1 or Ad-F4-Acsl1 increased [14C]16:0 incorporation into complex lipids by 40-60%. Thus, ER-targeted ACSL1 directed fatty acids towards complex lipid synthesis, but not oxidation. These data suggest that site of acyl-CoA synthesis influences the entry of their acyl-CoA products into specific downstream pathways.

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FERM-dependent E3 Ligase Recognition: A Conserved Mechanism for Targeted Degradation of Lipoprotein Receptors

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The E3 ubiquitin ligase IDOL (inducible degrader of the LDL receptor) regulates LDL receptor (LDLR)-dependent cholesterol uptake, but its mechanism of action, including the molecular basis for its stringent specificity, is poorly understood. Here, we show that IDOL uses a singular strategy among E3 ligases for target recognition. Through the use of mutational studies, we identify critical residues within the FERM F3b domain of IDOL that bind a newly identified recognition sequence in the cytoplasmic tails of its lipoprotein receptor targets, LDLR, VLDLR, and apoER2. Key residues defining the IDOL-LDLR interaction are functionally conserved in their insect homologs DNR1 and LpR, respectively. We confirm that target recognition by IDOL involves a tripartite interaction among the IDOL FERM domain, membrane phospholipids, and the lipoprotein receptor tail. Through the use of fluorescence polarization assays, we demonstrate that IDOL directly binds the lipoprotein tails of its targets, and this requires key residues identified in the IDOL recognition sequence. We also identify a nonsynonymous SNP encoding a functional variant within the FERM domain of IDOL that is associated with increased plasma cholesterol in humans. Finally, we demonstrate that IDOL controls its own stability through autoubiquitination of lysine residues situated in a unique FERM subdomain, F3c, not present in other FERM proteins. Mutation of these residues generates a molecule resistant to autodegradation but with intact E3 ligase activity, we termed "super IDOL." This molecule has become the basis of in vivo studies examining the effect of IDOL in metabolic tissues including the liver and adipose tissue. Our data identify the IDOL-LDLR interaction as an evolutionarily conserved mechanism for the regulation of lipid uptake and suggest that this interaction could potentially be exploited for the pharmacologic modulation of lipid metabolism.

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An Essential Requirement for the SCAP/SREBP Signaling Axis to Protect Cancer Cells from Lipotoxicity

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Sterol regulatory element-binding proteins (SREBPs) are key transcriptional regulators of lipid metabolism and cellular growth. It has been proposed that SREBP signaling regulates cellular growth through its ability to drive lipid biosynthesis. Unexpectedly, we find that loss of SREBP activity inhibits cancer cell growth and viability by uncoupling fatty acid synthesis from desaturation. Integrated lipid profiling and metabolic flux analysis revealed that cancer cells with attenuated SREBP activity maintain long chain saturated fatty acid synthesis while losing fatty acid desaturation capacity. We traced this defect to the uncoupling of fatty acid synthase activity from SCD1-mediated desaturation. This deficiency in desaturation drives an imbalance between the saturated and monounsaturated fatty acid pools, resulting in severe lipotoxicity. Importantly, replenishing the monounsaturated fatty acid pool restored growth to SREBP-inhibited cells. These studies highlight the importance of fatty acid desaturation in cancer growth and provide a novel mechanistic explanation for the role of SREBPs in cancer metabolism.

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Multimetabolic Characteristics of Early Onset Coronary Heart Disease and Low HDL Cholesterol Levels: A Pilot Study using Family Data

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Multimetabolic phenotyping with nonlinear statistical analysis is increasingly used in the research of complex diseases, such as coronary heart disease (CHD), to better understand their complicated pathogenesis with special emphasis on their intermediate quantitative risk factors, including lipoprotein subclasses. Here we used high throughput serum NMR metabonomics platform and application of self-organizing maps (SOMs) to characterize the lipoprotein metabolism. NMR provides quantitative data on lipoproteins, various low molecular weight metabolites, and individual lipid molecules together with their degree of (poly)(un)saturation. The SOM analysis provides coherent integration of the metabolite and clinical data allowing quick visual interpretations on the overall multivariate associations in the study population. Here, we focused on family members of Northern Finnish probands with early onset CHD and low serum HDL-c levels (n = 132). This is a good example of a highly selected set of individuals still likely to comprise extensive metabolic variation. Although CHD patients were characterized by a combination of atherogenic clinical and metabolic phenotypes, wide ranging variations were noted in many metabolic measures. This CHD-enriched population with low levels of HDL-c was also characterized by small size of HDL particles (diameter), low levels of large HDL, high levels of large VLDL, low levels of all of the LDL subclasses, low apoA1 and low apoE. The same population was characterized by low relative concentrations of ω-6 fatty acids (FAs) and high relative concentrations of ω-7, -9, and saturated FAs, high concentrations of pyruvate, and elevated concentrations of glycoprotein, isoleucine, leucine, and valine. Replication of the analyses using a larger sample size will show us whether this metabolic profile truly describes individuals with low HDL-c levels or early onset CHD or whether its components have value as predictors or biomarkers for CHD risk.

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Nitro Fatty Acids Are Inhibitors of 5-Lipoxygenase-mediated Inflammation

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Nitric oxide and nitrite-derived species readily react with polyunsaturated fatty acids yielding nitroalkene derivatives (NO2-FAs). The latter have been attributed anti-inflammatory properties via covalent electrophilic adduction to nucleophilic residues within target proteins. Electrophilic substances are potential inhibitors of the 5-lipoxygenase (5-LO); therefore, we assessed whether NO2-FAs inhibit the 5-LO in vitro and in vivo. 5-LO activity in human polymorphonuclear leukocytes (PMNLs) was concentration-dependently attenuated by nitrolinoleic acid (NLA) and nitro-oleic acid (NOA). Similar effects were observed using the recombinant human protein, indicating a direct effect on the enzyme. Neither NO2-FA affected the activity of 12-LO or 15-LO enzymes. The NO2-FA-induced inhibition of 5-LO was irreversibly mediated by nitroalkylation of histidine and cysteine residues resulting in iron release. Both the NO2-FA-mediated inhibition of the 5-LO as well as iron release were abolished by mutation of Cys418 to serine. In vivo, systemic administration of NOA to wild-type mice, but not 5-LO knockout mice, decreased neutrophil and monocyte mobilization in response to LPS and elicited effects similar to those of the 5-LO inhibitor zileuton. In summary, NO2-FAs directly and irreversibly inhibit the 5-LO by the nitroalkylation of functionally critical histidine and cysteine residues and attenuated acute inflammation and lung injury. Thus, NO2-FAs represent a novel therapeutic option for the treatment of 5-LO-associated inflammation.

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An SREBP-regulated Micro-RNA Operon Contributes a Regulatory Loop to Maintain Intracellular Lipid Homeostasis

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Sterol regulatory element binding proteins (SREBPs) have evolved as a focal point for linking lipid synthesis with other pathways that regulate cell growth and survival. Here, we have uncovered a polycistrionic micro-RNA (miR) locus that is activated directly by SREBP-2. Two of the encoded miRs, miR-182 and miR-96, negatively regulate expression of Fbxw7 and Insig-2 respectively, both of which are known to negatively affect nuclear SREBP accumulation. Direct manipulation of this miR pathway alters nuclear SREBP levels and the entire endogenous lipid synthesis pathway. Thus, we have uncovered a new mechanism for regulation of intracellular lipid metabolism that is mediated by the concerted action of a pair of miRs that are expressed from the same SREBP-2-regulated miR locus, and each targets a different protein of the multistep pathway that regulates SREBP function. These studies reveal a miR "operon" analogous to the classic model for genetic control in bacterial regulatory systems.

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Loss of ABCG1 Drives the Site-selective Expansion of B-1 B Cells and Secretion of Atheroprotective Natural Antibodies, in Response to Local Accumulation of Specific Oxidized Lipids

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The oxidation of LDL, a key event in the pathogenesis of atherosclerosis, generates multiple oxidation-specific neoepitopes (OSEs). These epitopes are recognized by natural germ line IgM antibodies that are secreted by B-1 B cells. Mice lacking the ATP-binding cassette (ABC) transporter, ABCG1, develop chronic inflammation in the lungs, associated with the accumulation of lipid (cholesterol, cholesterol ester, phospholipid)-filled cells and cholesterol crystal deposition, which are also characteristic of atherosclerotic lesions. However, hyperlipidemic mice lacking ABCG1 develop smaller atherosclerotic lesions compared with controls. We previously attributed this decrease to increased apoptosis of macrophages within the atherosclerotic lesions of mice lacking ABCG1. We now demonstrate that the lungs of Abcg1–/– mice have increased levels of specific oxidized phospholipids species. Here, we show that there is a significant increase in B cell number in the lungs, but not the spleens, of Abcg1–/– mice. Subsequent comparison of B cell subtypes revealed that B-1 B cells, which secrete natural antibodies (NAbs), are significantly expanded in the pleural cavity of Abcg1–/– mice, compared with wild-type mice. In addition, we show that the lungs and plasma of Abcg1–/– mice have increased titers of NAbs to OSEs and increased mRNA transcript levels for the known hypervariable (VH) CDR3 region of the atheroprotective EO6/T15 NAbs. These data are consistent with an antigen-specific enhanced local production of NAbs in the pleural cavity. These findings suggest that the accumulation of lipids resulting from loss of ABCG1 induces the specific expansion of B-1 B cells, which secrete NAbs that may help protect against the development of atherosclerosis. 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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Diet1 Functions in the FGF15/19 Enterohepatic Signaling Axis to Modulate Bile Acid and Lipid Levels

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Impaired regulation of bile acid production and cholesterol excretion is an underlying factor in the pathogenesis of several common clinical conditions such as hypercholesterolemia, cardiovascular disease, bile acid malabsorption, gallstone disease, and type 2 diabetes. Several years ago, Lusis and colleagues identified a substrain of C57BL/6 mice, C57BL/6ByJ (B6By), which had low circulating lipid levels and resistance to atherosclerosis. We found that the resistance to hypercholesterolemia in B6By mice was associated with increased bile acid excretion in the feces (2-fold) and urine (18-fold). We hypothesized that a mutation had occurred in B6By mice which leads to dysregulated bile acid metabolism. We have now identified a mutation in a novel gene, Diet1, which has a role in the regulation of signaling between intestine and liver for the regulation of bile acid synthesis. Diet1 encodes a 236-kDa protein consisting of tandem low density lipoprotein receptor and MAM (meprin-A5-protein tyrosine phosphatase mu) domains and is expressed in enterocytes of the small intestine. Diet1-deficient mice exhibited an elevated bile acid pool size and impaired feedback regulation of hepatic Cyp7a1, which encodes the rate-limiting enzyme in bile acid synthesis. In mouse intestine and in cultured human intestinal cells, Diet1 expression levels influenced the production of fibroblast growth factor 15/19 (FGF15/19), a hormone that signals from the intestine to liver to regulate Cyp7a1. Transgenic expression of Diet1, or adenoviral-mediated Fgf15 expression, restored normal Cyp7a1 regulation in Diet1-deficient mice. Diet1 and FGF19 proteins exhibited overlapping subcellular localization in cultured intestinal cells. These results establish Diet1 as a control point in enterohepatic bile acid signaling and lipid homeostasis.

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Ttc39b Deficiency Causes an Intestinal LXR Activation Phenotype with Increased Expression of ABCA1 and Increased HDL Cholesterol Levels

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TTC39B (T39) was identified in a GWAS as a novel gene influencing HDL cholesterol (HDL-c) levels. We have now verified increased HDL-c levels in T39–/– mice. On a chow diet HDL cholesterol levels were significantly increased by 22%, and there were increases in LXR protein but not mRNA, increased expression of ABCA1 mRNA and protein and increased secretion of HDL by small intestinal enterocytes. When mice were challenged with a high fat/high cholesterol/bile salt (Paigen) diet, there was a significant 42% increase in HDL-c and also decreased incorporation of dietary cholesterol and fat into chylomicrons and marked protection from steatohepatitis; in addition to intestinal changes, there was increased LXR protein and induction of Abcg5/8 in liver. Ldlr–/–T39–/– mice on the Western diet showed increased HDL-c, decreased V/LDL cholesterol, and decreased atherosclerosis. These studies show that T39 deficiency results in increased LXR primarily in enterocytes, beneficial lipoprotein changes, and reduced atherosclerosis. Moreover, T39–/– mice are protected from fatty liver, indicating that T39 inhibition could be an effective strategy for reducing atherosclerosis and fatty liver.

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A Synthetic POVPC-Peptide Is a Model Oxidized Phospholipid That Induces Expression of Inflammatory Genes in Macrophages and Endothelial Cells

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POVPC (1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine) is an oxidized phospholipid (OxPL) found in OxLDL and other inflammatory settings. It has proinflammatory effects on macrophages (MACs) and endothelial cells (ECs), promoting inflammation and atherosclerosis. POVPC possesses a reactive aldehyde in the sn-2 position, which renders it highly reactive and prone to structural changes and is therefore difficult to study. We developed a synthetic POVPC-peptide adduct at the sn-2 side chain, which is stable and not prone to further oxidation, to study inflammatory effects on MACs and ECs. The POVPC-peptide induced substantial expression of the proinflammatory cytokines Ccl2, Ccl3, Ccl4, Cxl2, and TNF-⍰ in murine bone marrow-derived macrophages and RAW264.7 cells. Moreover, we stimulated human monocyte-derived MACs and ECs with either POVPC-peptide or oxidized PAPC (OxPAPC), a mixture of OxPLs, and analyzed gene expression with microarray analysis. Both were more bioactive in ECs versus MACs, with POVPC-peptide > OxPAPC. In contrast, OxPAPC was more active in MACs. The gene expression patterns in human and murine macrophages were similar, including genes mentioned above, whereas expression patterns between human MACs and ECs were very different. The POVPC-peptide prominently increased the MAC and EC expression of COX-2, an enzyme known to play a central role in eicosanoid production. Thus, we studied the effect of POVPC-peptide on eicosanoid production in RAW macrophages. Although POVPC-peptide alone modestly induced eicosanoid secretion, co-stimulation along with ATP, which raises intracellular arachidonic acid levels and provides substrates for COX-2, robustly stimulated eicosanoid production. This work shows that the POVPC-peptide adduct is a stable model of an OxPL that is highly bioactive. It induced pro-inflammatory responses in MAC and EC similar to OxPAPC and should be valuable in further study of the role of OxPL in inflammation and atherosclerosis.

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β-Glucosylceramide Activates NKT Cells in Vivo and Prevents Tumor Metastasis in Mice

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Natural killer T (NKT) cells are well known to play important roles in both tumor rejection and the defense against infections. Therefore, the antitumor potential of NKT cell-activating antigens have been the focus for the development of NKT cell-based immunotherapies. Up until now, several studies have revealed that the administration of glycolipids (e.g. α-galactosylceramide) can successfully treat certain metastatic tumors. However, liver injuries appeared upon the application of these antigens. We previously examined the potential of using β-glucosylceramide to inhibit tumor metastasis to the liver. The aim of this study was to determine the antimetastatic effects of β-GlcCer and its impact on the activation of NKT cells. Intraperitoneal administration of β-GlcCer enhanced the production of interferon-γ from hepatic lymphocytes containing NKT cells and increased the cytotoxicity of hepatic lymphocytes against tumor cells. Moreover, β-GlcCer administration suppressed the hepatic metastasis of tumors in wild-type mice, but not in CD1d–/– or Ja18–/– mice. The drawback associated with the other glycolipids in liver injury was not noted in these.

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The Impact of Partial and Complete Loss-of-function Mutations in Endothelial Lipase on HDL Levels and Cardioprotective Functionality in Humans

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Endothelial lipase (EL; LIPG) is a phospholipase with activity against high density lipoprotein (HDL). Although a small number of mutations in LIPG have been described, the role of LIPG in protection against atherosclerosis is unclear. We identified eight loss-of-function (LOF) mutations in LIPG in individuals by sequencing a cohort of individuals with high HDLc (HDL >90th percentile). Functional analysis confirmed that most rare mutations abolish lipase activity in vitro, indicating complete LOF, whereas two more common mutations N396S and R476W reduce activity by ~50%, indicating partial LOF, and implying that ~50% and ~75% remaining EL function in heterozygous complete and partial LOF mutation carriers, respectively. Complete LOF mutation carriers had significantly higher plasma HDLc levels compared with partial LOF mutation carriers. HDL isolated from complete LOF carriers showed significantly enhanced cholesterol efflux acceptor capacity, HDL particle size, and large α-1 HDL particle concentration, compatible with atheroprotective HDL functionality, whereas only trends were observed in partial LOF carriers. Carriers of LIPG mutations exhibited trends toward reduced CAD in four independent cohorts (meta-analysis OR = 0.7, p = 0.04). Our data suggest that the impact of LIPG mutations is directly related to their effect on EL function and support that antagonism of EL function significantly improves the cardioprotective function of HDL.

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An Acyl-CoA Thioesterase and an Acyl-CoA Synthetase Are Both Induced in Inflammatory Macrophages and Exacerbate the Inflammatory Phenotype of These Cells

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Atherosclerosis is accelerated by conditions that enhance the inflammatory activity of macrophages, allowing them to accumulate in greater numbers in the artery wall. We have recently shown that an acyl-CoA synthetase (ACSL1), which converts free fatty acids into their acyl-CoA derivatives, is induced in macrophages by inflammatory stimuli and in mouse models of type 1 diabetes mellitus (T1DM). Myeloid cell ACSL1 expression is required for the inflammatory effect of diabetes in macrophages and diabetes-accelerated atherosclerosis. We hypothesized that acyl-CoA thioesterase 7 (ACOT7), which liberates fatty acids from the CoA moiety, acts in conjunction with ACSL1 to promote inflammatory activation of macrophages. Like ACSL1, ACOT7 was up-regulated in thioglycolate-elicited macrophages from diabetic mice, compared with nondiabetic controls ($p < 0.05$). Furthermore, lipopolysaccharide (LPS) stimulation increased ACOT7 expression (1.8-fold; $n = 6$; $p < 0.01$) concomitant with ACSL1 expression in macrophages, indicating the involvement of both enzymes in the inflammatory response. To determine whether ACOT7 promotes an inflammatory phenotype, ACOT7 was overexpressed in both J774 macrophages and primary mouse macrophages. LPS-induced secretion of IL1-β, IL-6, and TNF-α was significantly increased 1.5- to 5-fold in ACOT7-overexpressing cells, compared with control cells. Conversely, knockdown of ACOT7 in thioglycolate-elicited macrophages resulted in a modest decrease in Il6 mRNA after LPS stimulation ($p < 0.05$). Thus, ACOT7 and ACSL1 are co-induced in inflammatory macrophages after LPS stimulation and in the setting of T1DM. Furthermore, ACOT7 exacerbates the inflammatory phenotype of macrophages during LPS stimulation. Therefore, the bidirectional flux between free fatty acids and acyl-CoAs is required for maximal inflammatory responses of macrophages and may be a target for inhibition of the exaggerated inflammatory response associated with diabetes and other inflammatory diseases.

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The Proteomic and Functional Analysis of HDL Subclasses in Non-Alcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) defines a spectrum of disease manifestations that are associated with a liver fat content of >5%, with predisposing factors including obesity, insulin resistance, and dyslipidemia. Although a subset of individuals accumulate fat in their liver with no adverse complications, approximately 10-25% of NAFLD patients progresses to nonalcoholic steatohepatitis (NASH), characterized by liver inflammation and fibrosis. Due to the varied spectrum of disease manifestations, it is imperative to elucidate the underlying mechanisms that render some individuals susceptible to the development of NAFLD and the progression to NASH. One of the characteristic changes in the lipid profile of obese individuals that significantly contributes to the cardiovascular disease risk is a shift to smaller denser HDL particles, which lose their cardioprotective qualities. In previous studies, we have shown that there is a shift to smaller denser HDL particles in NAFLD patients, which correlates with quantitative changes in HDL-associated proteins. We have previously validated a technique of separating the larger HDL2 and the smaller HDL3 particles from human serum by size exclusion chromatography. Using nano-HPLC-ESI-tandem mass spectrometry, the proteomes of the particles can be quantitatively assessed.

In this study, we characterized differences between the HDL particles of NAFLD and NASH patients and control individuals. All individuals were morbidly obese females of Northern European descent. HDL particle sizes were significantly smaller in individuals with NAFLD compared with controls (8.65 versus 8.86 mm). We selected three representative individuals with NAFLD and age-matched controls for a comprehensive proteomic analysis. To assess the impact of these proteomic changes on cholesterol efflux function of HDL, we are currently examining HDL2 and HDL3 subclasses from NAFLD and control individuals using in vitro efflux assays.

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Lipoprotein(a) Levels, ApoA Isoform Size, and the Risk of Coronary Heart Disease in South Asians and Europeans

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Although evidence from genetic studies suggests a causal role for the Lp(a) pathway in the etiology of coronary heart disease (CHD), it remains unknown whether this pathway operates through Lp(a) levels, size of the apo(a) protein bound to Lp(a) particle, or both. We investigated the associations of circulating Lp(a) levels, apo(a) isoform size, and genotypes at the LPA locus with CHD risk in up to 34,000 participants of the Pakistan Risk of Myocardial Infarction Study (PROMIS) of South Asian origin and the European Investigation into Cancer (EPIC) Norfolk study. In all participants, Lp(a) levels were measured in serum using ELISAs, apo(a) isoform size was estimated using quantitative PCRs on genomic DNA and electrophoresis in serum, and genotypes at the LPA locus were assessed using the Illumina 660 GWAS chip or TaqMan assays. In multivariate analyses, in addition to major lipids and conventional risk factors, Lp(a) levels and apo(a) isoform size were mutually adjusted for each other. Apo(a) isoform size assessed through quantitative PCR correlated highly with the electrophoretic method. Lp(a) concentration and apo(a) isoform size were associated with CHD risk in both South Asians and Europeans independent of each other and of conventional risk factors and major lipids. In combined analyses, the mutually adjusted odds ratios for CHD were 1.12 (95% CI: 1.08 to 1.16) per standard deviation (SD) increase in Lp(a) concentration and 1.24 (95% CI: 1.09 to 1.42) for apo(a) isoform size (bottom quintile compared with the top quintile). The relative risk per 1.27 SD higher genetically elevated Lp(a) concentration was 1.22 (95% CI: 1.01 to 1.43), which was consistent with the expected relative risk for a 1.27 SD increase in circulating Lp(a) concentration from prospective studies (1.17 (95% CI: 1.12 to 1.23)). These results indicate that circulating Lp(a) levels and apo(a) isoform size are both independently and causally associated with CHD risk

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Fine-tuning of Cellular Cholesterol Mobilization by MicroRNA-33

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Increasing evidence suggests that autophagy and cholesterol homeostasis, both ancient and conserved cellular pathways, have co-evolved to share common regulatory elements. Autophagy plays a key role in cellular cholesterol efflux by regulating cholesterol mobilization from lipid droplets (LDs), a rate-limiting step in macrophage reverse cholesterol transport. Recent studies by our group and others identified microRNA-33 (miR-33) as a post-transcriptional regulator of reverse cholesterol transport via repression of multiple genes involved in cellular cholesterol efflux, HDL biogenesis, and bile transport. Notably, miR-33 is also predicted to target the 3′-UTR of a number of essential autophagy-related (ATG) proteins and autophagy regulators. We hypothesized that by exerting post-translational control over components of the autophagy machinery, miR-33 regulates the mobilization of LD cholesterol for efflux via ABCA1 and ABCG1. Quantitative PCR array profiling revealed that a high proportion of autophagy genes are down-regulated by miR-33 overexpression and conversely up-regulated by miR-33 inhibition in mouse peritoneal macrophages. We validated a subset of genes in the autophagy pathway as bona fide miR-33 targets using 3′-UTR luciferase assays and confirmed regulation of these targets by miR-33 using quantitative PCR and Western blot analysis. In macrophages treated with miR-33 inhibitors in vitro, we observed elevated autophagic flux by microscopy and Western blot analysis. Furthermore, anti-miR-33 treatment of Apoe–/– mice also enhanced autophagy in vivo in peritoneal macrophages, which was paralleled by cytosolic LD depletion. Moreover, anti-miR-33-treated Ldlr–/– mice showed enhanced autophagy in plaque macrophages coincident with atherosclerosis regression. These data identify a novel role for miR-33 in the regulation of autophagy and further highlight the coordinated regulation by miR-33 of pathways that regulate cellular cholesterol trafficking and efflux.

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Biomimetic, Spherical High Density Lipoproteins from a Gold Nanoparticle Template

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Coronary heart disease (CHD) is the leading cause of death worldwide and is inversely correlated with levels of high density lipoprotein (HDL). Efforts to raise HDL as a therapeutic strategy have met with mixed results for decreasing CHD. HDL is a natural nanostructure with a range of sizes and compositions, parameters known to influence HDL function. The limited success of therapies that raise HDL cholesterol indicates the need to better understand HDL structure-function relationships. Chemistry on the nanoscale provides the ability to controllably synthesize mimics of natural, spherical HDL. Using a gold nanoparticle (Au NP) as a template, we have synthesized spherical, biomimetic HDL nanostructures (HDL Au NP). HDL Au NPs are designed to mimic the surface composition and physical properties of naturally occurring HDL. The Au NP serves as a template for the assembly of phospholipids and apolipoprotein A-I. Cell culture experiments demonstrate that HDL Au NPs are nontoxic and function to efflux cholesterol through the same biochemical pathways as natural HDL. An in vitro co-culture assay shows the ability of HDL Au NPs to transport cholesterol from macrophages to hepatocytes. Overall, our data demonstrate the use of an Au NP template to synthesize functional, biomimetic structures of HDL. These unique materials have the potential to be used as a tool to study the structure-function relationship of spherical HDL and as novel therapeutics for CHD.

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Competitive Binding of CXCL12 to CXCR7 Reduces Atherosclerosis in ApoE-Deficient Mice

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Genome-wide association studies consistently link loci at chemokine CXCL12 to coronary artery disease. CXCL12 interacts with two chemokine receptors named CXCR4 and CXCR7. Genetic deletion or pharmacological inhibition of CXCR4 exacerbates atherosclerosis in mice (Circ. Res. 2008; 102: 209-217). However, the role of CXCR7 in atherosclerosis is unclear. It was aimed to examine the effect of a CXCR7-specific compound in a mouse model of atherosclerosis. PF-708 was synthesized, and shows an IC50 of 4nM in depleting binding of a radio-labeled CXCL12 to CXCR7, with no effect on the ligand binding to CXCR4. Single subcutaneous (s.c.) injection of PF-708 at 10mg/kg resulted in free drug exposure above 20 folds of in vitro IC50 at 24 hours post injection. Twelve-week treatment by s.c. injection of PF-708 at 10mg/kg/day significantly reduced atherosclerotic lesion in high-fat diet fed ApoE-deficient mice. This was associated with reduced aortic tissue expression of CXCL12. Remarkably, combined treatment of PF-708 with atorvastatin (Lipitor) further limited lesion size compared to atorvastatin-alone treatment. Targeting CXCR7 might provide novel opportunities to treat atherosclerosis-related disease.

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Genetic Loci Influencing Plasma Triglycerides and Risk for Coronary Artery Disease

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Plasma triglycerides (TGs) are transported in specific lipoproteins: very low density lipoproteins, chylomicrons, and remnants of their metabolism. Increased plasma TG levels correlate with higher risk for coronary artery disease (CAD) in observational epidemiologic studies; however, it is unclear whether this association reflects causal processes. Interpretation of genetic information can help assess causality and can be useful in dissecting the influences of correlated measures such as TGs, low density lipoprotein cholesterol (LDL-c), and high density lipoprotein cholesterol (HDL-c). We used 185 common variants recently mapped for plasma lipid traits ($p < 5 \times 10–8$ for each) to examine the role of TGs on CAD risk. First, we highlight loci with pleiotropic effects on both LDL-c and TG and show that the direction and magnitude of both of these effects are factors in determining risk for CAD. Second, we consider a subset of lipid loci that have a strong effect on TG but minimal effect on LDL-c and show that these loci remain associated with CAD. Finally, we demonstrate that the strength of a variant's effect on TG is correlated with the magnitude of its effect on CAD risk, even after accounting for the same variant's potential effect on LDL-c and/or HDL-c. These results suggest that TG-rich lipoproteins may causally influence risk for CAD and that prioritization of therapeutic targets for CAD may benefit from attention to plasma TG.

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Apolipoprotein A-V Gene Therapy as a Tool to Correct Hypertriglyceridemia

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Apolipoprotein (apo) A-V is a low abundance protein with profound effects on plasma triglyceride (TG) levels. Previously, we showed that adeno-associated virus (AAV2/8)-mediated gene transfer of APOA5 into apoa5–/– mice lowers plasma TG. Here, we study the metabolic effects of approximately 553 G>T single-nucleotide polymorphism (SNP). This SNP results in a Gly to Cys substitution at position 162 in mature apoA-V that correlates with elevated plasma TG in Asian populations. AAV2/8 particles harboring the coding sequence for wild-type apoA-V (AAV2/8-apoA-V), G162C apoA-V (AAV2/8-G162C) and LacZ (AAV2/8-LacZ) were injected (1 × 1012 viral genome) into the tail vein of apoa5–/– mice. Blood samples were collected weekly for 4 weeks and plasma TG and apoA-V levels measured. FPLC fractions corresponding to VLDL, LDL, HDL, and lipid-poor region were immunoblotted to detect apoA-V. Compared with AAV2/8-LacZ mice, AAV2/8-apoA-V injected animals had significantly lower TG levels (50 ± 5%). Mice injected with AAV2/8-G162C displayed little or no reduction in plasma TG. ApoA-V immunoblots of plasma samples from AAV2/8-apoA-V and AAV2/8-G162C mice revealed equivalent amounts of apoA-V. Immunoblots of FPLC fractions revealed that in AAV2/8-apoA-V mice, apoA-V resides mainly on VLDL, with a small amount on HDL. In AAV2/8-G162C mice, lipoprotein-associated apoA-V was dramatically reduced, with some detected on VLDL and none on HDL. A significant portion of G162C apoA-V was recovered in the lipid-poor fraction. ApoA-V represents an important gene therapy target. Reduced plasma TG observed in hypertriglyceridemic apoa5–/– mice following injection with AAV2/8-apoA-V supports this conclusion. Failure of AAV2/8-G162C to correct hypertriglyceridemia in this model is likely associated with its inability to normally bind VLDL thus reducing TG hydrolysis. The gene therapy model system established here provides a platform for studies of metabolic defects associated with common apoA-V SNPs.

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Tribbles1 (TRIB1) Is a Novel Regulator of in Vivo Hepatic Fatty Acid Lipogenesis in the Mouse

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Tribbles1 (TRIB1) was recently identified in genome-wide association studies as being strongly linked to plasma levels of VLDL, HDL, LDL, and TG as well as coronary artery disease in humans. Adeno-associated virus (AAV)-mediated hepatic overexpression of Trib1 in mice has confirmed this association. Here, we report a Trib1 liver-specific knockout mouse (Trib1_LSKO) created through AAV-mediated delivery of Cre recombinase into adult mice with a floxed version of Trib1. Trib1_LSKO mice exhibit 21% and 70% increases in TC and TG, respectively ($p = 0.01$ and 0.02) compared with floxed Trib1 littermates infected with null virus (controls). Trib1_LSKO animals also exhibited a 25% increase in liver weight ($p < 0.01$), and histological analysis revealed steatotic livers in LSKO mice. Real-time PCR analysis revealed >2-fold increases in the hepatic transcription of genes involved in fatty acid synthesis in Trib1_LSKO mice compared with controls. Trib1_LSKO mice also had a 78% increase in hepatic TG content ($p < 0.001$), but no significant change in hepatic cholesterol was observed. De novo lipogenesis was measured using [3H]acetate, and Trib1_LSKO animals exhibited increased production of TG (3.6-fold; $p < 0.001$), fatty acids (2.2-fold; $p = 0.02$), diacylglycerol (1.8-fold; $p < 0.01$), and phospholipids (2-fold; $p = 0.05$). Microarray analysis of Trib1_LSKO livers compared with controls revealed >1,600 genes significantly altered between the two groups (-fold change >1.5, FDR <10%). Pathway analysis suggested that the altered gene set was enriched for genes downstream of C/EBP and C/EBP. Western blot analysis of liver extracts showed increases in both C/EBP and C/EBP levels in Trib1_LSKO mice compared with controls. In conclusion, Trib1 is a novel regulator of de novo lipogenesis in mice, presumably through the regulation of lipogenic gene transcription. This transcriptional control may be regulated by increased levels of C/EBP and/or C/EBP, or an as yet undetermined target of Trib1.

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Effects of Hepatic DGAT2 Inhibition on Glucose and Lipid Metabolism in Obese Mice

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DGAT2 (acyl-CoA: diacylglycerol acyltransferase) catalyzes the final step in triglyceride (TG) synthesis and has been gaining attention for a proposed role in diabetes and metabolic syndrome. DGAT2 plays a role in regulating hepatic VLDL production, and its inhibition has been considered as a potential therapeutic strategy for atherosclerosis via decreased VLDL and LDL. Insulin resistance, hyperglycemia, hepatosteatosis, and dyslipidemia characterized by increased plasma TG and reduced HDL are contributing factors for accelerating atherosclerosis in diabetes and metabolic syndrome. To study the hypothesis that hepatic DGAT2 inhibition would reduce hepatic TG synthesis and improve hepatosteatosis and insulin sensitivity, we generated AAV-mediated DGAT2 knockdown (AAV-DGAT2shRNA) in a growing diet-induced obesity (gDIO) mouse model. The hepatic knockdown of DGAT2 significantly reduced newly synthesized TG and hepatic lipid contents and reduced plasma LDL cholesterol levels without changing body weight. Despite these dramatic lipid changes, AAV-DGAT2shRNA gDIO mice showed plasma insulin, glucose levels, and glucose tolerance test responses similar to those of controls. Further studies exploring the effects of DGAT2 modulation on glucose and lipid metabolism are warranted.

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Functional Characterization of GALNT2, a Novel Locus Influencing HDL Cholesterol and Triglycerides in Humans

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Recent genome-wide association studies (GWAS) for blood lipids have uncovered several novel genomic loci influencing these traits. One such locus associated with high density lipoprotein cholesterol (HDL-c) and triglycerides (TG) is that harboring GALNT2 on chromosome 1q42. GALNT2 encodes GalNAc-T2, an enzymatic mediator of the first step of mucin type O-glycosylation never previously implicated in lipoprotein metabolism. We demonstrated previously that GALNT2 overexpression in mice using liver-specific adeno-associated virus (AAV) significantly decreased plasma HDL-c and increased very low density lipoprotein (VLDL)-TG relative to control. Postprandial TG clearance was significantly delayed in GALNT2-overexpressing mice, and no differences in the rate of VLDL-TG secretion were observed. We also investigated the role of whole body and liver-specific Galnt2 deficiency using knockout (KO) mouse models harboring a deletion of exons 4-6 in the Galnt2 gene. Whole body GALNT2 deficiency resulted in elevated VLDL-TG levels compared with wild-type and a moderate reduction in HDL-C (20%; $p < 0.05$). Galnt2 KO mice did not exhibit differences in postprandial TG clearance or VLDL-TG secretion. Liver-specific deletion of Galnt2 reduced HDL-c (26% decrease relative to WT; $p < 0.01$) and reduced numbers of HDL particles (19% decrease relative to WT; $p < 0.01$) with minimal changes in plasma TG. Our data suggest a novel role for GALNT2 in the modulation of plasma lipid concentrations.

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Somatic Overexpression and Knockdown in Mice to Identify Causal Genes Underlying 26 Lipid-associated Loci

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Genome-wide association studies (GWAS) have identified 95 loci in the human genome that harbor common variants associated with plasma lipid traits. Of the 95 loci, 17 harbor genes known to cause monogenic lipid disorders, and collectively a third of them contain genes with characterized roles in lipid metabolism. Therefore in the majority of loci the causal genes are unknown. We selected 32 genes, not previously implicated in lipid metabolism and representing a total of 26 loci, to test for their ability to modify plasma lipid concentrations upon somatic overexpression in vivo. We utilized adeno-associated virus serotype 8 (AAV8) to overexpress the selected genes specifically in the livers of both C57BL/6 mice and in an appropriate humanized mouse model (either mice expressing human apolipoprotein A-I for HDL loci or Apobec1-knockout, Ldlr-haploinsufficient mice expressing human apolipoprotein B-100 for triglyceride and LDL loci). Approximately half of the genes tested reproducibly affected plasma lipids. For 13 of the interrogated loci the lipid-associated variants also correlated with expression variations of the respective genes in liver (liver expression quantitative trait loci eQTLs). We demonstrate a causal role for 7 of these 13 genes. The overexpression of these 7 genes not only affected the predicted lipid class, but additionally exerted its effect in the predicted direction in 6 of 7 cases (Tmem57, Slc39a8, Ppp1r3b, Vkorc1, Tbkbp1, and Ube2l3). Additionally, for a subset of the examined genes we proceeded to develop small interfering RNA (siRNA) nanoparticles that were particularly targeted to the liver. We were able to obtain robust knockdown for a significant number of genes and, in several cases, observe reciprocal effects on plasma lipids from our overexpression and knockdown studies. This work has identified several novel lipid regulators, whose further investigation can uncover novel mechanisms and pathways controlling plasma lipids.

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Small Molecule Inducers of TRIB1 Modulate Cholesterol Metabolism in HepG2 Cells

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Despite widespread use of statins, cardiovascular (CV) disease remains one of the leading causes of death worldwide. Epidemiological studies have repeatedly demonstrated that elevated levels of circulating cholesterol, specifically low density lipoprotein cholesterol (LDL-c), has a strong association with the development of atherosclerosis and coronary heart disease (CHD). Recent clinical GWAS have uncovered novel genes associated with the risk of CV disease (e.g. PCSK9, TRIB1, SORT1, and ANGPTL) and suggest novel approaches to developing improved therapeutics. Building on the observations that increased expression of the TRIB1 gene is associated with lower plasma levels of LDL-c and triglycerides, higher plasma levels of HDL cholesterol and a significantly reduced risk for CHD and myocardial infarction, we set up a quantitative RT-PCR-based phenotypic assay to identify compounds that induce TRIB1 expression in HepG2 human hepatocellular carcinoma cells. In the screen of a representative subset of the Broad's proprietary small molecule library, we identified several distinct series of TRIB1 inducers from the diversity-oriented synthesis (DOS) collection, including compounds that demonstrated stereochemically dependent activity. Upon further characterization of the TRIB1 inducer compounds in the HepG2 and Huh-7 hepatocellular carcinoma models, we found that they also modulate multiple components of hepatic cell cholesterol metabolism including elevating the expression of LDLR transcript and LDL receptor protein while reducing the levels of PCSK9 and MTTB transcripts and ApoB100 protein. The effects of the TRIB1 inducers are not masked by cholesterol depletion and are independent of the SREBP-2 regulatory circuit, suggesting that these compounds represent a novel class of modulators of hepatic LDL metabolism. Efforts to identify the molecular target(s) of the TRIB1 inducers are currently under way.

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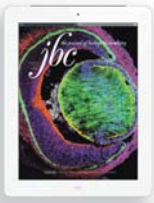

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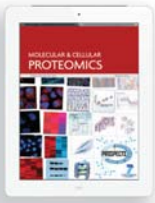

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