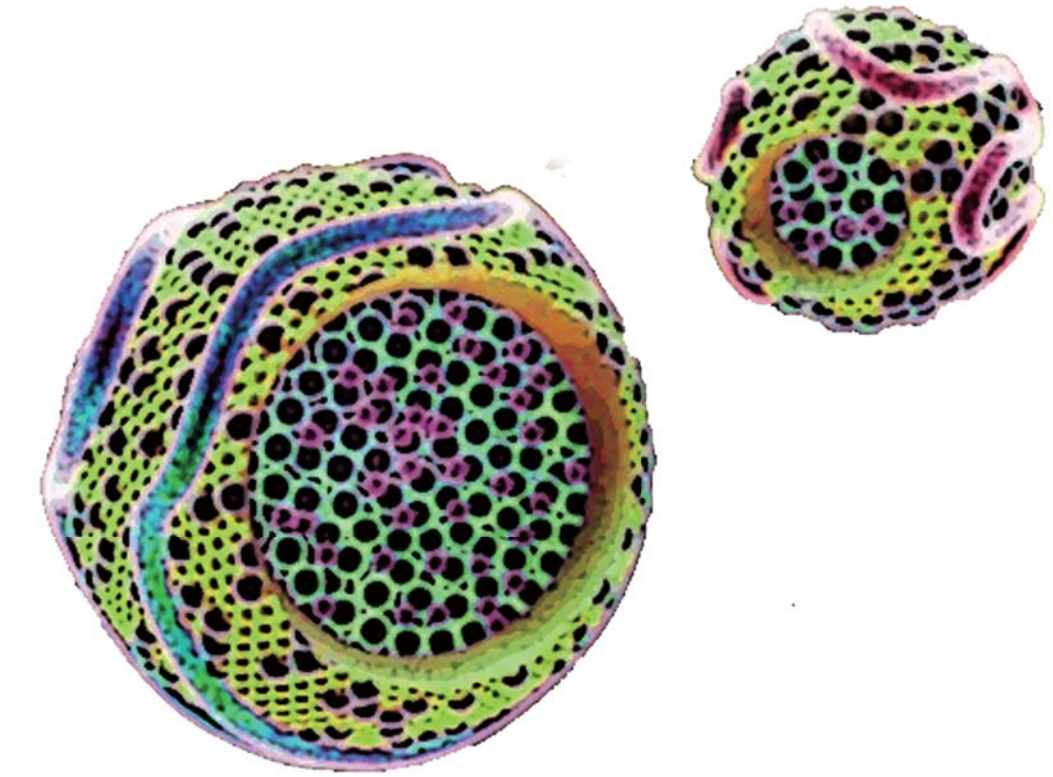
March 1-4, 2011

Napa Valley, California

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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## 2011 DEUEL Conference on Lipids



The Deuel Conference on Lipids was organized in 1955 by a small group of eminent West Coast investigators who were interested in lipid metabolism. Their goal was to establish a high-quality conference on lipids within the western part of the country, akin to forums provided by the Gordon Conferences on the east coast. Shortly after the Conference was organized, one of the founders, Dr. Harry Deuel, died—and the conference was named in his memory. The two-and-one-half day conference includes 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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# 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.

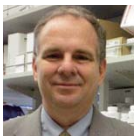
## Richard J. Havel Lecturers

2011 Christopher K. Glass, UCSD

"Oxysterol regulation of macrophage gene expression"

2010 David J. Mangelsdorf, UT Southwest

"Nuclear receptor control of lipid metabolism"



2009 Stephen G. Young, UCLA School of Medicine, Los Angeles, CA

"Adventures in Lipid Metabolism"

2008 Helen H. Hobbs, University of Texas Southwestern Medical Cnter, Dallas, TX

"Going to Extremes to Identify Genetic Variations Contributing to Cardiovascular Risk"

2007 Ronald Evans, The Salk Institute for Biological Sciences, La Jolla, CA

"PPARdelta and the Marathon Mouse: Running Around Physiology"

2006 David Russell , University of Texas Southwestern Medical Center, Dallas, TX

"The Enzymes of Cholesterol Breakdown"

2005 Johann Deisenhofer, HHMI/UTSW Medical Center, Dallas, TX

"Structure of the LDL receptor"

2004 Jeffrey M. Friedman, Rockefeller University, New York, NY

"The Function of Leptin in Nutrition, Weight and Physiology"

2003 Bruce Spiegelman, Harvard Medical School, Boston, MA

"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 1 | Wednesday, March 2 | Thursday, March 3 | Friday, March 4 |
|---|---|---|---|---|
| 7 AM | | Breakfast 7-8:30 | Breakfast 7-8:30 | Breakfast 7-8:30 |
| 8 AM | | | Board Meeting 7:30- 8:30 | |
| 9 AM | | Session I 8:30-10:00 | Session 2 8:30-9:50 | Session 4 8:30-9:50 |
| 10 AM | | Coffee Break 10:00 | Coffee Break 9:50 | Coffee Break 9:50 |
| 11 AM | | Session I, Continued 10:20-11:50 | Session 2, Continued 10:20-12:30 | Session 2, Continued 10:20-11:40 |
| 12 PM | | Free Time | | |
| 1 PM | | | Free Time | |
| 2 PM | | | | |
| 3 PM | Registration 3-6:30 | | | |
| 4 PM | | | Board Meeting 3:30 -5:00 | |
| 5 PM | | | Sponsor Reception 5:00-6:00 | |
| 6 PM | Welcome Reception and Dinner | Dinner 6:00- 7:30 | Dinner 6:00-7:30 | |
| 7 PM | | HAVEL LECTURE 7:30-9:30 | Session 3 7:30-9:30 | |
| 8 PM | | | | |
| 9 PM | | | | |
| 10 PM | | | | |

# Meeting Program

# 2011 Deuel Conference on Lipids, March 1-4, 2011
## Silverado Resort, Napa Valley, California

### *Wednesday, March 2*

*Wednesday, March 2, 8:30 AM to 12:00 PM*

**Session Chair: Jay Horton**

**Session 1** **Obesity, Metabolic Syndrome and Insulin Resistance**

8:30-9:15 "Surviving Starvation: Essential Role of the Ghrelin-Growth Hormone Axis"
**Joseph L. Goldstein**, *University of Texas Southwestern*, Dallas, TX

9:15-10:00 "Surviving Starvation: Essential Role of the Ghrelin-Growth Hormone Axis"
**Michael S. Brown**, *University of Texas Southwestern*, Dallas, TX

10:00-10:20 Coffee Break

10:20-11:00 "Regulation and Function of Adipose Lipolysis"
**Hei Sook Sul**, *University of California*, Berkeley, CA

11:00-11:40 "New Insights Regarding the Structure and Function of GPIHBP1, an Endothelial Cell Protein Required for Lipolysis"
**Anne Beigneux**, *University of California*, Los Angeles, CA

11:40-12:00 "Ubxd8, a Sensor for Unsaturated Fatty Acids, Regulates Cellular Responses to Fatty Acids"
**Jin Ye**, *University of Texas Southwestern Medical Center*, Dallas, TX

*Wednesday, March 2, 7:30 to 8:30 PM*

**Session Chair: Jay Heinecke**

### The Havel Lecture

"Oxysterol Regulation of Macrophage Gene Expression",
**Chris Glass**, *University of California*, San Diego, CA
**Wine Reception and Poster Session**

### *Thursday, March 3*

*Thursday, March 3, 8:30 AM to 12:00 PM*

**Session Chair: Jay Horton**

**Session 2** **Systems Biology**

8:30-9:10 "Exploring inflammatory links between obesity and type 2 diabetes"
**Alan Saltiel**, *University of Michigan,* Ann Arbor, MI

9:10-9:50 "Evolutionary Conservation and Adaptation in the Mechanisms that Regulate SREBP Action"
**Tim Osborne**, *Sanford-Burnham Medical Research Institute*, Orlando, FL

9:50-10:20 Coffee Break

10:20-11:00 "PNPLA3 to Pâté"
**Helen Hobbs**, *University of Texas Southwestern*, Dallas, TX

11:00-11:40

### The Journal of Lipid Research Lecture

Introduction to the lecture - Steve Young, University of California, Los Angeles, CA
"A Mouse Resource for Systems Genetics: Application to Atherosclerosis"
**Jake Lusis**, *University of California*, Los Angeles , CA

11:40-12:00 "Angptl4 Protects against Severe Proinflammatory Effects of Saturated Fat by Inhibiting Fatty Acid Uptake into Mesenteric Lymph Node Macrophages"
**Fritz Mattijssen**, *Wageningen University*, Netherlands

*Thursday, March 3, 7:30-9:30 PM*

**Session Chair: Jay Heinecke**

**Session 3** **Macrophages and Inflammation**

7:30-8:10 "Role of HDL, ABCA1 and ABCG1 Transporters in Cholesterol Efflux and Immune Responses"
**Alan Tall**, *Columbia University,* New York, NY

8:10-8:50 "Inflammation, Macrophages, and Insulin Resistance"
**Jerrold Olefsky**, *University of California*, San Diego, CA

8:50-9:30 "Fate of Monocytes During Reversal of Atherosclerosis"
**Gwen Randolph**, *Mount Sinai School of Medicine,* New York, NY

## *Friday, March 4*

*Friday, March 4, 8:30 AM to 12:00 PM*

**Session Chair: Jay Horton**

**Session 4** **Therapeutics/Diagnostics/Pharmacology**

8:30-9:10 "Development of Efficacious mAb against PCSK9 to Reduce LDL Levels in Humans"
**Mark Sleeman**, *Regeneron,* Tarrytown, NY

9:10-9:50 "Toward Metabolomic Signatures of Cardiovascular Disease"
**Robert Gerszten**, *Harvard University*, Charlestown, MA

9:50-10:20 Coffee Break

10:20-11:00 "Update on the CETP Inhibitor Anacetrapib (MK-859)"
**Yale Mitchel**, *Merck Research Labs,* Rahway, NJ

11:00-11:20 "Deletion of ATP-binding Cassette Transporter A1 (ABCA1) in Macrophages Protects Mice from Listeria Monocytogenes Infection but Impairs MHC Class I Restricted Antigen Presentation"
**Xuewei Zhu**, *Wake Forest University School of Medicine,* Winston-Salem, NC

11:20-11:40 Closing Remarks

# Poster Presentations

# 1
# Ubxd8, a Sensor for Unsaturated Fatty Acids, Regulates Cellular Responses to Fatty Acids

Jin Ye, Joon No Lee, Hyeonwoo Kim, and Hongbing Yao

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

Abstract:

In mammalian cells, fatty acids (FAs) are required for the synthesis of the phospholipid components of membranes and generation of energy. However, overaccumulation of FAs is toxic. When FAs accumulate in cells, they inhibit their own synthesis and enhance the incorporation of excess FAs into triglycerides (TGs) that are stored in lipid droplets. These regulatory functions are carried out by unsaturated but not saturated FAs. Here, we report that Ubxd8 is a sensor for unsaturated FAs that regulate cellular responses to FAs. In cells depleted of FAs, Ubxd8 binds to Insig-1, a reaction leads to proteasomal degradation of Insig-1. Inasmuch as Insig-1 inhibits proteolytic activation of sterol-regulatory element-binding protein (SREBP)-1, a transcription factor that activates all genes involved in FA synthesis, this reaction stimulates FA synthesis by promoting cleavage of SREBP-1. Ubxd8 also blocks TG synthesis by limiting the conversion of diacylglycerols (DAGs) to TGs in these cells as well. Thus, in cells deprived of FAs, the concerted regulatory actions of Ubxd8 make FAs available for incorporation into phospholipids by limiting their diversion into TGs. When long chain unsaturated FAs are supplied externally, these FAs change the structure of Ubxd8, promoting its polymerization, and inhibiting its activity. Consequently, Insig-1 is dissociated from Ubxd8 and stabilized so that FA synthesis is inhibited. Inhibition in TG synthesis is also relieved in these cells so that excess exogenous FAs are incorporated into TGs and stored in lipid droplets. Unlike unsaturated FAs, saturated FAs do not alter the structure of Ubxd8. As a result, saturated FAs are unable to inactivate Ubxd8 so that these FAs are channeled primarily into DAGs instead of TGs. Inasmuch as accumulation of DAGs is responsible for the development of insulin resistance, our studies suggest that Ubxd8 could be a novel drug target to relieve insulin resistance caused by excessive saturated FAs.

# 2

## miR-33a/b contributes to the regulation of fatty acid metabolism and insulin signaling

Leigh Goedeke1, Alberto Davalos1, Cristina Ramirez-Hidalgo1, Peter Smibert2, Nikhil Warrier1, Ursula Andreo1, Daniel Cirera-Salinas1, 3, Katey Rayner1, Enric Esplugues3, 4, Edward Fisher1, Kathryn Moore1, Yajaira Suarez1, Eric Lai2, and Carlos Fernandez-Hernando1

1Department of Medicine and Cell Biology, New York University School of Medicine, New York, NY; 2Department of Developmental Biology, Sloan-Kettering Institute, New York, NY; 3German Rheumatism Research Center (DRFZ), a Leibniz Institute, Berlin, Germany; 4Department of Immunobiology, Yale University School of Medicine, New Haven, CT

Abstract:

Cellular imbalances of cholesterol and fatty acid metabolism result in pathological processes, including atherosclerosis and metabolic syndrome. Recent work from our group and others has shown that the intronic microRNAs (miRNAs), hsa-miR-33a and hsa-miR-33b, are located within the sterol-regulatory element-binding protein (SREBP) 2 and 1 genes, respectively, and regulate cholesterol homeostasis in concert with the their host genes. Here, we show that miR-33a/b also regulate genes involved in fatty acid metabolism and insulin signaling. miR-33a/b target key enzymes involved in the regulation of fatty acid oxidation, including CROT, CPT1a, HADHB, SIRT6 and AMPKα. Moreover, miR-33a/b also target the insulin receptor substrate 2 (IRS2), an essential component of the insulin-signaling pathway in the liver. Overexpression of miR-33a/b reduces both fatty acid oxidation and insulin signaling in hepatic cell lines, whereas inhibition of endogenous miR-33a/b increases these two metabolic pathways. Together, these data establish that miR-33a/b regulate pathways controlling three of the risk factors of metabolic syndrome, namely, levels of HDL, triglycerides, and insulin signaling, and suggest that inhibitors of miR-33a/b may be useful in the treatment of this growing health concern.

# 3

## miR-758 Regulates Cholesterol Efflux through Posttranscriptional Repression of ABCA1

Cristina Ramírez-Hidalgo, Leigh Goedeke, Alberto Dávalos, Yajaira Suárez, and Carlos Fernández-Hernando

Departments of Medicine and Cell Biology, New York University School of Medicine, New York, NY

Abstract:

The ATP-binding cassette transporter A1 (ABCA1) is a major regulator of macrophage cholesterol efflux and protects cells from excess of intracellular cholesterol accumulation. The mechanism involved in posttranscriptional regulation of ABCA1 is poorly understood. Thus, here we investigate the potential contribution of microRNAs to regulate ABCA1 and macrophage cholesterol efflux posttranscriptionally. Based on an unbiased genome-wide screen of microRNA modulated by excess of cholesterol and quantitative real-time reverse-transcription PCR, we confirmed that miR-758 is repressed in cholesterol-loaded mouse peritoneal macrophages. Under physiologic conditions, fat dietary excess in mice repressed mir-758 both in peritoneal macrophage and in a lesser extent in liver. In mouse and human cells in vitro, miR-758 represses the expression of ABCA1, and conversely the inhibition of this microRNA by using anti-miR-758 increases ABCA1 expression. In mouse cells, mir-758 reduces cellular cholesterol efflux to apoA1, and anti-miR-758 increases it. miR-758 directly target the 3'-UTR of mouse ABCA1 assessed as the 3'-UTR luciferase activity and mutation of both putative miR-758 target sites in the ABCA1 3'-UTR completely abolished the inhibitory effects of miR-758. We identified miR-758 as a novel microRNA that posttranscriptionally controls ABCA1 levels in different cells and regulates macrophage cellular cholesterol efflux to apoA1, opening new avenues to increase apoA1 and raise HDL levels.

# 4

## Angptl4 Protects against Severe Proinflammatory Effects of Saturated Fat by Inhibiting Fatty Acid Uptake into Mesenteric Lymph Node Macrophages

Frits Mattijssen1, Laeticia Lichtenstein1, 2, Nicole de Wit1, 2, Anastasia Georgiadi1, Guido Hooiveld1, 2, Roelof van der Meer1, 2, 3, Yin He4, Ling Qi4, Anja Köster4, 5, Jouke Tamsma6, Nguan Soon Tan7, Michael Muller1, 2, and Sander Kersten1, 2, 4

1Nutrition, Metabolism, and Genomics Group, Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands; 2Nutrigenomics Consortium, TI Food and Nutrition, Wageningen, The Netherlands; 3NIZO Food Research, Ede, The Netherlands; 4Division of Nutritional Sciences, Cornell University, Ithaca, NY; 5Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN; 6Department of General Internal Medicine and Endocrinology, Leiden University Medical Centre, Leiden, The Netherlands; 7School of Biological Sciences, Nanyang Technological University, Singapore

Abstract:

Dietary saturated fat is linked to numerous chronic diseases, including cardiovascular disease. Here, we identify the lipoprotein lipase inhibitor Angptl4 as being critical for the response to dietary saturated fat. Strikingly, in mice lacking Angptl4, saturated fat induces a severe and ultimately lethal phenotype characterized by fibrinopurulent peritonitis, ascites, intestinal inflammation and fibrosis, and cachexia. These abnormalities are preceded by a massive acute phase response induced by saturated but not unsaturated fat or medium chain fat, originating in mesenteric lymph nodes (MLNs). MLNs undergo dramatic expansion in Angptl4–/– mice upon high saturated fat feeding and contain numerous Touton cells representing fused lipid-laden macrophages. In peritoneal macrophages incubated with chyle, Angptl4 dramatically reduced foam cell formation, inflammatory gene expression, and chyle-induced activation of endoplasmic reticulum stress. We conclude that induction of macrophage Angptl4 by fatty acids is part of a mechanism that serves to reduce postprandial lipid uptake from chyle into MLN-resident macrophages by inhibiting triglyceride hydrolysis, thereby preventing macrophage activation and foam cell formation and protecting against progressive, uncontrolled saturated fat-induced inflammation.

# 5

# Identification of Compounds Reducing the Expression of Proprotein Convertase Subtilisin-like/Kexin Type 9 (PCSK9) as Therapy in the Treatment of Hypercholesterolemia

Dong-Kook Min1, 2, Hyun-Sook Lee1, 3, Chan-Ju Lee1, 2, and Sahng Wook Park1, 2

1Department of Biochemistry and Molecular Biology, 2Center for Chronic Metabolic Diseases, Brain Korea 21 Project, 3Bio-Medical Science Institute, Yonsei University College of Medicine, Seoul, Korea

Abstract:

Proprotein convertase subtilisin/kexin type 9 (PCSK9), the ninth member of subtilisin serine protease, promotes the degradation of the LDL receptor, thereby increasing the plasma concentration of LDL-cholesterol. Several gain-of-function mutations of PCSK9 have been reported to cause a form of autosomal dominant hypercholesterolemia, whereas loss-of-function mutations cause hypocholesterolemia associated with a low incidence of coronary heart disease. These recent studies strongly suggest that inhibition of PCSK9 action is a potent therapeutic target of treatment of hypercholesterolemia. To inhibit PCSK9 action, we focused on screening the chemical library for identification of compounds that reduce the amount of PCSK9 with a reciprocal increase in that of the LDL receptor. We selected a set of chemicals that have the core scaffold structure. The decrease in the amount of PCSK9 protein by these chemicals is supposed to be achieved at the transcriptional level of the PCSK9 gene. They increased uptake of fluorescence-labeled LDL particles in HepG2 cells. These beneficial effects of increasing the uptake of LDL particles suggest that these chemicals could be implicated as a therapeutic modality to treat hypercholesterolemia. Evaluation of functionality in vivo is required for further study.

* This work was supported by National Research Foundation of Korea (NRF) Grants 2010-0028363 and 2010-0026376 funded by the Korean government (MEST).

# 6

## Patterns and Associations of LDL during and after Pregnancy

Donald Tanyanyiwa1, David Marais2, Sheena Jones2, and Pam Byrnes2

1Chris Hani Baragwanath Hospital, Division of Chemical Pathology, Johannesburg, South Africa; 2University of Cape Town, Faculty of Health Sciences, Cape Town, South Africa

Abstract:

The aim of this study was to report the prevalence of various species of LDL in pregnancy and the association of LDL species with triglyceride concentration and apolipoprotein E (apoE) genotype. 595 women were studied at antenatal clinics, after obtaining informed consent and excluding women with diabetes. Blood for lipids and lipoproteins was taken during and at least 6 weeks after pregnancy. Nondenaturing gradient gel electrophoresis was employed to classify LDL species into five categories named A, AI, I, IB, and B in decreasing sizes. Genotyping of apoE was done by polymerase chain reaction. Statistical analyses were done by nonparametric t tests and contingency tables; significance was taken as $p < 0.0001$. The total cholesterol was significantly higher during pregnancy, 4.1 median (4.1-4.3 95% confidence interval) and 3.6 (3.5-3.6) (5 mmol/liter) and severe (>15 mmol/liter) hypertriglyceridemia occurred in 0.7 and 0.6% during pregnancy but was observed in the GGE of the 15 subjects with apoE 2/2 status. A relationship between hypertriglyceridemia and LDL species was observed.

7

# Impact of Diabetes-associated LDL on Mitochondrial Respiration in Vascular Endothelial Cells

Garry Shen, Ganesh Sangle, Xueping Xie, and Subir Roy Chowdhury

University of Manitoba,Winnipeg, MB, Canada

Abstract:

Cardiovascular disease is the predominant cause of death in diabetic patients. Oxidative stress and endothelial dysfunction have been detected in vasculature of diabetic patients or animal models. Hyperglycemia and dyslipoproteinemia are biochemical markers for diabetes. Elevated levels of glycated LDL (gLDL) and oxidized LDL (oLDL) were frequently detected in diabetic patients. Our group reported that gLDL and oLDL increased the generation of reactive oxygen species (ROS) from vascular endothelial cells (EC). The present study demonstrated that gLDL and oLDL reduced oxygen consumption in mitochondrial electron transfer chain (mETC) Complex I and IV in porcine aortic EC. Treatment with gLDL or oLDL reduced mitochondrial membrane potential in EC, and inhibited the activities of NADH dehydrogenase (ND, Complex I), succinate cytochrome c reductase (Complex II), ubiquinol cytochrome c reductase (Complex III), and cytochrome c oxidase (Complex IV) in EC. Abundanced of ND1 and cytochrome b (a subunit of Complex III enzyme) in EC were reduced following incubation with gLDL or oLDL. Treatment with gLDL or oLDL increased the abundance of ROS-associated with mitochondria in EC detected using immunohistochemistry and confocal microscopy. The results suggest that diabetes-associated LDL may inhibit activities of mETC enzymes through reducing abundances of multiple subunits in mETC enzymes, which was associated with increase of ROS in mitochondria of EC. Impairment in mitochondrial respiration in EC induced by diabetes-associated LDL may contribute to oxidative stress and endothelial dysfunction in vasculature of diabetes.

# 8

# Adnectin Proprotein Convertase Subtilisin-like/Kexin Type 9 (PCSK9) Antagonists Inhibit PCSK9 Function and Rapidly Reduce LDL-C in hPCSK9 Transgenic Mice and Cynomolgus Monkeys

Rex Parker1 and Tracy Mitchell2

1Bristol-Myers Squibb R & D, Princeton, NJ; 2Adnexus, a Bristol-Myers Squibb R & D Company, Waltham, MA

Abstract:

Adnectins are 12-kDa proteins derived from the 10th type 3 domain of human fibronectin. We use mRNA display to engineer Adnectins to bind pharmaceutical targets with high affinity and specificity and express and purify the Adnectin proteins from Escherichia coli. Using these techniques, we identified potent Adnectins that bind PCSK9 and antagonize PCSK9/EGFA (LDLR) interactions. These Adnectins were optimized for high affinity, biophysical properties, and low in silico immunogenicity. Adnectin 1 (ADN1) bound PCSK9 with KD <1 nM (human) and ~10 nM (cynomolgus) by SPR and did not bind mouse PCSK9 appreciably. ADN1 competitively displaced EGFA in FRET-based assays and inhibited PCSK9 activity in cell-based LDLR functional assays, with EC50 values consistent with the law of mass action for human and cyno PCSK9 (i.e., EC50 related to KD and target concentration in the assays). In a hypercholesterolemic, hyperexpressing transgenic hPCSK9 mouse model (from UT-SW), PEGylated ADN1 (ADN1-PEG given i.p.) reduced TC and LDL-C by ~35% within 3 h as free PCSK9 levels fell to near-zero and liver LDLR increased ~2-fold. In a genomic transgenic hPCSK9 mouse (BMS) expressing normal hPCSK9 levels, ADN1-PEG (i.p.) reduced free circulating hPCSK9 to near-zero within 30 min with EC50 ~0.01 mg/kg (i.p.), whereas total plasma hPCSK9 levels rose ~2-fold by 48 h. In normal cynomolgus monkeys, ADN1-PEG (5 mg/kg, i.v. or s.c.) rapidly reduced LDL-C and free PCSK9 in a dose-dependent manner, whereas other lipids were unaffected. LDL-C decreased ~50% within 48 h and returned to base line by 3 weeks, mirroring the free PCSK9 profile. Plasma total PCSK9 levels rose 3-5-fold and returned to base line by 3 weeks, suggesting that liver LDLR binding and endocytosis is a key physiological route of clearance for circulating PCSK9. These studies indicate that PCSK9 Adnectins are potent and effective PCSK9 antagonists in vitro and in vivo and demonstrate the dynamic role of circulating PCSK9 in LDL metabolism.

# 10

# Apolipoprotein E Mediates Enhanced Plasma Cholesterol Clearance by Low Dose Streptococcal Serum Opacity Factor via Hepatic LDL Receptors in Vivo

Corina Rosales1, Daming Tang1, Baiba Gillard1, Harry Courtney2, and Henry Pownall

1Department of Medicine, Baylor College of Medicine, Houston, TX; 2Veterans Affairs Medical Center and Department of Medicine, University of Tennessee Health Science Center, Memphis, TN

Abstract:

Serum opacity factor (SOF), a virulence determinant produced by the group A streptococcus, Streptococcus pyogenes, opacifies human serum and is expressed by approximately half of the clinical isolates of S. pyogenes, an important human pathogen that causes pharyngitis, tonsilitis, impetigo, necrotizing fasciitis, and toxic shock syndrome. SOF opacifies serum by disrupting HDL, its exclusive target, and forming a large cholesteryl ester-rich microemulsion (CERM; r ~100-200 nm), lipid-free (LF) apolipoprotein (apo)A-I and small neo-HDL that are cholesterol-poor and phospholipid-rich. The CERM contains apoE and its heterodimer with apoA-II as its sole proteins and the neutral lipids of ~400,000 HDL particles. Given the occurrence of apoE on the CERM, we tested the hypothesis that rSOF injection into mice would reduce total plasma cholesterol clearance via apoE-dependent hepatic LDL receptors (LDLR). rSOF (4 µg) injection into wild-type C57BL mice forms neo-HDL, CERM, and LF apoA-I, as observed in vitro, and reduced plasma total cholesterol (–43%, t1/2 = 44 ± 18 min) whereas control saline injections had a negligible effect. Similar experiments with apoE–/– and LDLR–/– mice, respectively, reduced plasma total cholesterol ~0 and 9%. rSOF is potent; injection of 0.18 µg of rSOF produces 50% of maximum reduction of plasma cholesterol 3 h after injection, corresponding to a ~0.5-mg human dose. Most cholesterol is cleared hepatically (>99%), with rSOF treatment increasing clearance by 65%. We conclude that intravenous injection of rSOF into mice forms a CERM that is readily cleared via hepatic LDLR that recognize apoE. Therapies based on the rSOF reaction have the potential to atheroprotect via enhanced reverse cholesterol transport.

# 11
# Regulation of Alternative Splicing of the LDL Receptor Gene

Feng Gao, Marisa Medina, and Ronald Krauss

Children's Hospital Oakland Research Institute, Oakland, CA

Abstract:

The LDL receptor (LDLR), a cell surface glycoprotein, is responsible for the binding and uptake of plasma LDL particles and plays an important role in maintaining cellular cholesterol homeostasis. LDLR transcription is regulated by SREBP-2 in response to sterol availability. It has been reported that rs688, a common polymorphism in LDLR, is associated with LDLR exon 12 splicing efficiency. However, the regulation of LDLR alternative splicing is not well understood. This study describes sterol regulation of alternative splicing of LDLR. Cellular sterol depletion suppresses and sterol loading induces the amounts of alternatively spliced LDLR transcripts in relation to levels of the full-length LDLR transcripts. rs688 attenuates change in alternative splicing, indicating there is an interaction between genetic and nongenetic regulation of this process. A general splicing factor (PTBP1) has been identified to mediate sterol-regulated alternative splicing of LDLR as PTBP1 is transcriptionally responsive to sterol, and knockdown of PTBP1 eliminates sterol-induced changes in LDLR alternative splicing. In addition, it has been found in this study that rs688 decreases total LDLR mRNA levels, and remarkably reduces LDLR cell surface protein amount, suggesting that it has multiple functional effects on modulation of plasma cholesterol. These results implicate that regulation of alternative splicing of LDLR may contribute to cellular homeostasis and plasma LDL levels.

# 12

## Reactive Oxygen Species Are Implicated in the Mitogenic Effect of Ceramide 1-Phosphate

Antonio Gomez-Munoz, Lide Arana, Alberto Ouro, and Patricia Gangoiti

University of the Basque Country, Bilbao, Spain

Abstract:

We reported previously that ceramide 1-phosphate (C1P) is mitogenic and antiapoptotic in macrophages. Major pathways involved in the stimulation of cell proliferation by C1P include mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinases (ERK1/2), phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB, or Akt), c-Jun N-terminal kinase (JNK), and protein kinase C-. Here, we demonstrate that C1P induces reactive oxygen species (ROS) production through a mechanism involving NADPH oxidase activation. C1P-stimulated ROS generation was inhibited by apocynin, a potent NADPH oxidase inhibitor, and by the cell-permeable ROS scavenger N-acetylcysteine (NAC). In addition, C1P-stimulated ROS production was blocked by the protein kinase C (PKC) inhibitor Go6976, the PKC- inhibitor rottlerin, and by long term treatment with phorbol esters. Interestingly, a specific cytosolic phospholipase A2- inhibitor also blocked C1P-stimulated ROS production, and all of the ROS inhibitors blocked C1P-stimulated macrophage proliferation, suggesting that ROS are implicated in the mitogenic effect of C1P in macrophages.

* This work was supported by Grants BFU2009-13314 from "Ministerio de Ciencia e Innovación" (Madrid, Spain) and SA-2010/00013 from the "Departamento de Industria, Innovación, Comercio y Turismo del Gobierno Vasco" (Basque Country, Spain).

# 14

## Targeted Disruption of the Idol Gene Alters Cellular Regulation of the LDL Receptor by Sterols and LXR Agonists

Elena Scotti1, 2, 6, Cynthia Hong1, 2, Yuko Yoshinaga5, Yiping Tu4, Yan Hu4, Noam Zelcer1, 2, Rima Boyadjian1, 2, Pieter J. de Jong5, Stephen G. Young3, 4, Loren Fong4, and Peter Tontonoz1, 2

1Howard Hughes Medical Institute, 2Department of Pathology and Laboratory Medicine, 3Department of Human Genetics, 4Department of Medicine, UCLA, Los Angeles, CA; 5Children's Hospital Oakland Research Institute, Oakland, CA; 6Dipartimento di Scienze Farmacologiche, Università degli Studi di Milano, Milan, Italy

Abstract:

Previously, we identified the E3 ubiquitin ligase Idol (inducible degrader of the LDL receptor) as a post-transcriptional regulator of the LDLR pathway. Idol stimulates LDLR degradation through ubiquitination of its C-terminal domain, thereby limiting cholesterol uptake. Here, we report the generation and characterization of mouse embryonic stem cells homozygous for a null mutation in the Idol gene. Cells lacking Idol exhibit markedly elevated levels of LDLR protein and increased rates of LDL degradation. Furthermore, despite an intact SREBP pathway, Idol-null cells exhibit an altered response to multiple regulators of sterol metabolism, including serum, oxysterols, and synthetic LXR agonists. The ability of oxysterols and lipoprotein-containing serum to suppress LDLR protein levels is reduced, and the time course of suppression is delayed, in cells lacking Idol. LXR ligands have no effect on LDLR levels in Idol-null cells, indicating that Idol is required for LXR-dependent inhibition of the LDLR pathway. In line with these results, the half-life of the LDLR protein is prolonged in the absence of Idol. Finally, the ability of statins and PCSK9 to alter LDLR levels is independent of, and additive with, the LXR–Idol pathway. These results demonstrate that the LXR–Idol pathway is an important contributor to feedback inhibition of the LDLR by sterols and a biological determinant of cellular LDL uptake.

# 15

## Improved Sensitivity to Ezetimibe and Rosuvastatin following siRNA-mediated Knockdown of Proprotein Convertase Subtilisin-like/Kexin Type 9 (PCSK9) in Mice

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Abstract:

Elevated LDL cholesterol (LDL-c) is a risk factor for cardiovascular disease. Current treatments that reduce circulating LDL-c elevate serum PCSK9 (proprotein convertase subtilisin/kexin type 9a) in patients, which may attenuate their efficacy by reducing the amount of LDL-c cleared from circulation. Using mice engineered to exhibit a human-like lipid profile, we show that both ezetimibe and rosuvastatin induce PCSK9 (serum protein and hepatic mRNA) along with many other genes within the hepatic SREBP-2 cholesterol biosynthesis pathway. We utilized small interfering RNAs (siRNAs) to knock down Pcsk9 together with ezetimibe and rosuvastatin and found that Pcsk9 knockdown in combination with either treatment led to greater reductions in serum non-HDL cholesterol. We further show that the combined rosuvastatin/ezetimibe/Pcsk9 siRNA treatment led to an even greater reduction in serum non-HDL cholesterol levels and exhibited significant reductions in serum apolipoprotein B protein and triglyceride levels relative to the other treatment combinations. Taken together, these data provide evidence that PCSK9 inhibitors in combination with current therapies have the potential to achieve greater reductions in both serum cholesterol and triglycerides.

# 16

## Hepatic Deletion of CTP:phosphocholine Cytidylyltransferase-α Reduces Hepatic Phosphatidylcholine, Which Leads to the Development of Nonalcoholic Steatohepatitis

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Abstract:

In all nucleated mammalian cells, phosphatidylcholine (PC) is synthesized via the CDP-choline pathway. Flux through the pathway is controlled by CTP:phosphocholine cytidylyltransferase (CT). Previous experiments have shown that hepatic deletion of CTα results in decreased VLDL secretion, reduced lipid efflux, and mild steatosis. To investigate further the importance of PC in lipid metabolism, we challenged the mice with a high fat diet. After 1 week of the high fat diet, CTα-deficient livers developed nonalcoholic steatohepatitis as indicated by the presence of lobular and portal inflammation and hepatocyte ballooning. CTα-deficient livers had a 3-fold increase in triacylglycerol (TG) mass and a 2-fold increase in diacylglycerol mass while the concentration of PC was reduced by 20%. Despite a 2-fold increase in plasma ketone bodies and a 30% decrease in fatty acid uptake, TG still accumulated in CTα-deficient livers. Furthermore, adenoviral expression of CTα normalized both hepatic and plasma TG levels in liver-specific CTα-knockout mice. This suggests that decreased VLDL secretion primarily causes TG accumulation. To understand whether the reduction in hepatic PC contributes to the development of steatohepatitis, liver-specific CTα-knockout mice fed the high fat diet were treated with a daily dose of CDP-choline. CDP-choline treatment normalized hepatic PC levels and showed that PC mass correlates with the severity of nonalcoholic fatty liver disease (NAFLD); however, the increase in PC alone was not sufficient to attenuate steatohepatitis. In conclusion, hepatic deletion of CTα causes alterations in hepatic lipid metabolism, which lead to the development of nonalcoholic steatohepatitis. Despite the correlation between hepatic PC and the severity of NAFLD, improving PC alone is not sufficient to prevent nonalcoholic steatohepatitis.

# 17

## HDL Is an Important Source for Hepatic Phosphatidylcholine and Triacylglycerol

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Abstract:

Hepatic phosphatidylcholine (PC) is synthesized via the CDP-choline pathway, controlled by the activity of CTP:phosphocholine cytidylyltransferase (CT), and via sequential methylation of phosphatidylethanolamine through the action of phosphatidylethanolamine N-methyltransferase (PEMT). The liver can also obtain PC by uptake of circulating lipoproteins. In mice, the majority of circulating PC is associated with HDL. The current study has quantitated the importance of hepatic uptake of HDL-PC in vivo as well as its subsequent metabolism. We intravenously injected PEMT–/–, liver-specific CTα–/–, and their wild-type control mice with [3H]PC-labeled HDL. Hepatic uptake of HDL-PC was about 10 μmol/day in all mouse models, which is of the same order as hepatic de novo PC synthesis. In agreement, specific activities of PC in plasma and liver indicated that 50% of hepatic PC is derived from the circulation. Surprisingly, neither the absolute uptake of HDL-PC nor its relative contribution to total hepatic PC is affected by PEMT or CTα deficiency. Analysis of 3H radiolabel in the different lipid fractions of the liver showed that 33% of HDL-derived PC was converted into triacylglycerols. This process was unaffected in liver-specific Ctα–/– mice but increased to 49% in mice lacking PEMT. Importantly, approximately 65% of the total hepatic pool of triacylglycerol appears to be derived from PC. These data clearly show the quantitative importance of HDL-PC to the hepatic pool of PC as well as its significance as a precursor for hepatic triacylglycerol.

# 18

## Vitamin E-binding Protein Afamin Is Associated with the Metabolic Syndrome and Infertility

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Abstract:

The pathogenesis of the metabolic syndrome is multifactorial and polygenic. Several heritability studies indicated a major role of genetic susceptibility to the metabolic syndrome. 1-year-old transgenic mice overexpressing the gene for the vitamin E-binding protein afamin had significantly higher body weight and plasma concentrations of glucose, cholesterol, and triglycerides compared with their wild-type littermates, suggesting a role of afamin in carbohydrate and lipid metabolism. To test whether these findings also hold true for human populations, we measured afamin plasma concentrations in the prospective population-based Bruneck study (n = 826) and found significant associations with waist-to-hip ratio, body mass index, obesity, systolic and diastolic blood pressure, diabetes, and plasma concentrations of LDL- and HDL-cholesterol, triglycerides, free fatty acids, glucose and Hba1c. In addition, basal afamin concentrations were also positively correlated with increasing numbers of these parameters in a 10-year follow-up prospective observation. These results (particularly those from transgenic mice and prospective observations) indicate not only an association between afamin and the metabolic syndrome but suggest causality and a high predictive potential of afamin for developing this modern epidemic disease. In contrast, genetically modified mice in which the afamin gene was deleted were infertile already at the chimeric (heterozygous) level. Male animals exhibited a grossly altered testis histology showing severely degenerated testis tissue and almost absent spermiogenesis. Application of exogenous recombinant mouse afamin (via implanted diffusion pump) could completely restore testicular tissue histology and fertility. In summary, afamin seems to play major roles in the development of cardiovascular and infertility disorders.

# 19

## X-ray Crystal Structure of Proprotein Convertase Subtilisin-like/ Kexin Type 9 (PCSK9) in Complex with the LDL Receptor

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Abstract:

LDL cholesterol (LDL-c) homeostasis in vertebrates is largely governed by the LDL receptor (LDLR). A number of effector molecules are able to control the concentration of LDLR at the cell surface and thus affect plasma LDL-c levels. One such molecule, PCSK9, has been shown to disrupt LDLR recycling and redistribute LDLR for degradation in the lysosome. Naturally occurring mutants of PCSK9 have been identified which correlate with plasma levels of LDL-c and consequently with occurrence or resistance to coronary heart disease. Understanding the nature of the interaction between PCSK9 and LDLR, how LDLR is inhibited, and the tertiary structure of cell surface LDLR are among the major goals of cardiovascular research. Here, we report the crystal structure of a complex of PCSK9 with LDLR and the first of LDLR at a cell surface pH. The crystal structure defines the quaternary structure of the complex, and data collected on crystals at both acidic and basic pH reveal this conformation to be stable throughout the extracellular and endosomal pH range. The structure enhances the current model of LDLR inhibition by PCSK9, showing that PCSK9 potentially stabilizes the open LDLR form by locking the conformation of the β-propeller/EGFC domains preventing ring closure of LDLR at endosomal pH.

# 20

## Cardiac Dysfunction and Aberrant Phosphatidate Metabolism in the Lipin-1-deficient Mouse

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Abstract:

The heart uses diverse substrates to produce energy, but when substrate utilization becomes inflexible, heart function inevitably deteriorates. For example, there are increased fatty acid (FA) oxidation and triglyceride (TG) levels in diabetes, which are associated with cardiac dysfunction. We are studying how the heart might coordinate the control of TG synthesis and FA oxidation through lipin-1. This protein provides the major cardiac phosphatidate phosphatase (PAP) activity, which is essential for synthesizing TG. Lipin-1 also acts with PGC-1α and PPARα to increase expression of FA oxidation genes. Despite the importance of lipin-1, little is known about its role in the heart. We hypothesize that lipin-1 deficiency in fatty liver dystrophy (fld) mouse hearts would cause dysfunction due to decreased capabilities to esterify or oxidize FAs. Echocardiographic studies revealed cardiac dysfunction, specifically decreased ejection fraction and fractional shortening. We then measured FA esterification and oxidation in ex vivo working perfused fld hearts. Surprisingly, fld hearts perfused with radiolabeled oleate have TG accumulation rates similar to those of controls. Decreased TG lipolysis due to decreased HSL activation and ATGL levels, in combination with decreased TG synthesis due to reduced PAP activities (20% of controls), explain this result. Rates of FA and glucose oxidation were not significantly different. Interestingly, there was also increased accumulation of oleate in phosphatidate (PA), the substrate for lipin-1, as well as diversion of oleate into phosphatidylinositol synthesis. Moreover, S6 ribosomal protein activation was increased, which could be promoted by increased PA signaling through mTOR and p70S6 kinase. We concluded that there was cardiac dysfunction in fld mice in the surprising absence of gross changes in FA oxidation and esterification. Some of the functional defects could be related to changes in cellular PA signaling.

# 21

## Control of One-carbon Cycle Genes by SREBP-1 Links S-Adenosylmethionine Production, Phosphatidylcholine Biosynthesis, and Hepatic Lipogenesis

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Abstract:

Altered methionine/1-carbon cycle (1CC) metabolism has been linked to elevated hepatic lipogenesis and the development of liver steatosis; however, the mechanisms are unclear. We have found that the sterol regulatory element-binding protein-1 (SREBP-1), a transcriptional activator of fatty acid and phospholipid biosynthesis, also controls the expression of 1CC genes in Caenorhabditis elegans and mammals and influences the levels of the key 1CC product S-adenosylmethionine (SAMe), the major cellular methyl donor. SAMe is important for production of the membrane phospholipid phosphatidylcholine (PC), and we find that blocking SAMe production or PC biogenesis in C. elegans, mouse liver, and human cells causes depletion of PC and induces SREBP-1-dependent lipogenic transcription and accumulation of lipid droplets. In addition, we find that in HepG2 cells, blocking SAMe or PC production promotes loss of Golgi-specific localization of the SREBP-activating Site-1 and Site-2 proteases. This suggests that similar to brefeldin A-induced SREBP activation, reduction of PC levels in intracellular membranes may promote SREBP-1 activation by allowing the proteases access to SREBP-1 while in the endoplasmic reticulum. Although SREBP-2 and cholesterogenic genes are regulated by cholesterol in a negative feedback manner, our data suggest that a conserved regulatory feedback loop involving SAMe and PC controls the levels of nuclear, active SREBP-1 and lipogenesis in metazoans.

# 22

## New Insight into an Intrahepatic Role of Human Apolipoprotein C-III in Hyperlipidemia and Hepatic Stress Induced by Lipotoxicity

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Abstract:

The plasma concentration of human apolipoprotein C-III (apoC-III) is positively associated with VLDL apoB-100 and triglyceride (TG); elevated apoC-III is invariably linked to hypertriglyceridemia in humans and in transgenic mice. Although the hyperlipidemic role of apoC-III was ascribed to its action in inhibiting lipoprotein lipase-catalyzed TG hydrolysis and attenuating receptor-mediated endocytosis of TG-rich lipoproteins, several in vivo studies using stable isotopes have suggested a role of apoC-III in hepatic VLDL production in humans. Our transfection experiments with cultured hepatic cells showed that expression of human apoC-III enhanced assembly and secretion of apoB-100 and TG associated with VLDL1 (Sf > 100) under lipid-rich conditions (i.e. media supplemented with oleic acid). Mutational analysis with two naturally occurring human apoC-III mutants, namely A23T and K58E, originally identified in hypotriglyceridemia and hyperalphalipoproteinemia subjects, has revealed a loss-of-function phenotype of apoC-III in VLDL1 assembly/secretion. Our structural-functional analysis has provided evidence for a two-domain model depicting apoC-III action: the C-terminal lipid-binding domain (a typical type A amphipathic □-helix) is responsible for the formation of luminal lipid droplets (LLD) that are the precursors of VLDL1, whereas the N-terminal domain may play a role in promoting fusion of LLD with primordial VLDL. Kinetic studies have demonstrated that apoC-III, working in tandem with MTP, promotes formation of LLD. The human apoC-III-stimulated VLDL1-apoB-100 production was readily observed (via adenovirus-mediated gene transfer) in apoC3-null mice fed with high fat diet or palm oil gavage. Further, cells stably expressing human apoC-III displayed resistance to endoplasmic reticulum stress (as determined by eIF2a phosphorylation) induced by palmitic acid. The data suggest that apoC-III may protect lipotoxicity-induced hepatic stress by rapidly purging lipids as TG-rich VLDL1.

# 23

## Deletion of ATP-binding Cassette Transporter A1 (ABCA1) in Macrophages Protects Mice from Listeria monocytogenes Infection but Impairs Major Histocompatibility Complex Class I-restricted Antigen Presentation

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Abstract:

ATP-binding cassette transporter A1 (ABCA1), a plasma membrane protein, is a primary gatekeeper for eliminating excess free cholesterol (FC) from tissues by effluxing cellular FC and phospholipids to lipid-poor apolipoprotein A-I. Macrophage ABCA1 also dampens proinflammatory MyD88-dependent Toll-like receptor signaling by reducing cellular membrane FC and lipid raft content. In this study, we investigated whether ABCA1 expression impacts important macrophage functions, including microbial killing, phagocytosis, and antigen presentation. Macrophage-specific ABCA1 knockout (MSKO) vs. wild-type (WT) mice were highly resistant to infection with the intracellular bacterium Listeria monocytogenes (Lm), with significantly less body weight loss, less Lm burden in liver and spleen, and milder liver pathologic damage 3 days after infection. Compared with WT mice, Lm-infected MSKO mice showed significantly increased IL-12p40, but similar IFN-☒ and marginally decreased IL-6 and TNF-☒ in plasma. Macrophages from MSKO mice were better at phagocytosing apoptotic thymocytes compared with those from WT mice. Lm growth in MSKO macrophages was retarded at late stage of infection, suggesting that MSKO macrophages provide a suboptimal growth environment for Lm. Finally, MSKO macrophages showed impaired capacity to present ovalbumin antigen to CD8+ T cell hybridomas via MHC class I-restricted pathway after infection with a recombinant, ovalbumin-expressing Lm strain. WT macrophages loaded with cholesterol using acLDL mimicked MSKO macrophages with impaired capability to present Lm ovalbumin antigen to CD8+ T cells. Thus, deletion of ABCA1 in macrophages favors Lm clearance during systemic infection in mice and limits Lm growth in macrophages; however, it impairs antigen presentation mediated by MHC class I molecule. Taken together, we conclude that macrophage ABCA1 impacts both innate and adaptive immunity.

# 24

## Antagonism of miR-33 in Mice Promotes Reverse Cholesterol Transport and Regression of Atherosclerosis

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Abstract:

Plasma HDL levels have a protective role in atherosclerosis, yet clinical therapies to raise HDL have remained elusive. Recent advances in the understanding of lipid metabolism have revealed that miR-33, an intronic microRNA located within the SREBP2 gene, suppresses expression of the cholesterol transporter ABCA1 and lowers HDL. Conversely, mechanisms that inhibit miR-33 increase ABCA1 and circulating HDL, suggesting that antagonism of miR-33 may be atheroprotective. Because the regression of atherosclerosis is clinically attractive, we assessed the impact of miR-33 inhibition on Ldlr–/– mice with established atherosclerotic plaques. Mice treated with anti-miR33 for 4 weeks showed an increase in circulating HDL and enhanced reverse cholesterol transport to the plasma, liver, and feces. Consistent with this, anti-miR33-treated mice showed a reduction in plaque size, increased markers of plaque stability, and decreased inflammatory gene expression. Notably, in addition to raising ABCA1 in the liver, anti-miR33 oligonucleotides directly target the plaque macrophage, where they enhance ABCA1 expression and cholesterol removal. These studies establish that raising HDL by anti-miR33 oligonucleotides promotes reverse cholesterol transport and regresses atherosclerosis and support targeting of miR-33 as a therapeutic strategy to treat atherosclerotic vascular disease.

# 25
# Molecular Mechanisms of Lupus Dyslipidemia in Mice

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Abstract:

Lupus dyslipidemia is characterized by increased total cholesterol, triglycerides, LDL, VLDL, and decreased HDL. In this study, we generated mice that have dyslipidemia and lupus phenotype on chow diet. LDLr- and Fas(lpr/lpr)-deficient mice on the B6 background were purchased from Jackson Laboratories (Bar Harbor, ME). These mice were interbred to produce mice homozygous for both alleles. Thirteen female mice from each group were killed at the age of 24-28 weeks. LDLrLpr mice developed a lupus phenotype demonstrated by autoantibody production, splenomegaly, hepatomegaly, and generalized lymphadenopathy compared with LDLr mice. LDLrLpr mice had a significantly increased proteinuria and kidney disease compared with LDLr mice. The lymphocyte population in the spleen measured by flow cytometry resulted in significantly increased CD4+ T cells double-negative T cells, and CD138+ plasma cells and decreased CD8+ T cells in LDLrLpr mice compared with LDLr mice, similar to human lupus. Lipid analyses in LDLrLpr mice compared with LDLr mice on chow diet demonstrated significantly elevated total cholesterol (mg/dl) ($347.0 \pm 31.65$ vs. $220.9 \pm 8.235$; $p < 0.0008$), triglycerides ($118.8 \pm 18.51$ vs. $42.85 \pm 3.665$; $p < 0.0005$), VLDL ($66.85 \pm 17.54$ vs. $3.385 \pm 0.4875$; $p < 0.0014$), and LDL cholesterol ($214.9 \pm 22.22$ vs. $148.1 \pm 8.049$; $p < 0.0093$) without a significant difference in HDL level. The triglycerides level was increased as measured by Triton block experiment, and hepatic and lipoprotein lipase activity decreased in LDLrLpr mice. The increased plasma cholesterol due to increased cholesterol synthesis and decreased fatty oxidation in liver in LDLrlpr mice were measured by gene expression analysis. We conclude that increased cholesterol synthesis, decreased fatty acid oxidation, increased triglyceride secretion, and decreased lipase activity are the molecular mechanisms of dyslipidemia in lupus mice.

# 26

## Transport of Lipoprotein Lipase by GPIHBP1

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Abstract:

The lipolytic processing of triglyceride-rich lipoproteins by lipoprotein lipase (LPL) is the central event in plasma lipid metabolism, providing lipids for storage in adipose tissue and fuel for vital organs such as the heart. LPL is synthesized and secreted by myocytes and adipocytes but then must find its way into the lumen of capillaries, where it hydrolyzes the triglycerides within plasma lipoproteins. Recently, we found that GPIHBP1, a GPI-anchored protein of endothelial cells, is responsible for the transport of LPL into capillaries. We found that GPIHBP1 actively transports LPL across monolayers of endothelial cells and that LPL is mislocalized to the interstitial spaces surrounding myocytes and adipocytes in Gpihbp1-deficient mice. These experiments defined the function of GPIHBP1 in triglyceride metabolism and delineated a means for LPL transport into capillaries. Further experiments have begun to illuminate mechanics, kinetics, and regulation of this transport.

# 27

# Cholesteryl Ester Transfer Protein Inhibition by Anacetrapib Results in Increased HDL and Bulk Cholesterol Excretion in Syrian Golden Hamsters

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Abstract:

Anacetrapib (ANA), a reversible inhibitor of cholesteryl ester transfer protein (CETP), raises HDL cholesterol and lowers LDL cholesterol in dyslipidemic patients. The purpose of the current study was to characterize the plasma and fecal lipid compositional changes that occur in lipoprotein particles with ANA treatment in dyslipidemic hamsters to determine the utility of the model to study cholesterol excretion in response to CETP inhibition. Treatment of hamsters with ANA (60 mg/kg per day, 2 weeks in feed) resulted in increased in HDL-c (53%; $p < 0.001$ vs. vehicle). An analytical LC-MS platform was developed to investigate plasma and fecal sterols and lipid composition in lipoprotein fractions. In the HDL fraction, the major cholesteryl esters (18:1, 18:2, 18:0, 20:4) were increased with ANA treatment vs. vehicle ($p < 0.05$), with cholesteryl linoleate (18:2) being the most abundant cholesterol ester in HDL. The largest increases in cholesterol were observed in the large HDL subfractions (91%; $p < 0.001$) compared with intermediate HDL (29%; $p < 0.01$). Further, all major classes of triglyceride were reduced in the HDL fraction with a reciprocal increase in triglyceride classes in LDL fractions. Following ANA treatment, fecal total cholesterol (24-h collection) was increased 29%, and cholic acid was increased 19% compared with vehicle ($p < 0.05$), indicating increased cholesterol excretion with ANA treatment. Furthermore, serum from ANA-treated hamsters stimulated cholesterol efflux in cultured macrophages expressing either endogenous SR-B1 or recombinant ABCA1 (loaded with radiolabeled cholesterol). These data indicate that ANA stimulates cholesterol efflux and cholesterol accumulation in HDL in Syrian golden hamsters, which is associated with increased fecal cholesterol excretion. These results indicate that the Syrian golden hamster can be used to study the effects of CETP inhibition on lipoprotein cholesterol composition and support the hypothesis that ANA promotes reverse cholesterol transport.

# 28

## Myeloid-specific Estrogen Receptor-α Deficiency Impairs Metabolic Homeostasis and Accelerates Atherosclerotic Lesion Development

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Abstract:

Estrogen receptor-α (ER-α) is readily expressed in macrophages and other immune cells, which are shown to exert dramatic effects on whole body glucose homeostasis and atherosclerosis development. Here, we investigated the impact of ER-α expression on macrophage function to determine whether hematopoietic or myeloid cell-specific ER-α deletion promotes obesity-induced insulin resistance. Mice harboring a hematopoietic or myeloid cell-specific deletion of Esr1 exhibit altered circulating adipokine levels, glucose intolerance, muscle insulin resistance, and increased adipose tissue mass. A similar obese phenotype and increased atherosclerotic lesion area were observed in LDL receptor (LDLR) knock-out mice transplanted with Esr1–/– bone marrow. In isolated macrophages, we find that ER-α is necessary for the maintenance of oxidative metabolism, IL-4-mediated induction of alternative activation, and phagocytic capacity in response to oxidized LDL and lipopolysaccharide. In addition, we found that ER-α is an important and direct regulator of macrophage transglutaminase 2 expression, a potent atheroprotective multifunctional enzyme. Taken together, our findings suggest that diminished ER-α expression in hematopoietic/myeloid cells is causal for aspects of the metabolic syndrome and accelerates the development of atherosclerosis.

# 29

## Structural and Functional Characterization of Spot 14

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Abstract:

Spot14 (S14) is highly expressed in liver and is regulated by SREBP-1c, a transcription factor that activates all genes involved in fatty acid synthesis. S14 knock-out mice manifest decreased rates of fatty acid synthesis in mammary glands and increased rates of fatty acid synthesis in liver. The mechanism for the ability of S14 to modulate lipogenesis has not been elucidated. In a first step in further defining the function and mechanism of S14 action, we have determined the crystal structure of S14 to 2.65 Å. Additional biochemical studies reveal that S14 forms a heterodimer with MIG12, the only protein in the genome that shares significant homology with S14. MIG12 can bind acetyl-CoA carboxylase (ACC), the rate-limiting enzyme in fatty acid synthesis. The binding of MIG12 to ACC induces ACC polymerization and enzyme activity. In vivo, we show that MIG12 dimerizes with S14. Coexpression of S14 and MIG12 in CHO-K1 cells leads to heterodimers and reduced ACC polymerization and activity. Conversely, reducing S14 levels in rat primary hepatocytes using small interfering RNA led to increased ACC polymerization and activity. Therefore, we suggest that S14 can regulate fatty acid synthesis by modulating ACC activity through heterodimer formation with MIG12.

# 30

## Evaluating Hepatic Steatosis as a Risk Factor for 70% Partial Hepatectomy

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Abstract:

The process of liver regeneration is important for increasing the success rate of small-for-size liver transplants, grafts, and resections. A major risk factor for liver surgery is hepatic steatosis, which has been shown to increase the occurrence of postoperative morbidity and mortality. Hepatic steatosis is related to wide spectrum of diseases including obesity and diabetes. With the increasing prevalence of these diseases in the Western population today, it becomes important to perfect the current evaluation methods of livers slated for surgery and to develop therapies to improve the postoperative outcome in patients with hepatic steatosis. We used mice models of hepatic steatosis to answer these questions. Mice deficient in phosphatidylethanolamine N-methyl transferase (PEMT) and mice deficient specifically in hepatic phosphocholine cytidylyltransferase α (LCT-α) were fed a high fat diet for various lengths of time to stimulate varying grades of steatosis. 70% partial hepatectomy (PH) was then performed in these mice, and postoperative survival was monitored. Interestingly, we found the ratio of hepatic phosphatidylcholine (PC) to phosphatidylethanolamine (PE) to have a much higher correlation to the rate of survival after surgery than hepatic triacylglycerol (TG) levels. Histopathological evaluation further suggests that inflammation more than steatosis may increase the risk of post-PH mortality. Finally, supplementation of additional choline to the diet in already steatotic PEMT-deficient mice improved survival rate, decreased inflammation, and increased hepatic PC:PE ratio but did not affect hepatic TG levels. In conclusion, other factors in addition to hepatic steatosis need to be considered in determining the risk of post-surgery complications. Furthermore, choline supplementation may be a viable therapy to improve liver regeneration in instances of elevated inflammation or PEMT and choline deficiency.

# 31

## Syndecan-1 Shedding Reduces Clearance of Triglyceride-rich Lipoproteins by Human Hepatocytes and Causes Hypertriglyceridemia

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Abstract:

The heparan sulfate proteoglycan syndecan-1 mediates hepatic clearance of triglyceride-rich lipoproteins in mice based on systemic deletion of Sdc1 and hepatocyte-specific inactivation of heparan sulfate biosynthesis (MacArthur et al. (2007) J. Clin. Invest. 117, 153-164; Stanford et al. (2009) J. Clin. Invest. 119, 3236-3245; Stanford et al. (2010) J. Biol. Chem. 285, 286-294). Here, we show that syndecan-1 is expressed on primary human hepatocytes and Hep3B human hepatoma cells and can mediate binding and uptake of VLDL, based on heparin lyase inhibition and small interfering RNA (siRNA) directed against SDC1. We also show that syndecan-1 is spontaneously shed from primary human and murine hepatocytes and Hep3B cells. In human cells, syndecan-1 shedding was induced with phorbol myristic acid (PMA), resulting in 70-75% reduction of syndecan-1 expression on the cell surface and accumulation of syndecan-1 ectodomains in the medium. Shedding occurred through a protein kinase C-dependent activation of ADAM-17 (a disintegrin and metalloproteinase-17) based on pharmacological inhibition studies and siRNA-mediated silencing. PMA stimulation significantly decreased diD-VLDL binding. Furthermore, the shed syndecan-1 ectodomains bound to VLDL based on an assay in which association of VLDL with 35S-labeled ectodomains caused a decrease in the buoyant density of the proteoglycan. Induction of Sdc1 shedding in mice by injection of lipopolysaccharide resulted in loss of hepatic Sdc1, accumulation of ectodomains in the plasma, and hypertriglyceridemia. Thus, shedding of syndecan-1 provides a mechanism that might explain hypertriglyceridemia in patients with sepsis or in patients undergoing therapeutic regimens that result in idiopathic hyperlipidemia.

# 32

## CD36 and SR-A Contribute Similarly and Independently to Nonalcoholic Steatohepatitis in Hyperlipidemic Mice

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Abstract:

The major feature of nonalcoholic steatohepatitis (NASH) is hepatic lipid accumulation in combination with inflammation, which can further progress into fibrosis and cirrhosis. Recently, we demonstrated that combined deletion of the two main scavenger receptors, CD36 and macrophage scavenger receptor 1 (MSR1), which are important for the uptake of modified cholesterol-rich lipoproteins, reduced the initiation and progression of NASH. So far, the individual contributions of these receptors to NASH and the intracellular mechanisms by which they contribute to inflammation have not been established. We hypothesize that CD36 and MSR1 contribute similarly and independently to the progression of diet-induced NASH. Ldlr–/– mice were lethally irradiated and transplanted with wild-type (WT), Cd36–/–, or Msr1–/– bone marrow and fed a Western diet for 3 months. Results showed that Cd36–/– and Msr1–/– transplanted (tp) mice showed a reduction in hepatic inflammation and fibrosis similar to those in WT-tp mice. In plasma, the protective IgM autoantibodies were increased in both Cd36–/– and Msr1–/– tp mice compared with WT-tp mice. Although the total amount of cholesterol inside Kupffer cells (KCs) was similar in all groups, electron microscopy analysis revealed that the KCs of WT-tp mice showed increased lysosomal cholesterol accumulation, whereas the Cd36–/– tp and Msr1–/– tp mice showed increased cytoplasmic cholesterol accumulation inside KCs. Moreover, lysosomal enzyme activity in the livers of both Cd36–/– tp and Msr1–/– tp, but not in WT-tp mice was disturbed. We conclude that CD36 and MSR1 contribute similarly and independently to the progression of NASH in the presence of high levels of plasma-modified lipoproteins. Internalization of lipids by these two receptors leads to abnormal cholesterol metabolism and trafficking in KCs and consequently disturbed lysosomal function and hepatic inflammation. These observations provide a new basis for the prevention and treatment of NASH.

# 33

## Ezetimibe Exacerbates the Hypertriglyceridemia in Gpihbp1–/– Mice

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Abstract:

Adult GPIHBP1-deficient mice (Gpihbp1–/–) have severe hypertriglyceridemia; however, the plasma triglyceride levels are only mildly elevated during the suckling phase when lipoprotein lipase (Lpl) is expressed at high levels in the liver. Lpl expression in the liver can be induced in adult mice with dietary cholesterol. We therefore hypothesized that plasma triglyceride levels in adult Gpihbp1–/– mice would be sensitive to cholesterol intake. After 4–8 weeks on a Western diet containing 0.15% cholesterol, plasma triglyceride levels in Gpihbp1–/– mice were 10,000–12,000 mg/dl. When 0.005% ezetimibe was added to the diet to block cholesterol absorption, Lpl expression in the liver was reduced significantly, and the plasma triglyceride levels were significantly higher (>15,000 mg/dl). We also assessed plasma triglyceride levels in Gpihbp1–/– mice fed Western diets containing either high (1.3%) or low (0.05%) amounts of cholesterol. The high cholesterol diet significantly increased Lpl expression in the liver and lowered plasma triglyceride levels. We conclude that treatment of Gpihbp1–/– mice with ezetimibe lowers Lpl expression in the liver and increases plasma triglyceride levels. A high cholesterol diet had the opposite effects. Thus, cholesterol intake modulates plasma triglyceride levels in Gpihbp1–/– mice.

# 34

## Identification of Ubxd8 Protein as a Sensor for Unsaturated Fatty Acids and Regulator of Triglyceride Synthesis

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Abstract:

Fatty acids (FAs) are essential for cell survival, yet their overaccumulation causes lipotoxicity. To prevent lipotoxicity, cells store excess FAs as triglycerides (TGs). In cultured cells, TG synthesis is activated by excess unsaturated but not saturated FAs. Here, we identify Ubxd8 as a sensor for unsaturated FAs and regulator of TG synthesis. In cultured cells depleted of FAs, Ubxd8 inhibits TG synthesis by blocking conversion of diacylglycerols (DAGs) to TGs. Excess unsaturated but not saturated FAs relieve this inhibition. As a result, unsaturated FAs are incorporated into TGs, whereas saturated FAs are incorporated into DAGs. In vitro, unsaturated but not saturated FAs alter the structure of purified recombinant Ubxd8 as monitored by changes in its thermal stability, trypsin cleavage pattern, and oligomerization. These results suggest that Ubxd8 acts as a brake that limits TG synthesis, and this brake is released when its structure is altered by exposure to unsaturated FAs.

# 35

## Loss of ABCG1 Elicits a Natural Immune Response That May Protect from Atherosclerosis

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Abstract:

The oxidation of LDL, a key event in the pathogenesis of atherosclerosis, generates multiple oxidation-specific neoepitopes, including malondialdehyde-modified (MDA-modified) LDL (MDA-LDL), and the phosphorylcholine (PC) head group of oxidized phospholipids (oxPL). These epitopes are recognized by natural germ line IgM antibodies that are secreted by B cells. We previously showed that mice lacking the ABC transporter, ABCG1, display signs of severe inflammation in the lungs, associated with the accumulation of lipid-laden foam cells and cholesterol crystals, which are also characteristic of atherosclerotic lesions. We have also published that Abcg1–/–ApoE–/– mice develop smaller atherosclerotic lesions compared with ApoE–/– controls and have previously attributed this decrease in lesion size to increased numbers of apoptotic Abcg1–/– macrophages observed within the lesions of Abcg1–/–ApoE–/– mice. Here, we show that the lungs of Abcg1–/– mice have increased levels of specific oxidized cholesterol derivatives and specific PL species. Abcg1–/– mice also display elevated levels of specific immunoglobulins and an expanded B-1 B cell population, reflective of an enhanced immune response. In addition, we show that the lungs and plasma of Abcg1–/– mice have increased titers of antibodies to specific lipid epitopes and increased mRNA transcript levels for the known hypervariable (VH) CDR3 region of the EO6/T15 idiotype. Finally, ELISpot assays show that there is a lipid that accumulates specifically in Abcg1–/–, but not wild-type lungs, that reacts with an IgM antibody expressed in Abcg1–/– lungs. These findings suggest that the accumulation of lipids resulting from loss of ABCG1 induces the specific expansion of B-1 B cells and that these cells secrete natural antibodies that may protect against the development of atherosclerosis.

# 36

## Functional and Dysfunctional Structures of Apolipoprotein A-I in HDL

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Abstract:

Apolipoprotein A-I (apoA-I) is the major protein component of HDLs and a critical element in vascular health. The three-dimensional organization of this exchangeable lipoprotein is highly flexible and includes several structural subspecies that vary in oligomeric state and lipidation profile. We have previously used electron paramagnetic resonance (EPR) spectroscopy to describe the structural organization of the N-terminal domain of apoA-I in the lipid-free state (Lagerstedt et al. (2007) J. Biol. Chem. 282, 9143-9149). Here, we report on the structure of residues 6-98 on 9.6-nm rHDL and, at select sites, on 7.8- and 8.4-nm rHDL. Our analyses of 9.6-nm rHDL reveal a secondary structure composed of random coil and β-strand positioned between two α-helices. Further, we have identified N-terminal residues important in apoA-I structural rearrangement that occur in response to changes in HDL lipid cargo and particle size. The structural conversion of this region in initial lipid loading will be discussed. In addition to functional apoA-I, several variants of apoA-I have been shown to form tissue-specific amyloid deposits in vivo. We previously characterized the N-terminal G26R variant, which possesses decreased stability and increased □-structure typical of amyloid proteins (Lagerstedt et al. (2007) Biochemistry 46, 9693-9699). Currently, we are analyzing the structural properties of the C-terminal amyloid variant L178H (associated with cardiac and larynx amyloidosis) which, in similarity to G26R, exhibits decreased stability and an altered conformation. However, in contrast to G26R, the L178H variant acquires helical structure upon fibril formation and may thus represent a novel aggregation pathway.

# 37

# Anacetrapib Stimulates Macrophage-to-feces Reverse Cholesterol Transport in the Dyslipidemic Syrian Golden Hamster

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Abstract:

Cholesteryl ester transfer protein (CETP) mediates transfer of cholesteryl ester and triglyceride between HDL and apoB-containing lipoproteins such as LDL and is therefore an attractive target for increasing HDL-cholesterol (HDL-c) and reducing LDL-cholesterol (LDL-c). Anacetrapib (ANA), a reversible inhibitor of CETP, raises HDL-c and lowers LDL-c in dyslipidemic patients. Although we have shown that ANA increases HDL-c in a dyslipidemic hamster model designed to mimic human dyslipidemia, whether ANA promotes macrophage-to-feces reverse cholesterol transport (RCT) has not been demonstrated. To test whether ANA (60 mg/kg per day in feed, 2 weeks) would promote RCT in dyslipidemic hamsters, [3H]cholesterol macrophages were injected after treatment to measure [3H]tracer appearance in HDL and feces over 72 h. Another set of hamsters was injected with [3H]cholesteryl oleate-HDL to evaluate the effect of CETP inhibition with ANA on HDL-cholesteryl esters kinetics. Compared with vehicle-treated animals, ANA treatment over 2 weeks inhibited CETP activity by ~90% ($p < 0.001$) and increased HDL-c by 50% ($p < 0.001$). After injection of [3H]cholesterol-loaded macrophages and monitoring for up to 72 h, [3H]tracer appearance in HDL was significantly increased at 24, 48, and 72 h compared with vehicle, suggesting increased cholesterol efflux from macrophages to HDL particles. At 72 h, [3H]tracer recovery in fecal cholesterol and bile acids was also increased by 90 and 57%, respectively (both $p < 0.01$), indicating increased macrophage-to-feces RCT with ANA treatment. Injection of [3H]cholesteryl ester-labeled HDL showed a 47% reduction of the HDL fractional catabolic rate ($p < 0.01$), indicating that ANA raises HDL-c levels by decreasing the catabolism of HDL particles. In conclusion, CETP inhibition with ANA promotes macrophage-to-feces RCT, further supporting its use as a novel lipid therapy for the treatment of dyslipidemia and resultant coronary artery disease.

# 38

## Characterization of Proprotein Convertase Subtilisin-like/Kexin Type 9 (PCSK9) Secretion Mutant S462P: Fate of the Protein

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Abstract:

Proprotein convertase subtilisin-like/kexin type 9 (PCSK9) is an attractive target for treatment of dyslipidemia. It secretes and binds to LDL receptors (LDLRs) and subsequently directs the LDLR to lysosomes for degradation in the liver, therefore controlling the level of LDL in plasma. Various naturally occurring PCSK9 gain-of-function (GOF) or loss-of-function (LOF) mutants have been identified. Among them, S462P, a LOF mutant, is defective on PCSK9 secretion. In the current study, we investigated the possible mechanism of action of the S462P mutant utilizing biochemistry and imaging approaches as well as protein modeling tools. Stable cell lines expressing wild-type PCSK9, S462P, or S462A mutant were established in HEK293 cells. S462 is located in the loop of the C-terminal domain that interfaces with the catalytic domain. The side chain OH of Ser-462 appears to stabilize the local region via hydrogen bonds to the backbone NH of Ala-522 and backbone C=O of Ala-423. Mutation of Ser to Pro would eliminate those two strong hydrogen bonds and introduce a bulky Pro ring that may cause misfolding; mutation of Ser to Ala also causes elimination of hydrogen bonds; however, sterically it may be possible to maintain the folding structure. Quantitative Western blotting revealed that the S462P mutant is cleaved to the same extent as the wild-type and S462A mutant; however, most S462P protein stayed in the cytosol; only a small amount is secreted. Imaging analysis indicated that the S462P protein is mostly trapped in the endoplasmic reticulum during protein trafficking. Protein purified from the medium showed in vitro functional activity similar to that of the wild-type PCSK9. Difficulty in purifying the active form of protein from the cell lysate suggests improper folding. Further characterization is ongoing to investigate markers of endoplasmic reticulum stress in association with S462P-containing HEK293 cells.

# 39

## Measuring the Effect of the Membrane Environment on Cholesterol Activity

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Abstract:

Maintaining the proper distribution of sterols throughout the cell is vital for many cellular functions because cholesterol affects the fluidity and permeability of the membrane and regulates integral membrane proteins by altering the membrane environment. Although it is not known how lipids and sterols become distributed throughout cellular membranes, it is clear that the membrane lipids set a natural threshold at which the membrane can contain cholesterol. Cholesterol added above that threshold has a higher chemical activity, or escape tendency, than the complexed cholesterol and is rapidly trafficked throughout the cell. It has been proposed that it is only the active cholesterol that is available for cholesterol sensing and regulation. Thus, we examined how changes in the lipid environment, including oxysterol regulators of cholesterol homeostasis, modulate the cholesterol activity in a membrane. For these studies, we used perfringolysin O, a cholesterol-dependent cytolysin that binds only to active cholesterol, as a sensor of membrane cholesterol activity. Using this assay, we show that 25-hydroxycholesterol (25-HC), a regulator of cholesterol homeostasis with known membrane-disordering properties, increases the activity of cholesterol. Studies with the enantiomer of 25-HC demonstrate that the activating effects on cholesterol activity are non-enantioselective and thus due to direct oxysterol-membrane interactions. We also show that LY295427, an antagonist of the regulatory effects of 25-HC, decreases the activity of cholesterol in a non-enantioselective manner. Saturated lipids blunt both the activity of cholesterol as well as the ability of 25-HC to activate cholesterol, consistent with our findings in cultured cells. These studies show that known effectors of cholesterol homeostasis can regulate cholesterol activity, and thus the proportion of cholesterol available to be sensed and trafficked, by modulating the membrane environment.

# 40

# Evidence for Nondetectable Cholesteryl Ester Transfer Protein Expression in Parenchymal Cells of Human Liver and Adipose Tissues using Immunohistochemistry

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Abstract:

Cholesteryl ester transfer protein (CETP) mediates transfer of cholesteryl ester and triglyceride between HDLs and LDLs and currently is a target for dyslipidemia and coronary artery disease. Although CETP is known to circulate in plasma, historically the study of tissue distribution of CETP expression is limited to studies examining mRNA measurement in in vitro systems. Such studies suggested that nonhuman primate CETP mRNA expression in the liver occurs in nonparenchymal (nonhepatocyte) cells. In the current study, we sought to determine the cell type responsible for CETP expression in human liver and adipose tissue, using immunohistochemical methods. Immunohistochemistry was performed using three specific mouse anti-human CETP monoclonal antibodies, TP1, TP2, and TP20, which recognize different segments of human CETP. In normal human liver, CETP was expressed in nonparenchymal cells of the liver sinusoid, whereas no CETP was found in parenchymal cells and biliary epithelial cells. Double immunofluorescence staining demonstrated that CETP-expressing cells were co-localized with CD68, a macrophage/Kupffer cell marker, in the liver. In human adipose tissue, CETP expression was not detected in adipocytes but rather in stromal cells, although additional studies are required to identify the type of stromal cell. These studies describe the in situ expression pattern for CETP in human liver and adipose tissue. Once the location of CETP tissue expression is determined, future studies may focus on the role of resident tissue CETP activity and its contribution to cholesterol homeostasis both under normal and diseased states.

# 42

## Proprotein Convertase Subtilisin-like/Kexin Type 9 (PCSK9)-mediated Regulation of LDL Receptor in the Mouse Enterocyte

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Abstract:

Proprotein convertase subtilisin-like/kexin type 9 (PCSK9) has been demonstrated by others to be present in the gut as well as in the liver. We examined the role of PCSK9 on LDL receptor regulation in the mouse small intestine. First, we studied immunohistochemical examination of the ileum in the wild-type C57BL/6 mouse and the PCSK9–/– mice. In the normal, wild-type mouse, the LDLR is localized predominantly on the basolateral membrane. In the PCSK9–/– mouse, a significant up-regulation of the LDLR by immunohistochemical staining in the absence of endogenous PCSK9 was observed. Increased staining, although found predominantly on the basolateral membrane, is also found in increased amounts intracellularly and on the apical membrane as well. Much like hepatic LDLR regulation, the changes observed in the intestine appear to be post-transcriptional as because did not see changes of LDLR mRNA in either the liver or ileum of the PCSK9–/– mouse. Second, to study functional interactions of intestinal LDLR and PCSK9, mouse enterocytes were isolated from the ileum. Cells studied were those that had "enterocyte-like" crescent moon-shaped brush-border membrane staining as characterized by AF488-phalloidin, a molecule that binds F-actin in brush borders. The impact of PCSK9 on LDL uptake in the isolated enterocytes was examined. PCSK9 decreased LDL uptake in a dose-related manner; conversely, simvastatin treatment increased LDL uptake in these cells. In summary, in mice, LDLR in the ileum appears to be modulated by PCSK9 in a manner similar to LDLR in the liver.

# 43

## Effect of Anacetrapib Treatment on HDL Function: Anti-inflammatory Response on Endothelial Cells and Promoting ☒-Cell Function

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Abstract:

HDL cholesterol levels are inversely related to the development of coronary heart disease. Cholesteryl ester transfer protein (CETP) is a plasma protein that facilitates the transport of cholesteryl esters from HDL to apoB-containing lipoproteins. Thus, CETP inhibition has been identified as a potential strategy of raising HDL cholesterol levels for the treatment of atherosclerotic vascular disease. Recent studies suggest a number of potential atheroprotective functions of HDL. The objective of this study was to evaluate two potential aspects of HDL: anti-inflammatory effects on endothelial cells and improving pancreatic β-cell function. Specifically, we wanted to determine whether CETP inhibitor-treated HDL maintains proper function. Human, hamster, and anacetrapib-treated hamster HDL samples were isolated by ultracentrifugation, and their ability to suppress endothelial inflammation and promote β-cell function was determined. HDL demonstrated anti-inflammatory effects of reducing expression of cell adhesion molecules in human artery endothelial cells (HAEC). Both human and hamster HDL inhibited TNFα-induced expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin. Moreover, HDL from anacetrapib-treated hamsters maintained the ability to suppress inflammatory responses in HAEC. The effect of HDL to promote insulin secretion was tested using primary β-cells. HDL increased glucose-stimulated insulin secretion by both hamster and human islets. These studies demonstrate that anacetrapib treatment maintains the potent ability of HDL to suppress an endothelial cell inflammatory response. In addition, both human and hamster HDL promoted β-cell function. Additional studies to determine the effect of anacetrapib treatment on the ability of HDL to promote β-cell function are currently ongoing. The present studies indicate that anacetrapib treatment maintains proper HDL function.

# 44

## Coordinately Regulated Alternative Splicing of Genes Involved in Cholesterol Biosynthesis and Uptake

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Abstract:

Genes involved in cholesterol biosynthesis and uptake are transcriptionally regulated in response to cellular sterol content in a coordinated manner. A number of these genes, including 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and LDL receptor (LDLR), have been reported to undergo alternative splicing in a manner that results in reductions of enzyme or protein activity. Here, we demonstrate that cellular sterol depletion suppresses, and sterol loading induces, alternative splicing of multiple genes involved in the maintenance of cholesterol homeostasis including HMGCR and LDLR, the key regulators of cellular cholesterol biosynthesis and uptake, respectively. These changes were observed in both in vitro studies of the HepG2 human hepatoma-derived cell line, as well as in vivo studies of St. Kitts vervets. These effects are mediated in part by sterol regulation of polypyrimidine tract-binding protein 1 (PTBP1) because knockdown of PTBP1 eliminates sterol-induced changes in alternative splicing of several of these genes. Single-nucleotide polymorphisms (SNPs) that influence HMGCR and LDLR alternative splicing (rs3846662 and rs688, respectively), have been associated with variation in plasma LDL-cholesterol levels. Sterol-induced changes in alternative splicing are blunted in carriers of the minor alleles for each of these SNPs, indicating an interaction between genetic and nongenetic regulation of this process. Our results implicate alternative splicing as a novel mechanism of enhancing the robust transcriptional response to conditions of cellular cholesterol depletion or accumulation. Thus, coordinated regulation of alternative splicing may contribute to cellular cholesterol homeostasis as well as plasma LDL levels.

# 45

## Does VLDL or Chylomicron Secretion Contribute to Lowered Plasma Lipids in Apolipoprotein A-IV Knock-out Animals?

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Abstract:

Many functions have been attributed to intestinal apolipoprotein A-IV (apoA-IV), an intestinally derived apolipoprotein secreted in response to fat in the diet, including its role as an antioxidant, an anti-inflammatory factor, and a mediator of reverse-cholesterol transport. Although these are important functions of apoA-IV, these are also ascribed to other apolipoproteins. We have demonstrated that apoA-IV plays a role in mediating triglyceride (TG) and cholesterol homeostasis in plasma. Loss of apoA-IV causes a significant decrease in plasma lipid levels in mice maintained on a chow diet, confirming the findings of Breslow et al. (1997). Further, apoA-IV knock-out mice are resistant to high fat diet-induced increases in plasma TG and cholesterol. The lowered plasma lipids in apoA-IV knock-out mice are not due to differences in body weight, because apoA-IV knock-out and wild-type animals have comparable body weights. Additionally, apoA-IV knock-out mice have a total fat mass to body weight ratio that is comparable to that of wild-type mice on the same diet. Loss of apoA-IV does not cause mice to develop fatty liver on chow, low fat, or high fat diets. Finally, apoA-IV knock-out mice do not have altered TG-rich lipoprotein secretion from the liver. This function for intestinally derived apoA-IV strongly suggests that apoA-IV plays a previously unknown role in the regulation of plasma lipid levels. We have recently determined that the loss of apoA-IV does not affect absorption of TG or cholesterol from the intestine into lymph. Whether apoA-IV affects chylomicron metabolism is the focus of ongoing experiments.

# 46

## Effects of Small Interfering RNA-mediated Hepatic Glucagon Receptor Inhibition on Glucose and Lipid Metabolism in db/db Mice

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Abstract:

Hepatic glucose overproduction is one of the major characteristics of type 2 diabetes. Glucagon is a key regulator for glucose homeostasis. Inhibition of glucagon activity by inhibition of glucagon receptor (GCGR) has been considered as one of the therapeutic strategies for the treatment of diabetes mellitus. To study the effects of hepatic GCGR inhibition on glucose metabolism in a diabetic mouse model, small interfering RNA (siRNA) targeting GCGR was introduced to db/db mice by tail vein injection. Knocking down of GCGR reduced plasma glucose levels but also increased plasma cholesterol levels. Hepatic lipid contents in si-GCGR-treated animals were also increased. Detailed lipid analysis showed that increased plasma cholesterol was due to an increase of LDL, but VLDL or HDL fractions were not changed. Taqman analysis of liver samples indicated that the mRNA expression levels of gluconeogenic genes were reduced, whereas the mRNA levels of the genes associated with fatty acid and cholesterol biosynthesis were increased in si-GCGR-treated mice. Because alterations in glucagon levels have been associated with changes in serum lipid levels, these findings provided additional insight on the role of GCGR in hepatic glucose output. Additional studies around lipid synthesis and clearance in these siRNA-treated mice are currently ongoing. Further understanding of glucagon/GCGR effects in glucose and lipid metabolism could lead to the identification of novel therapeutic strategies to treat hyperglycemia without causing lipid abnormalities.

# 47

# Insights into Triglyceride-rich Lipoprotein Assembly from Drop Tensiometry

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Abstract:

Apolipoprotein B-100 (apoB) is the principal protein component of LDL, commonly known as "bad cholesterol." The N-terminal ~1,000 amino acids of apoB, called the βa1 superdomain, co-translationally recruit neutral lipids for the assembly of triglyceride-rich lipoproteins. The βa1 superdomain contains four subdomains and is predicted to interact directly with lipids while retaining its tertiary structure. Using drop tensiometry, we examined the interfacial properties of the second and third domains, called the ⍁-helical (amino acids 316-636) and C-sheet (amino acids 636-787) domains, and their subdomains to establish their structure-function relationship at a hydrophobic interface. We studied the adsorption, stress response, viscoelasticity, exchangeability, and pressure-area relationship at both a TO/W and TO/POPC/W interface. The ⍁-helical domain spontaneously adsorbs to a TO/W interface and forms a viscoelastic surface. It is anchored to the surface by helix 6 and remodels on the surface as a function of surface pressure. The C-sheet forms an elastic film on a TO/W interface and is irreversibly anchored to a lipid surface, which is consistent with the behavior of an amphipathic β-sheet. The exclusion pressure at a TO/POPC/W interface of the ⍁-helical domain is 22 ± 1.5 mN/m, whereas the C-sheet is ~17 ± 1 mN/m. When both domains are adsorbed together to the surface, the C-sheet shields a portion of the ⍁-helical domain from the surface, which may retain its globular structure. We used these as well as other insights to develop a molecular level model of co-translational triglyceride recruitment by apoB.

# 48

## Birth of Lipid Droplets: A Mammalian System for Induced Lipid Droplet Formation

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Abstract:

The lipid droplet (LD) is a dynamic and ubiquitous organelle that is central to cellular energy homeostasis. Despite their importance, almost nothing is known about how LDs form. The working model suggests that neutral lipids (sterol esters and triacylglycerols) coalesce locally in subdomains of the endoplasmic reticulum (ER) bilayer. These collections of neutral lipids then separate from the ER and localize to the cytosol where they are coated by a phospholipid monolayer and a number of specific proteins. This model raises two key questions: (1) What cellular components organize sites of LD formation? (2) How is the LD monolayer separated from a bilayer while maintaining the integrity of the donor membrane? To address these questions directly, we have developed a new system in which LD formation can be induced. We have previously generated adipocytes lacking triacylglycerols due to genetic ablation of the two triacylglycerol synthesis enzymes, DGAT1 and DGAT2. These adipocytes lack visible LDs yet retain many functional features of adipocytes. To mimic this situation, we differentiate DGAT2 knock-out fibroblasts in the presence of a DGAT1 inhibitor. These cells also express adipocyte-specific proteins and undergo morphological changes indicative of differentiation yet lack cytosolic LDs. After washing out the chemical inhibitor, LD formation is rapidly induced. We find that many LDs are visible in the cell as early as 30 min after washout. Using the vital dye BODIPY 493/503, nascent LDs appear in a disperse pattern that suggests tubular ER localization. We also co-localize perilipin-1, a surface marker of LDs, with these BODIPY-positive foci. Using cellular markers, we find that LD foci form independently of mitochondria and lysosomal compartments, but in close proximity to ER markers. Using this model in combination with light and electron microscopy, we aim to determine the membrane topology of nascent LDs and identify proteins responsible for the formation and maturation of LDs.

# 49

## CYP27: A Novel Player in Diet-induced Nonalcoholic Steatohepatitis

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Abstract:

Nonalcoholic steatohepatitis (NASH) is a disorder that is characterized by hepatic fat accumulation (steatosis) combined with inflammation. Unlike steatosis, inflammation can lead to further irreversible liver damage. Currently, the mechanisms that trigger hepatic inflammation are poorly understood. Recently, we demonstrated a clear association between hepatic inflammation and lysosomal cholesterol accumulation inside Kupffer cells (KCs). However, it is still not clear whether lysosomal cholesterol accumulation is causally involved in hepatic inflammation. 27-hydroxycholesterol (27-HC), a derivate of cholesterol formed by CYP27, was previously shown to mobilize cholesterol from the lysosomes to the cytoplasm of the cell in vitro. We hypothesize that lysosomal cholesterol accumulation is a trigger for hepatic inflammation. Therefore, CYP27 can modulate hepatic inflammation by redirecting the cholesterol pool from the lysosomes to the cytoplasm of the KCs in vivo. Ldlr–/– mice were lethally irradiated and transplanted (tp) with wild-type (WT) or Cyp27–/– bone marrow and fed a control vs. Western diet for 3 months. Electron microscopy analysis of KCs revealed increased lysosomal cholesterol accumulation in Cyp27–/– tp mice compared with WT-tp mice upon HFC diet. Consequently, the amount of cholesterol precipitation inside the KCs of Cyp27–/– tp mice was increased, and lysosomal enzyme activity was disturbed in Cyp27–/– tp compared with WT-tp mice. In line with our hypothesis, Cyp27–/– tp mice had increased hepatic inflammation compared with WT-tp mice as indicated by the elevated numbers of infiltrated macrophages, neutrophils, and T cells. These findings were confirmed by hepatic expression of TNF, Il-6, and Il-1b. We concluded that lysosomal cholesterol accumulation in KCs is an important trigger for hepatic inflammation. Moreover, our discovery points toward the potential of 27-HC to be used as a novel therapy for NASH.

# 50

## Acyl-CoA Synthetase 1 (ACSL1) Promotes Apoptosis and Release of Proinflammatory Mediators in Mouse Macrophage J774 Cells

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Abstract:

Epidemiological studies have shown that the risk of cardiovascular events is increased in patients with diabetes. Macrophages are believed to play a central role in all stages of atherosclerosis. Recently we have demonstrated that ACSL1, a member of the long chain acyl-CoA synthetase (ACSL) family, which mediates the thioesterification of long chain fatty acids into their acyl-CoA derivatives in the initial step of fatty acid metabolism, is up-regulated both in macrophages from diabetic mice and in classically activated inflammatory macrophages in vitro. To investigate the biological effect of increased ACSL1 expression in macrophages, we overexpressed this protein in a mouse macrophage-derived cell line, J774, using a retroviral system. Overexpression of ACSL1 markedly increased levels of 20:4-CoA > 22:6-CoA > 18:2-CoA > 18:0-CoA > 16:0-CoA > 18:1-CoA (all $p < 0.05$; $n = 9$). Interestingly, the cell number was significantly reduced by ACSL1 overexpression, and caspase-3 activity was elevated 1.7-fold ($p < 0.001$), suggesting that ACSL1 promotes apoptosis. Furthermore, ACSL1-overexpressing cells released 1.6-fold more TNF-□ after LPS stimulation compared with control cells ($p < 0.05$). There was no increase in long chain or short chain acylcarnitines or reactive oxygen species production in ACSL1-overexpressing cells, suggesting that neither mitochondrial activity nor oxidative stress is likely to be involved in ACSL1-exacerbated apoptosis or inflammatory changes. Taken together, our data reveal a novel effect of ACSL1 in macrophage apoptosis and inflammatory phenotype, which may contribute to the complications of diabetes or of other disease states characterized by an inflammatory macrophage phenotype.

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## Apolipoprotein E Reduces Atherosclerosis Independently of Lowering Plasma Cholesterol by Suppressing Inflammation in Circulating Leukocytes and Vascular Endothelium

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Abstract:

We sought to investigate mechanisms by which apolipoprotein (apoE) E can suppress atherosclerosis beyond reducing plasma cholesterol. To this end, we bred hypomorphic apoE (Apoeh/h) mice to Ldlr–/– mice to derive Apoeh/hLdlr–/– mice. When fed a chow diet, Apoeh/hLdlr–/– mice displayed plasma cholesterol levels that were similar to those of chow-fed Apoe–/–Ldlr–/– mice (597.5 ± 24.3 mg/dl vs. 662.4 ± 26.4 mg/dl, respectively), despite accumulating 4-fold more plasma apoE than wild-type mice. By 20 weeks of age, Apoeh/hLdlr–/– mice developed ~4-fold less Oil Red O- and ~3-fold less macrophage-positive surface area in the aortic root than Apoe–/–Ldlr–/– mice. Apoeh/hLdlr–/– mice displayed reduced endothelial activation, with reduced expression of ICAM-1 (1.28-fold less), PECAM-1 (4.5-fold less), and JAM-A (11-fold less) as assessed by confocal microscopy of en face preparations of the aortic arch derived from 14-week-old mice of both groups. By 20 weeks of age, Apoeh/hLdlr–/– mice displayed 30% fewer circulating blood leukocytes and 10% fewer proinflammatory Ly6C-hi monocytes. They also displayed 16% less intracellular neutral lipid in circulating monocytes that correlated with reduced cell surface expression of adhesion molecules including ICAM-1 (8% less), VLA-4 (17% less), and L-selectin (32% less). Fractionation of Apoeh/hLdlr–/– mouse plasma revealed that apoE distributed mainly to apoB lipoproteins. In contrast to Apoe–/–Ldlr–/– mouse plasma in which apoA1 distributed equally among apoB lipoproteins and HDL, apoA1 levels in Apoeh/hLdlr–/– mice were increased by almost 2-fold and distributed mainly to HDL that were also found to be 2.3-fold more potent at promoting cellular cholesterol efflux. Our findings demonstrate that apoE reduces atherosclerosis in the setting of dyslipidemia by increasing plasma apoA1-HDL, which likely contributes to reduce intracellular lipid accumulation and thereby the activation of circulating leukocytes and the vascular endothelium.

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## Lipoprotein Sizing Comparison among Different Analytical Platforms: Is There a Technique That Fits All?

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Abstract:

Recently, there has been much discussion as to which analytical platform is the most appropriate for lipoprotein sizing/measurement. Because the outputs from these measurements are routinely used for the diagnosis and management of hypercholesterolemia and coronary cardiovascular events, this has been a very hot topic in the cardiovascular disease arena. Different methods have been utilized for the measurement of lipoproteins such as ultracentrifugation (UC), vertical autoprofiling (VAP), fast protein liquid chromatography (FPLC), gradient gel electrophoresis (GGE, (Lipoprint®)), and nuclear magnetic resonance (NMR). In the current study, we selected human plasma samples (n = 20) which were segregated by individuals having low and high HDL-c content (n = 10 per group). The samples from these human volunteers were analyzed for VLDL-c, LDL-c, and HDL-c utilizing all of the different analytical platforms for lipoprotein analysis mentioned above. Unsupervised multivariate statistical analysis, principal component analysis (PCA) was utilized to compare all the different lipoprotein sizing techniques. Preliminary computational analysis of the data revealed that UC was predominantly the outlier technique for all lipoprotein measurements compared with the other analytical platforms. Acceptable correlation (R2 ranging from 0.6355 to 0.9013) was achieved for LDL and HDL cholesterol measurements among the Lipoprint®, FPLC, NMR, and VAP methodologies. Comprehensive head-to-head comparisons were also performed for each lipoprotein measurement and sizing calculation as well. This study demonstrates that for human plasma samples from patients with a range of HDL-c values, certain methods provide similar quantitative information for lipoprotein parameters. This sets the stage for comparison of these methods among other sample conditions (patients treated with novel lipid-modifying therapies, different disease states, etc.).

# 53

## Systems Genetics-based Approach to Study Obesity in Mice

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Abstract:

Obesity is a polygenic disease with a complex etiology involving multiple genetic and environmental factors. In addition to these factors, recent studies have highlighted important roles for both the immune system and the microbiome in contributing to obesity in humans and mice. To understand the complex molecular interactions contributing to obesity we are using a powerful systems genetics resource, the Hybrid Mouse Diversity Panel (HMDP), which enables high resolution genetic mapping and integration of multiple high-throughput "-omics" data sets, such as transcriptomics and metabolomics. The HMDP is composed of commercially available, classical, and recombinant inbred strains of mice (~100 strains total), which takes advantage of common genetic variations that exists among inbred mice strains. Obesity will be assessed in mice fed a high fat or normal chow diet for 8 weeks (starting at 8 weeks of age), followed by extensive phenotyping (body weight, lean/fat mass, plasma lipids, etc.). To address immune system and microbiota contributions to obesity we are performing high throughput flow cytometry analysis of lymphoid and myeloid cell types in the periphery and within visceral adipose tissue. Gut microbial diversity is being measured using 454 pyrosequencing, which enables precise determination of the microbial community. Data collected thus far show robust weight gain with a high fat diet and significant strain variation for all traits measured. Collectively, these data will provide the first comprehensive analysis of gene by environmental interactions contributing to obesity across multiple scales of biology (DNA, RNA, protein, metabolites, microbiota) and will allow the dissection of complex interactions in obese and lean settings.

# 55

# Apolipoprotein E4 "Domain Interaction" Accelerates Diet-induced Atherosclerosis in Mice

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Abstract:

Apolipoprotein (apo) E4 is an established risk factor for atherosclerosis-related cardiovascular diseases. However, the mechanisms underlying this association remain unclear. ApoE4 displays a unique biophysical property called "domain interaction" (DI), causing its C- and N-globular domains to interact abnormally. To address whether DI is responsible for apoE4-induced atherosclerosis in vivo, we created mouse models in which (1) DI was introduced into mouse apoE by substituting a threonine for an arginine in position 61: ApoeT61 (wild-type) to ApoeR61 (DI), and (2) apoE expression levels were reduced in corresponding hypomorphic mice: ApoeT61h/h and ApoeR61h/h. On a chow diet, these hypomorphic mice expressed low levels of plasma apoE (≈5-10% of wild type), predisposing them to dyslipidemia when fed a high cholesterol diet (HCD). To test whether apoE4 DI promotes atherosclerosis, ApoeR61h/h and ApoeT61h/h mice were fed a HCD, and their phenotypes were compared. Both male and female ApoeR61h/h mice developed larger aortic root lesions than ApoeT61h/h mice. Male ApoeR61h/h mice showed a 62% increase in atherosclerotic lesion area after 15 weeks of HCD despite similar plasma lipid levels and lipoprotein profiles. Moreover, after 9 and 15 weeks of HCD, female ApoeR61h/h mice had 45% and 11% increases in atherosclerotic lesion area, respectively, with a slight increase in plasma lipid levels and VLDL cholesterol. Results of in vitro studies suggest that apoE4 DI contributes to macrophage dysfunction as peritoneal macrophages derived from ApoeR61 mice displayed enhanced susceptibility to inflammation and endoplasmic reticulum stress. Results of our in vivo and in vitro studies demonstrate that DI contributes significantly to the pathological effects of apoE4 in promoting atherosclerosis. The synthesis of pharmacological chaperones inhibiting DI could hold great promise for the therapeutic treatment of atherosclerosis in apoE4 individuals.

# 56

## Effects of Postprandial Triglyceride-rich Lipoprotein Lipolysis Products on Kidney Injury

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Abstract:

Hypertriglyceridemia is often observed in chronic kidney disease patients, but its effect on the renal system remains unclear. Our previous work has established that postprandial triglyceride-rich lipoprotein (TGRL) lipolysis products induce inflammation and apoptosis in human aortic endothelial cells. We hypothesized that circulating postprandial TGRL lipolysis products injure the renal glomerulus. Using quantitative polymerase chain reaction, our results with human mesangial cells in culture show that TGRL lipolysis products up-regulate inflammatory markers such as IL-6 and IL-8. Western blot analysis also shows increased phosphorylation of SMAD, and supernatant analysis demonstrates increased TGF-β activation after lipolysis products treatment. These results suggest that the injury mechanism involves the TGF-β pathway. Our animal experiments corroborate these in vitro observations. After vascular infusion of TGRL lipolysis products into C57BL/6 mice, a significant increase in renal TGF-β production was detected 2 h after treatment. No significant effect was seen after infusion of medium or TGRL. Although further studies are needed to pinpoint the mechanism, these preliminary observations suggest that postprandial TGRL lipolysis products may be a novel mechanism in chronic, repetitive glomerular injury.

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## LDL-c Lowering in Rhesus Monkeys by Subcutaneous Administration of a Monoclonal Anti-proprotein Convertase Subtilisin-like/Kexin Type 9 (PCSK9) Antibody

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Abstract:

Proprotein convertase subtilisin-like/kexin type 9 (PCSK9) has recently emerged as a major regulator of plasma LDL cholesterol (LDL-c) and consequently as a promising therapeutic target for treating coronary heart disease (CHD) (1-4). Inhibitors of the cell surface PCSK9-LDLR interaction are expected to enhance LDL-c clearance and lower circulating levels of LDL-c. Several lines of preclinical and clinical data validate the development of an anti-PCSK9 monoclonal antibody (mAb) as an effective therapeutic agent for LDL-c lowering in the clinic (5-8). We identified a human monoclonal antibody (mAb8) that binds to human, murine, and Rhesus PCSK9 with low nanomolar affinity (6.8, 2.8, and 2.2 nM, respectively) and that potently inhibits the PCSK9 functional effects in a HepG2 cell-based LDL-c uptake assay (EC50: 9 nM). mAb8 efficiently lowered LDL-c in CETP/LDLR+/– mice upon administration of a single, 3 mpk dose (>20% for 5 days). Efficient lowering of plasma LDL-c (>25%) was also obtained by subcutaneous administration of 1 mpk mAb8 in Rhesus monkeys, which lasted for 45 days. A subsequent study in normal Rhesus monkeys showed a shorter duration of action for this mAb, in the same dosing paradigm. Analysis of LDL-c levels showed differences in base-line levels in these Rhesus monkeys that correlated with the duration of LDL-c lowering caused by mAb8. Our results show efficacy of the monoclonal antibody mAb8 in Rhesus monkeys and suggest that base-line LDL-c levels may be of importance for the duration of efficacy.

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# Notes

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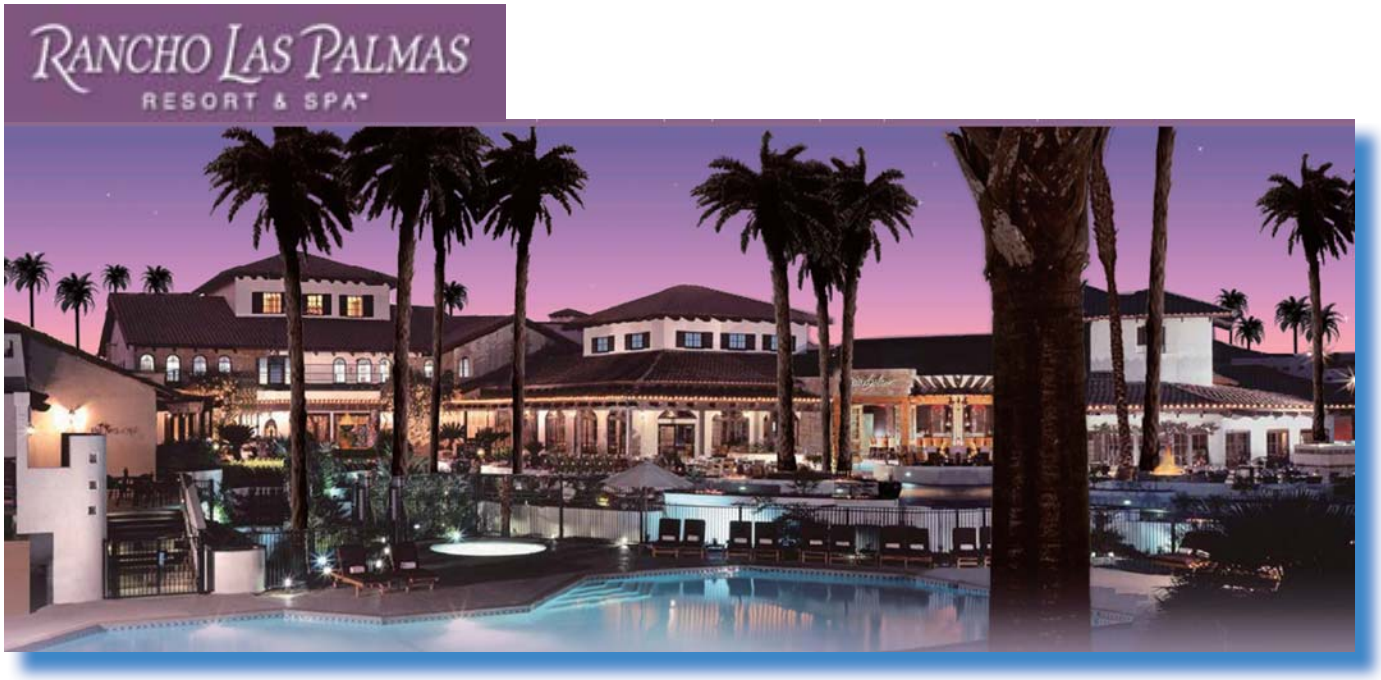
**2012 DEUEL Conference on Lipids**

**Rancho Mirage, California**



2011 DEUEL Conference on Lipids
March 1-4, 2011
Napa Valley, California