

The Metabolism of Lipids: Implications in Human Diseases

May 22nd – 25th, 2011

34th

Steenbock Symposium
University of Wisconsin-Madison USA

Organizer:
James M Ntambi

Cardiovascular
Disease

Obesity

Hypertension

Insulin
Resistance

Type 2
Diabetes

Atherosclerosis

Cancer

Hepatic
Steatosis

Inflammation

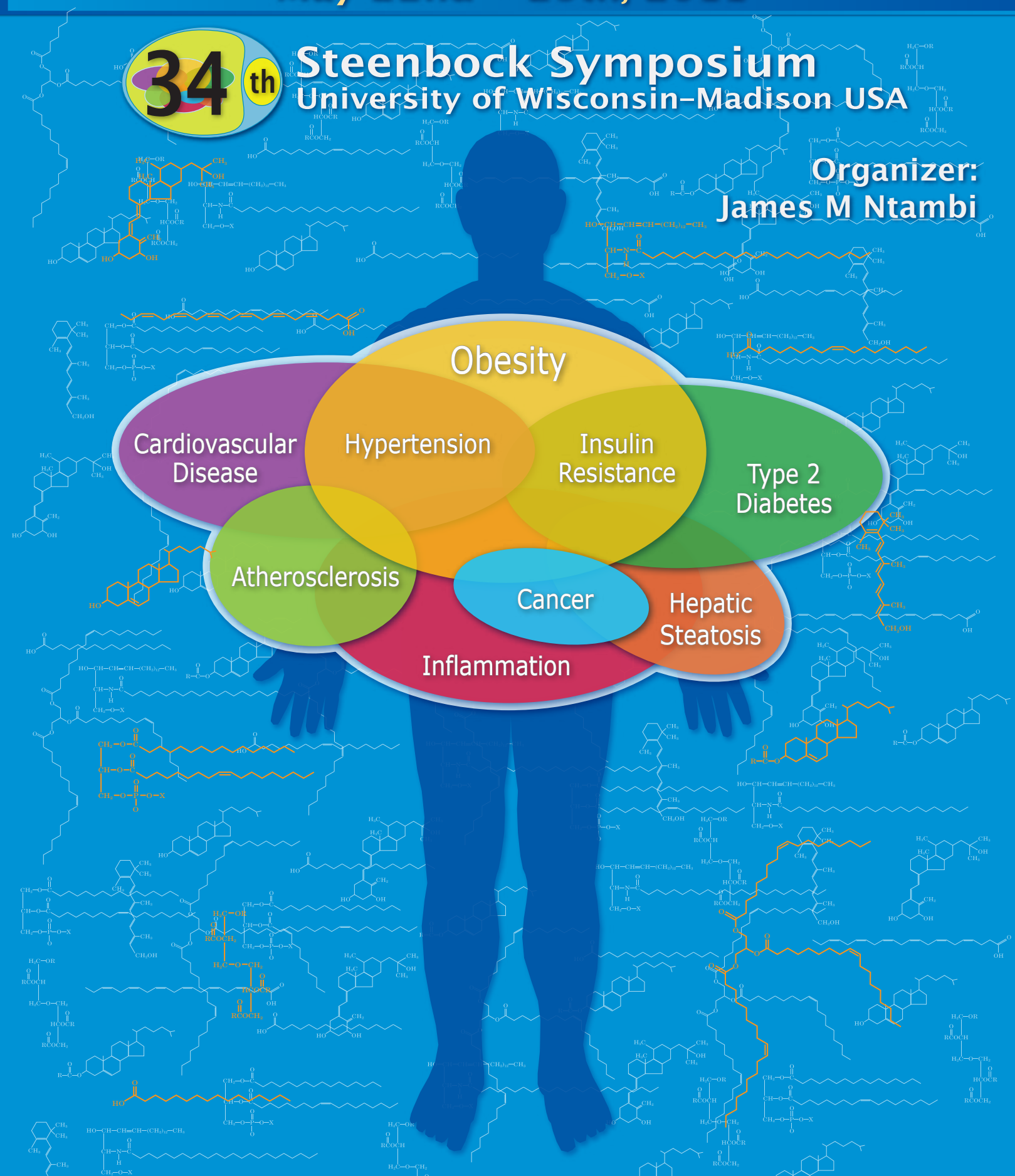


Table of Contents

Page	1 General Information
	2 Support Staff
	3 About Harry Steenbock
	3 Campus Map
	4 About the Organizer
	5 Sponsors
	6 - 7 Schedule
	8 - 9 Speaker Index
	10 - 85 Speakers & Abstracts
	86 - 87 Poster Presenter Index
	88 - 124 Poster Abstracts
	126 Attendee Addresses

The 34th Steenbock Symposium

The Metabolism of Lipids: Implications in Human Diseases

The symposium will bring together leading scientists to present and discuss their research on the roles of lipids in health and disease. Participants and speakers will reflect international excellence in this medically relevant area of science, and a balance between youth and wisdom.

Lectures will cover a broad range of topics, including:

- brain function
- adipokines
- lipokines
- lipid droplets
- lipolysis
- adipocyte differentiation
- nuclear receptors
- liver steatosis
- insulin signaling
- atherosclerosis
- inflammation

The approaches are very broad, using genomics, proteomics, lipidomics and metabolomics in a wide variety of *in vitro* and *in vivo* strategies.

General Information

Name Badges

Because several groups will be using the meeting and dining facilities, Steenbock participants are requested to wear the name badge during all conference activities

Registration

Pick up conference materials on Sunday, May 22nd, 6:30-8:00 PM
in the Wisconsin Institutes for Discovery Atrium, 330 N. Orchard Street

Reception

Sunday, May 22nd, 6:30-8:00 PM
in the Wisconsin Institutes for Discovery Atrium, 330 N. Orchard Street

Scientific Talks

All talks will be in the Wisconsin Institutes for Discovery Forum, 330 N. Orchard Street
Talks begin at 8:00 AM on Monday and conclude by 12:00 PM on Wednesday

Lunches

Monday and Tuesday
Registration fee includes breaks and lunch in Wisconsin Institutes for Discovery Atrium.
Please wear your name badge.

Poster Session

Posters can be set up beginning Monday at 9:00 AM and should be removed by Monday at 7:30 PM.
Boards are numbered and reserved. Please see the reservation list in this booklet and by the boards.

Banquet

Tuesday, May 24th, in the Wisconsin Institutes for Discovery Atrium, 330 N. Orchard Street

The Metabolism of Lipids: Implications in Human Diseases

**34th Steenbock Symposium
University of Wisconsin – Madison**

Symposium Organizer

James M. Ntambi
Departments of Biochemistry
and Nutritional Sciences
University of Wisconsin – Madison

Symposium Coordinators

Flavia Arana
Department of Biochemistry
University of Wisconsin – Madison

Brad Clark
Department of Biochemistry
University of Wisconsin – Madison

Leah Leighty
CALS Conference Services
University of Wisconsin – Madison

Linda McNiel
Department of Biochemistry
University of Wisconsin – Madison

Symposium Advisors

Faculty
Department of Biochemistry
University of Wisconsin – Madison

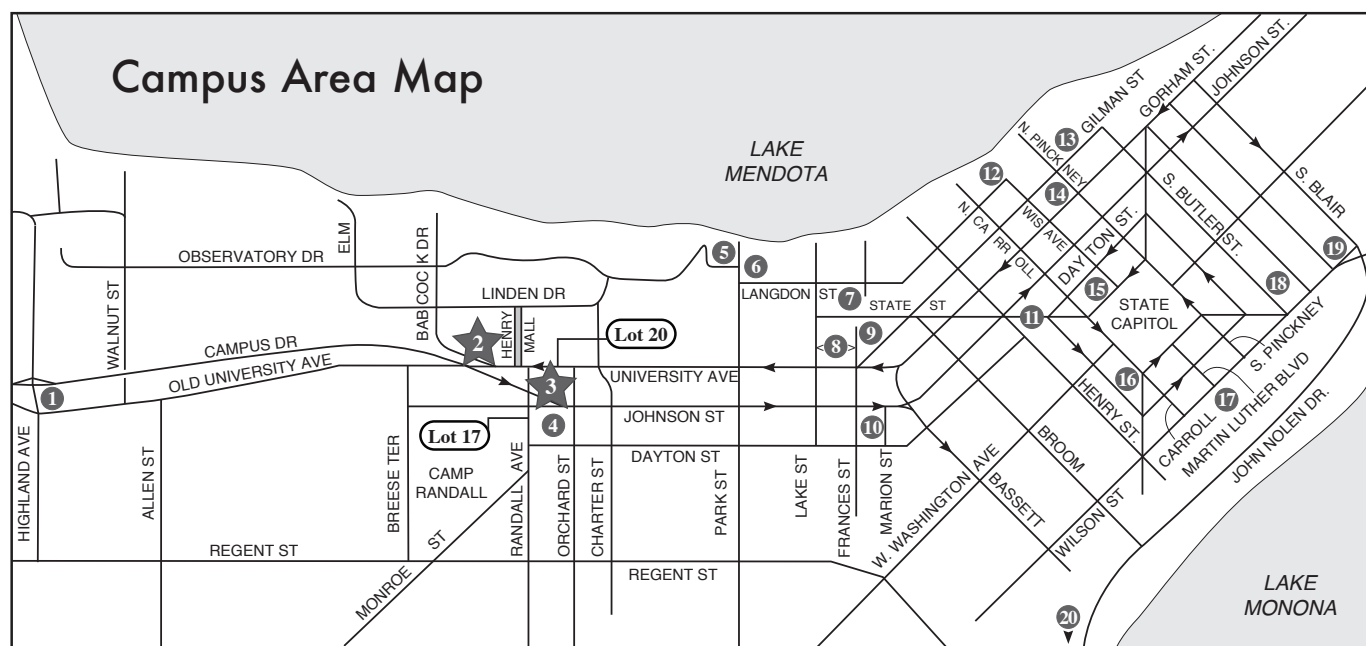
Mary Cantu
Original Cover & Poster design

Laura Vanderploeg & Robin Davies
Symposium Web Site, Abstract Book & Illustrations

Harry Steenbock 1886–1967

A distinguished Professor of Biochemistry at the University of Wisconsin-Madison. His interests and contributions spanned many areas of nutrition and biochemistry. Of special note is his discovery of the conversion of carotenes to vitamin A, the production of vitamin D by ultraviolet irradiation, and his central participation in demonstrating that copper and iron cure nutritional anemia. The production of vitamin D by ultraviolet irradiation virtually abolished rickets, a widespread and debilitating disease. Professor Steenbock patented the irradiation process for producing vitamin D and conceived the idea of the Wisconsin Alumni Research Foundation, a nonprofit foundation which invests the proceeds from patents for support of research in the natural sciences at the University of Wisconsin-Madison. Professor Steenbock assigned his valuable patents to the Foundation, and revenue from them has provided uniquely flexible resources for fostering sustained scientific development on the Madison campus.

To honor Professor Steenbock, the Steenbock Endowment sponsors a symposium at the University of Wisconsin-Madison.

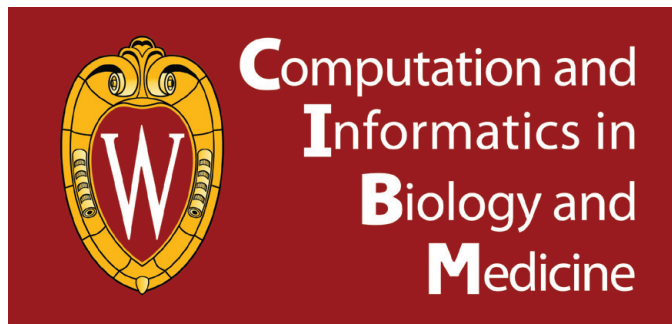


- | | | | |
|--|--|---|----------------------------|
| 1. Best Western InnTowner Hotel | 6. Memorial Union | 11. State Street (shopping & restaurants) | 17. Hilton Hotel |
| 2. Department of Biochemistry | 7. Campus Inn | 12. Edgewater Hotel | 18. The Madison Hostel |
| 3. Wisconsin Institutes for Discovery
(Site of symposium) | 8. Lake St. & Frances St.
Parking Ramps | 13. Gilman Street Rag | 19. Hotel Ruby Marie |
| 4. Union South | 9. University Inn | 14. Mansion Hill Inn | 20. Sheraton Madison Hotel |
| 5. H.C. White Library (Parking) | 10. Doubletree | 15. The Madison Concourse Hotel | |
| | | 16. Best Western Inn on the Park | |

Symposium Sponsors

The Steenbock Endowment

Department of Biochemistry
University of Wisconsin – Madison





James M. Ntambi is currently a Professor of Biochemistry and a Steenbock Professor of Nutritional Sciences. He is also the new chair of the Department of Nutritional Sciences. He received his BSc and MSc degrees in Biochemistry and Microbial Biochemistry respectively from Makerere University Kampala, Uganda. In 1980 he received a Fulbright Fellowship to study for a PhD at the Johns Hopkins University School of Medicine in Baltimore MD. He was admitted into the Biochemistry, Cellular and Molecular Biology (BCMB) program of the Department of Biological Chemistry. Here he trained under Dr. Paul Englund who introduced him to molecular biology techniques that led to the identification of the origin of mitochondrial DNA replication in African trypanosomes. After obtaining his PhD and as he was preparing to return to Africa to study the differentiation and antigenic variation in Africa trypanosomes, Dr. M. Daniel Lane then chair of the Department of Biological Chemistry

invited him to join his lab as a postdoctoral fellow to study the differentiation of mouse 3T3-L1 preadipocytes. The plan was to clone the gene corresponding to a differentially expressed pAL122 cDNA that David Bernlohr and Bill Angus had pulled out by subtractive hybridization from the 3T3-L1 cDNA library. Ntambi rapidly cloned the gene, sequenced it and discovered that it encoded the mouse stearoyl-CoA desaturase enzyme (SCD). This enzyme catalyzes the regulated step in the synthesis of monounsaturated fatty acids mainly oleate and palmitoleate from the corresponding saturated fatty acid substrates. Several SCD genes (SCD 1-4) have since been isolated and characterized. The SCD-1 isoform is the most highly induced during preadipocyte differentiation and is very highly regulated by dietary and hormonal factors. The research that Ntambi did in Dr. Lane's lab marked the beginning of his interest in the genetic regulation of lipid metabolism.

In 1989 he moved to Georgetown University School of Medicine in Washington DC as Assistant Professor of Biochemistry and Molecular Biology where he continued to study the dietary and hormonal regulation of the SCD genes.

His stay at Georgetown University was short-lived. When he moved to the University of Wisconsin-Madison in 1992 with joint appointments in the Departments of Biochemistry and of Nutritional Sciences, he began to address the physiological roles of the SCD genes in lipid metabolism. His research group of talented graduate students and postdoctoral fellows first generated mice with a targeted disruption, or whole-body knockout, of the SCD-1 isoform. Over the past several years, Ntambi's group has shown that SCD-1 deficient mice are remarkably protected from obesity and insulin resistance and that SCD is a critical metabolic control point partitioning fats towards storage when activity is high and towards oxidation when activity is low. Studies from Ntambi's lab and studies from other laboratories have gathered considerable scientific evidence that link SCD-1 expression to various aspects of the metabolic syndrome. Ntambi has utilized the Cre-lox system to create mice with a tissue-specific (liver, adipose, muscle, brain and skin) disruption of SCD-1. The goal is to use these mice to determine which tissues are most responsible for the phenotypes observed in whole-body SCD-1-deficient mice. Recent studies have shown that skin SCD-1 deficiency elicits a hypermetabolic phenotype that protects mice from obesity and insulin resistance. Ntambi has also shown that liver SCD-1 deficiency impairs carbohydrate-induced up regulation of hepatic lipogenesis and protects mice from carbohydrate-induced obesity. The role for SCD-1 in other tissues, as well as the role of the other SCD isoforms, is still under investigation.

The Ntambi lab continues to be interested in the molecular mechanisms of cellular differentiation and gene expression using the 3T3-L1 preadipocyte cell line as model. They are using molecular biology approaches to characterize the mechanisms that regulate the expression of the SCD genes during fat cell differentiation. Ntambi's research group is also using genetic tools to knockdown the expression of the different SCD isoforms and assesses their importance for normal adipocyte differentiation. Based upon the skin-related phenotypes observed in mice lacking SCD-1 in the skin, Ntambi is also interested in the role of SCD in the differentiation of skin sebocytes, which are the major lipid producing cells of the skin.

For additional details of Ntambi's research work see: www.biochem.wisc.edu/faculty/ntambi

For the last 10 years Ntambi has led an extraordinary and invaluable program for undergraduates and graduate students taking them to Uganda to learn about nutrition and health in a developing country. This experience brings new meaning to the students' overall education. He has also been involved in doing research on obesity and diabetes in Uganda and other developing countries. His goal is to establish the relationship between obesity, genetics, nutrition, environment and metabolic diseases that are now very prevalent among adults and children in developing countries.

For additional details see: <http://www.villagehealthproject.org>

James Ntambi has received numerous awards including the Osborne and Mendel Award, Steenbock Career Development Award, the Forgarty International Biomedical Research Award, the Fulbright Research Award, the Arthur J. Maurer Extra Mile Award, Excellence in International Activities Award and the Chancellor's Distinguished Teaching Award. In 2003 he became the Katherine Berns Von Donk Steenbock Professor of Nutritional Sciences and in 2009 became a member of the Uganda National Academy of Sciences. He serves on numerous scientific committees and advisory boards.

Symposium Schedule

Sunday, May 22nd, 2011

6:30 - 8:00 PM	Reception and Registration. Wisconsin Institutes for Discovery Atrium, 330 N. Orchard Street
----------------	--

**** All talks will be in The Forum, Wisconsin Institutes for Discovery, 330 N. Orchard Street ****

Monday, May 23rd, 2011

7:30 AM	Continental Breakfast. Wisconsin Institutes for Discovery Atrium, 330 N. Orchard Street
8:00 AM	Welcoming Remarks - James M. Ntambi , Katherine Berns Von Donk Steenbock Professor
8:10 AM	Opening remarks - Hector DeLuca , Harry Steenbock Research Professor
Session 1	<i>Regulation of Lipid Metabolism</i>
8:25 AM	<i>Chair:</i> Rosalind A. Coleman
8:30 AM	Rosalind A. Coleman
9:00 AM	Michael P. Czech
9:30 AM	Dawn L. Brasaemle
10:00 - 10:20	Coffee Break
10:20 AM	Robert V. Farese, Jr.
10:50 AM	Hei Sook Sul
11:20 AM	Karen Reue
11:50 AM	Eric Yen
12:20 - 1:10	Lunch & the Poster Session (<i>Posters on display 12:30-7:30</i>)
Session 2	<i>Signaling in Adipocytes and Regulation of Body Weight</i>
1:15 PM	<i>Chair:</i> Xiaoli Chen
1:20 PM	Ormond A. MacDougald
1:50 PM	Susanne Mandrup
2:20 PM	Barbara B. Kahn
2:50 - 3:10	First Afternoon Break
3:15 PM	Xiaoli Chen
3:45 PM	Robert H. Eckel
4:15 PM	M. Daniel Lane
4:45 - 5:05	Second Afternoon Break
Session 3	<i>Metabolism in Heart and Muscle</i>
5:10 PM	<i>Chair:</i> Agnieszka Dobrzyn
5:15 PM	Aldons J. Lusis
5:45 PM	Ira J. Goldberg
6:15 PM	Agnieszka Dobrzyn
6:45 PM	Deborah M. Muoio

Symposium Schedule

Tuesday, May 24th, 2011

8:00 AM	Continental Breakfast. Wisconsin Institutes for Discovery Atrium, 330 N. Orchard Street	
Session 4	<i>Liver and Skin Metabolism</i>	
8:25 AM	<i>Chair:</i> Sudha Biddinger	
8:30 AM	Sudha Biddinger	
9:00 AM	Jay D. Horton	
9:30 AM	Philipp E. Scherer	
10:00 AM	Pere Puigserver	
10:30 - 11:00	Coffee Break	
11:00 AM	Chih-Hao Lee	
11:30 AM	Apostolos Pappas	
12:00 PM	James M. Ntambi	
12:30 PM	Frank J. Gonzalez	
1:00 - 1:50	Lunch	
Session 5	<i>Diabetes</i>	
1:55 PM	<i>Chair:</i> Alan D. Attie	
2:00 PM	Alan D. Attie	
2:30 PM	Christopher B. Newgard	
3:00 PM	Daniel S. Greenspan	
3:30 - 3:45	Break	
3:45 PM	Joyce J. Repa	
4:15 PM	David A. Bernlohr	
4:45 PM	Dennis E. Vance	
5:15 PM	Ronald M. Krauss	
6:00 PM	Social Time	Wisconsin Institutes for Discovery Atrium, 330 N. Orchard Street
7:00 PM	<i>Dinner Banquet</i>	

Wednesday, May 25th, 2011

8:00 AM	Continental Breakfast. Wisconsin Institutes for Discovery Atrium, 330 N. Orchard Street	
Session 6	<i>Cholesterol Metabolism; Stem cells; Atherosclerosis</i>	
8:25 AM	<i>Chair:</i> Richard J. Deckelbaum	
8:30 AM	Makoto Miyazaki	
9:00 AM	C. Ronald Kahn	
9:30 AM	Peter Tontonoz	
10:00 AM	Christopher Glass	
10:30 AM	Alan Tall	
11:00 AM	Richard J. Deckelbaum	
11:30 AM	James M. Ntambi	

Speaker Index

Speaker	Title of Talk	Abstract Page	Day Time
Alan D. Attie	Gene Loci for Type 2 Diabetes in Mice	11	Tuesday 2:00 pm
David A. Bernlohr	Inflammation and Macrophage Leukotriene Synthesis	13	Tuesday 4:15 pm
Sudha Biddinger	The Control of Lipid Metabolism in Insulin Resistant States	15	Tuesday 8:30 am
Dawn L. Brasaemle	Perilipin Control of Lypolysis	17	Monday 9:30 am
Xiaoli Chen	Lipocalin 2 as an Important Regulator of Energy Metabolism	19	Monday 3:15 pm
Rosalind A. Coleman	Lipid Metabolism: Location, Location, Location	21	Monday 8:30 am
Michael P. Czech	Metabolic Control by Lipid Droplet Proteins	23	Monday 9:00 am
Richard J. Deckelbaum	Omega-3 Fatty Acids: Preventive and Therapeutic Molecules for Liver, Heart, and Brain	25	Wednesday 11:00 am
Agnieszka Dobrzyn	Unsaturated Fatty Acids and Regulation of Cardia Energy Metabolism	27	Monday 6:15 pm
Robert H. Eckel	Lipoprotein Lipase in the Brain Regulates Energy Balance and Body Weight	29	Monday 3:45 pm
Robert V. Farese, Jr.	How Cells Fatten Up: Fat Synthesis and Lipid Droplets	31	Monday 10:20 am
Christopher Glass	Sterol/Oxysterol Regulation of Macrophage Gene Expression	33	Wednesday 10:00 am
Ira J. Goldberg	Correcting Cardiac Lipotoxicity	35	Monday 5:45 pm
Frank J. Gonzalez	Investigating the Roles of Lipids in Disease and Chemical Toxicities using Metabolomics	37	Tuesday 12:30 pm
Daniel S. Greenspan	$\alpha 3(V)$ Collagen Ablation in Mice Induces Diabetes-related Symptoms via Effects on Pancreatic Islets and Peripheral Tissues	39	Tuesday 3:00 pm
Jay D. Horton	Molecular Mediators of Hepatic Steatosis	41	Tuesday 9:00 am
Barbara B. Kahn	Adipose ChREBP, Lipogenesis, and Insulin Sensitivity	43	Monday 2:20 pm
C. Ronald Kahn	Insulin, Diabetes and the Control of Brain Cholesterol Metabolism	45	Wednesday 9:00 am
Ronald M. Krauss	Blood Lipids and Body Fat – Making the Connection	47	Tuesday 5:15 pm

Speaker Index

Speaker	Title of Talk	Abstract Page	Day Time
M. Daniel Lane	How Glucose and Fructose Alter Hypothalamic Signaling and Feeding Behavior	49	Monday 4:15 pm
Chih-Hao Lee	Metabolic Regulation by Immune Signaling	51	Tuesday 11:00 am
Aldons J. Lusis	Systems Genetics Approaches to Complex Cardiovascular Traits	53	Monday 5:15 pm
Ormond A. MacDougald	Role of Wnt Signaling in Adipocyte Differentiation and Metabolism	55	Monday 1:20 pm
Susanne Mandrup	Transcriptional Networks of Fat Cell Development	57	Monday 1:50 pm
Makoto Miyazaki	Bile Acid Signaling in Regulation of Atherosclerosis and Vascular Calcification	59	Wednesday 8:30 am
Deborah M. Muoio	Mitochondrial Stress and Metabolic Dysfunction in Skeletal Muscle	61	Monday 6:45 pm
Christopher B. Newgard	Metabolomics Used for Understanding Chronic Disease Mechanisms	63	Tuesday 2:30 pm
James M. Ntambi	A Role for Skin Stearoyl-CoA Desaturase-1 in Obesity and Diabetes	65	Tuesday 12:00 pm
Apostolos Pappas	Lipids in Skin Physiology, Health and Disease	67	Tuesday 11:30 am
Pere Puigserver	A Nutrient Sensor Mechanism for Control of Lipid Oxidation	69	Tuesday 10:00 am
Joyce J. Repa	Lipid-sensing Transcription Factors and Beta-cell Biology	71	Tuesday 3:45 pm
Karen Reue	Deciphering the Roles of Lipin Family Members in Tissue Lipid Homeostasis	73	Monday 11:20 am
Philipp E. Scherer	Multiple Effects of Xbp1s on Hepatic Lipid Metabolism	75	Tuesday 9:30 am
Hei Sook Sul	Regulation and Function of Adipose Lipolysis	77	Monday 10:50 am
Alan Tall	ABC Transporters and ApoE in the Regulation of Hematopoietic Stem Cell Proliferation, Monocytosis and Atherogenesis	79	Wednesday 10:30 am
Peter Tontonoz	Control of Cholesterol Homeostasis by LXRs	81	Wednesday 9:30 am
Dennis E. Vance	Phospholipid Methylation, Diabetes and Obesity	83	Tuesday 4:45 pm
C.-L. Eric Yen	Intestinal Triacylglycerol Metabolism and Energy Balance	85	Monday 11:50 am



Alan D. Attie

Alan Attie carried out his graduate and post-doctoral research at the University of California-San Diego working on lipid and lipoprotein metabolism. He then joined the faculty of the Biochemistry Department at the University of Wisconsin-Madison, initially working on the cellular and molecular biology of lipoprotein production, intracellular cholesterol transport, and mutations that affect cholesterol and lipoprotein metabolism in mutant animal models.

In mid-career, he refocused his research to study the genetics of type 2 diabetes using mouse model systems. The work has involved positional cloning of diabetes susceptibility loci and genomic studies focused on the pathways leading to insulin resistance and beta cell dysfunction.

Gene Loci for Type 2 Diabetes in Mice

Alan D. Attie*, Sushant Bhatnagar, Angie Oler, Mary Rabaglia, Kathryn Schueler, Lyndsa Schneider, Brendan Floyd, Donald Stapleton, Chen-Yu Wang, Summer Raines, Oliver Richards, Mark P. Keller

Department of Biochemistry, University of Wisconsin-Madison, Madison, Wisconsin

Through QTL mapping, we identified multiple gene loci linked to diabetes-related traits in a cross between C57BL/6 (B6) and BTBR mice made obese with the *Leptin^{ob}* mutation. A locus on Chr 16 was linked to fasting glucose and insulin levels. Through a series of interval-specific congenic mouse strains and DNA sequencing, we narrowed the position of the gene underlying this locus to <1 Mb, a segment containing just 13 genes. The *Stxbp5l* gene, encoding Tomosyn-2 emerged as our strongest candidate. It contains a coding SNP, its expression level is elevated in islets from BTBR mice, and our functional studies show that it is an inhibitor of insulin secretion. We previously reported the positional cloning of another QTL, on Chr 19, the *Sorcs1* gene. *Sorcs1* belongs to the vacuolar protein sorting-10 (vps10) gene family, which is associated with a wide range of biological functions, reflecting the fact that the proteins bind to many ligands and are involved in protein trafficking. Genetic studies in humans link *SORCS1* to type 2 diabetes, diabetes complications, and Alzheimer's Disease. A recent positional cloning project in rats linked *Sorcs1* to diabetic nephropathy. We have recently derived *Sorcs1* knockout mice. Our preliminary characterization of the mice shows that they are leptin-resistant and have an increase in food intake and fat mass.



David A. Bernlohr

David Bernlohr carried out his PhD work at the University of Illinois in Champaign-Urbana and did postdoctoral work with M. Daniel Lane at The Johns Hopkins School of Medicine. In 1985 he joined the faculty at the University of Minnesota and currently is the Distinguished McKnight University Professor and Head of the Department of Biochemistry, Molecular Biology and Biophysics. He holds the Cargill Chair in Systems Biology and studies inflammation and obesity as determinants in the development of Type 2 diabetes and atherosclerosis. His laboratory utilizes a combination of metabolomic, proteomic and molecular genetic approaches towards an understanding of oxidative stress, mitochondrial function and lipid signaling in adipocytes and macrophages. His work has profiled the role(s) of fatty acid binding proteins in cellular metabolism and their contributions to inflammation and metabolic disease. More recently his work has emphasized the covalent modification of proteins by reactive lipids and their influence on mitochondrial metabolism and signaling.

Inflammation and Macrophage Leukotriene Synthesis

David A. Bernlohr*, Ann V. Hertz, Eric Long, Kristina Hellberg, Rocio Foncea.

Department of Biochemistry, Molecular Biology and Biophysics. The University of Minnesota-Twin Cities. 321 Church St. SE, Suite 6-155. Minneapolis, MN 55455 USA bernl001@umn.edu

A variety of analyses have established the importance of macrophage inflammation leading to peripheral insulin resistance. In each of these systems, the end products of inflammation (e.g., TNF α , IL-6, IL-1 β) bind to receptors on endothelial cells and/or adipocytes and contribute to the insulin-resistant state. The chronic production of macrophage-derived factors lead to changes in insulin signaling, increased basal lipolysis and serum FFA, and altered adipokine synthesis and secretion thereby linking local events in visceral adipose tissue to systemic effects in peripheral (muscle, liver and β -cells) and central (hypothalamus) metabolism. In addition to inflammatory cytokines, macrophages also produce pro-inflammatory leukotrienes and the abundance of such factors increases with obesity. Consistent with this, targeted deletion of 5-lipoxygenase or pharmacologic inhibition of the 5-lipoxygenase activating protein reduces leukotriene synthesis and attenuates inflammation and insulin resistance. Treatment of either RAW 264.7 or primary murine peritoneal macrophages with saturated, n-6 or n-3 polyunsaturated fatty acids led to a rapid sustained increase in cytoplasmic calcium derived from both release of intracellular stores and influx from extracellular pools. Coincident with increased cytoplasmic calcium is the translocation of cPLA₂ to membranes in response to saturated and n-6 (but not n-3) fatty acids. LC-MS/MS analysis revealed that fatty acids (saturated $\gg$ n-6 = n-3) treatment of RAW 264.7 cells led to time and concentration-dependent synthesis and secretion of leukotriene C₄ while primary peritoneal macrophages produced LTC₄ only in response to palmitate. Consistent with inflammatory lipid synthesis in macrophage cell lines, high fat fed C57BL/6J mice exhibited elevated levels of adipose LTB₄, LTE₄, PGE₂, PGD₂, 5(S)-HETE and 15(S)-HETE (but not 12(S)-HETE) relative to chow fed control mice. Fatty acid binding proteins (FABP) increase the stability of LTA₄ >100 -fold and LTB₄ and LTC₄ biosynthesis was significantly attenuated in FABP4/aP2 null mice. The level of 5,6-diHETE was increased in FABP null cells indicating the leukotriene pathway was dependent upon FABP action. RAW264.7 cells expressing LTD₄ receptors respond to inflammatory leukotrienes by increasing MCP-1 synthesis while 3T3-L1 adipocytes respond to LTE₄ by decreasing insulin-stimulated glucose transport. These results suggest that adipocyte-derived fatty acids initiate a signaling cascade in macrophages producing leukotrienes that contribute to the inflammatory status of the tissue.



Sudha Biddinger

Sudha Biddinger completed her undergraduate studies at Princeton University, where she majored in Molecular Biology. She obtained her MD/PhD from the Johns Hopkins School of Medicine. Her PhD was performed with Dr. Richard Haganir, studying the signaling events underlying synapse formation. She completed her internship, pediatrics residency, and fellowship in pediatric endocrinology at the Children's Hospital Boston. During her fellowship, she worked with Dr. C. Ronald Kahn, studying the pathogenesis of the metabolic syndrome, particularly the mechanisms by which hepatic insulin resistance promotes dyslipidemia, atherosclerosis and cholesterol gallstones. In 2009, she joined the faculty of the Children's Hospital Boston, where she continues to study the mechanisms by which insulin regulates lipid and lipoprotein metabolism.

The Control of Lipid Metabolism in Insulin Resistant States

Joel Haas¹, Yanning Wang², Enpeng Zhao², Ji Miao², Mark Graham³, Roseanne Crooke³, and Sudha Biddinger*

¹Department of Biochemistry & Biophysics, University of California, San Francisco, California, USA

²Division of Endocrinology, Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts, USA

³Isis Pharmaceuticals, Carlsbad, California, USA

The master transcriptional regulator, Sterol Regulatory Element Binding Protein (SREBP)-1c, is sufficient to induce lipogenic gene expression, lipogenesis and hepatic steatosis. It is regulated at multiple levels, including transcription, maturation, and degradation. SREBP-1c is induced by insulin, but paradoxically increased in obesity, a state of insulin resistance, hyperinsulinemia, and nutrient excess. To dissect the roles of insulin and nutrients in the control of SREBP-1c, we have used Liver Insulin Receptor Knockout (LIRKO) mice; the livers of these mice are entirely incapable of responding to insulin. Surprisingly, some aspects of nutrient regulation, though blunted, were preserved in LIRKO livers; thus, fasting significantly decreased SREBP-1c mRNA and active nuclear protein in both control and LIRKO livers, leading to markedly reduced levels of downstream lipogenic targets such as *Scd1* and *Fas*. Hepatic triglyceride levels, in parallel with serum free fatty acid levels, were similar in control and LIRKO mice under fed and fasted conditions. However, the ability of nutrients to induce nuclear SREBP-1c protein, either by re-feeding a high carbohydrate diet for twenty-four hours or feeding a high fructose diet for one week, was markedly compromised in LIRKO livers. The dominant role of insulin versus nutrients in inducing nuclear SREBP-1c is further demonstrated by the fact that antisense oligonucleotides against the insulin receptor markedly decreased expression of SREBP-1c and its targets in *ob/ob* mice, despite their hyperphagia. Taken together these data suggest that though nutrients can induce SREBP-1c transcription independently of insulin, the accumulation of active, nuclear SREBP-1c protein requires insulin signaling.



Dawn L. Brasaemle

Dawn Brasaemle earned a Ph.D. in Biochemistry at the University of Wisconsin-Madison studying intracellular cholesterol transport in the laboratory of Alan Attie. She then conducted postdoctoral research on hepatic lipase processing of circulating lipoproteins in the laboratory of Andre Bensadoun at Cornell University. Seeking a novel area of inquiry, she then took a position as a Senior Staff Fellow in the National Institute of Diabetes and Digestive and Kidney Diseases on the NIH campus in Bethesda, MD working on perilipin, the first identified mammalian lipid droplet-associated protein, in the laboratory of Constantine (Dean) Londos. While at NIH, she studied the function of perilipin and identified adipophilin/ADRP/perilipin 2 as the second lipid droplet-associated protein. She is currently an Associate Professor at Rutgers University, where she continues functional studies of perilipin and other lipid droplet-associated proteins.

Perilipin Control of Lipolysis

Dawn L. Brasaemle

Rutgers Center for Lipid Research and Department of Nutritional Sciences,
Rutgers University, New Brunswick, NJ

The perilipins are a family of proteins encoded by 5 genes in chordates. Perilipins are abundant structural proteins associated with lipid droplets in the majority of cells and tissues. A growing body of evidence shows that perilipins serve as organizing scaffolds at the surfaces of lipid droplets to coordinate the activity of lipases and other factors. The expression of perilipin 1 is limited to adipocytes and steroidogenic cells, where this perilipin serves a critical function in the control of lipolysis. Under basal conditions, perilipin 1 restricts the access of cytosolic lipases to lipid droplets, thus promoting the storage of triacylglycerols in lipid droplets. Following binding of catecholamines to cell surface receptors of adipocytes, phosphorylation of perilipin 1 by protein kinase A promotes lipolysis through multiple mechanisms including facilitating recruitment and binding of hormone-sensitive lipase to lipid droplets, release of CGI-58 (a co-activator of adipose triglyceride lipase, ATGL) from the perilipin scaffold, and fragmentation and scattering of lipid micro-droplets. Perilipin 5 is highly expressed in oxidative tissues including heart and skeletal muscle. Perilipin 5 binds both CGI-58 and ATGL at lipid droplet surfaces, and serves as a negative regulator of lipolysis under basal conditions. Perilipin 5 is phosphorylated by PKA leading to increased lipolysis through an as yet unknown mechanism.



Xiaoli Chen

Xiaoli Chen carried out her graduate research on the regulation of adipocyte differentiation and development at the University of Georgia, Athens, USA and received her PhD in 1998. Following her PhD study, she undertook postdoctoral research in the field of glucose metabolism, insulin resistance, and type 2 diabetes in the Diabetes Branch at NIDDK/NIH in Bethesda. During her postdoctoral training at NIH, Dr. Chen became interested in adipose tissue secretory function; she applied high-throughput proteomics and genomic approaches to identify adipose-derived molecular mediators of insulin resistance. Her research led to the identification of novel adipocyte-secreted proteome dysregulated in obesity and insulin resistance. In 2005, she started her faculty career in the Department of Food Science and Nutrition at the University of Minnesota. Her current research interests focus on adipocyte biology, adipose tissue inflammation and dysfunction in obesity, and obesity-linked insulin resistance and type 2 diabetes. She is currently investigating the functions of lipocalin 2 as a new adipokine in the regulation of energy and lipid metabolism.

Lipocalin 2 as an Important Regulator of Energy Metabolism

Hong Guo, Daozhong Jin, Yuanyuan Zhang, Wendy Hahn, David Brockman, Bazuine M, Jennifer Hannaford, So Young Bu, Douglas G. Mashek, David A. Bernlohr, Xiaoli Chen*

Department of Food Science and Nutrition, University of Minnesota-Twin Cities, Minneapolis-St. Paul, Minnesota, 55108, USA.

Using high-throughput proteomics and microarray analyses, we previously identified lipocalin 2 (LCN2) as an adipose-derived cytokine – the expression of which is up-regulated in adipose tissue in obese rodents. LCN2 belongs to the lipocalin subfamily of low molecular mass secreted proteins that bind small hydrophobic molecules. We recently characterized the role of *Lcn2* in energy and metabolic homeostasis. When fed HFD, *Lcn2*^{-/-} mice gain more body fat mass and develop more severe fatty liver disease than do wild-type (WT) mice. Our data show that HFD induces more significant elevation of fasting blood glucose and insulin levels and reduction in adiponectin levels in *Lcn2*^{-/-} mice than that in WT mice. Additionally, *Lcn2*^{-/-} mice on RCD have significantly higher unfed-state levels of blood triglycerides (TGs), cholesterol, and free fatty acids (FFAs). In comparison with WT controls, *Lcn2*^{-/-} mice have a significantly lower basal body temperature under thermal stress and display cold intolerance. When acutely exposed to 4°C for 5 h, *Lcn2*^{-/-} mice develop severe hypothermia and hypoglycemia, but develop significantly higher levels of blood FFAs and lactate. Our results suggest that *Lcn2* deficiency impairs brown adipose tissue (BAT) thermogenic function and lipid homeostasis. These metabolic defects are completely rescued by TZD treatment. Rosiglitazone (Rosi) greatly improves HFD-induced insulin resistance in *Lcn2*^{-/-} mice; unfed-state levels of blood glucose, FFAs, and TGs are significantly reduced after a 25-d treatment of Rosi in *Lcn2*^{-/-} mice. Interestingly, *Lcn2* deficiency alters Rosi action on fat deposition and lipid homeostasis. Rosi-induced body weight and subcutaneous fat gain, liver lipid accumulation, and *de novo* lipogenesis in white adipose tissue are markedly lessened in *Lcn2*^{-/-} mice. In BAT, the expression of the genes functioning in TG hydrolysis and mitochondrial oxidation is highly up-regulated by Rosi in *Lcn2*^{-/-}, but not in WT mice. Most intriguingly, Rosi stimulates significantly higher levels of uncoupling protein-1 expression in BAT, and completely reverses BAT thermogenic dysfunction in *Lcn2*^{-/-} mice. It appears that increased BAT activity is the main mechanism by which TZD corrects insulin resistance and dyslipidemia in *Lcn2*^{-/-} mice. We conclude that LCN2 has a critical role in regulation of BAT activity and function, energy expenditure, and lipid homeostasis.

Grant Support: NIDDK (R01DK080743)



Rosalind A. Coleman

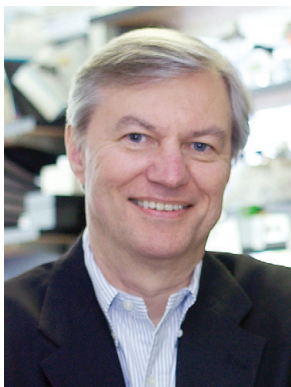
Rosalind Coleman received a BA *cum laude* from Harvard University and an MD from Case Western Reserve University. After completing residency training in pediatrics at New York Hospital-Cornell Medical Center and Duke University, she trained in endocrinology and metabolism and did a postdoctoral fellowship in biochemistry at Duke University with Robert M. Bell. As a Duke University faculty member, she diagnosed and treated children with inborn error of metabolism while continuing her study of enzymes involved in triacylglycerol synthesis and their regulation. Currently in the Department of Nutrition at the University of North Carolina, her research centers on triacylglycerol metabolism in whole animal studies designed to understand the development of insulin resistance, diabetes, and cancer.

Lipid metabolism: location, location, location

Rosalind A. Coleman*, Jessica M. Ellis, Trisha Grevengoed, Lei O. Li, and Chongben Zhang

Department of Nutrition, University of North Carolina, Chapel Hill, NC, USA, 27599

The link between obesity and insulin resistance has prompted consideration of diverse pathways that alter glucose metabolism and insulin signaling. To understand how this might occur mechanistically, we have studied the enzymes that initiate the synthesis of triacylglycerol, acyl-CoA synthetase (ACSL) and glycerol-3-phosphate acyltransferase (GPAT), each of which is represented by multiple isoforms in different locations. In liver, the functional phenotype resulting from the deficiency of ACSL1 is minimal, but in heart, muscle and adipose, the deficiency of ACSL1 abrogates fatty acid oxidation. The metabolic consequences of tissue reliance on glucose for energy production include activation of mTOR as well as the “starvation response,” whereas altering GPAT1 activity to increase or diminish the flux of intermediates through this pathway can also interfere with mTOR and insulin signaling. The role of glycerolipid intermediates as causative in these effects will be discussed. (Supported by NIH DK59935 and DK56598)



Michael P. Czech

Michael P. Czech is the Isadore and Fannie Foxman Professor and founding Chair of the Program in Molecular Medicine, which includes 40 faculty research laboratories, several Howard Hughes Investigators and about 400 scientists and staff at the University of Massachusetts Medical School. Dr. Czech's laboratory group addresses mechanisms of insulin signal transduction, metabolic regulation and insulin resistance in type 2 diabetes and obesity. His laboratory has also recently applied RNAi technologies to discover novel drug targets and to develop therapeutic strategies for alleviating inflammation and metabolic diseases. Dr. Czech has authored approximately 275 publications, serves on several editorial boards, and has served on NIH Study Sections and Review Panels of the Howard Hughes Medical Institute. He has received the Outstanding Scientific Achievement Award of the American Diabetes Association, 1982; the David Rumbough Scientific Award of the Juvenile Diabetes Foundation, 1985; an NIH MERIT Award, 1997-2005; the Elliot P. Joslin Medal, 1998; the 2000 Banting Medal and 2004 Albert Renold Award of the American Diabetes Association, and the Jacobaeus Prize presented in Umea Sweden, 2009.

Metabolic Control by Lipid Droplet Proteins

Michael P. Czech*, Emile Boutet, Kimberly Negrin and Sarah M. Nicoloso
Program in Molecular Medicine, University of Massachusetts Medical School,
Worcester, MA 01605 USA

Regulation of insulin sensitivity in several key human tissues including adipose tissue, skeletal muscle and liver is important to maintain glucose and lipid homeostasis and to reduce the risk of diabetes and cardiovascular disease. Many studies have suggested that a high capacity of adipose tissue to catalyze esterification of fatty acids into triglyceride and to store neutral lipid in lipid droplets is a key element in promoting normal insulin sensitivity in muscle and liver. Through RNAi-based screens for genes that control adipose tissue lipogenesis, we discovered that the CIDE domain-containing Fat Specific Protein (FSP)27 is a positive regulator of triglyceride deposition. Expression of FSP27 in a variety of cultured cell types revealed the FSP27 protein localizes to the surface of lipid droplets and markedly increases the size of such droplets when cells are incubated with fatty acids. FSP27 silencing in 3T3-L1 adipocytes causes increased basal lipolysis and decreases the size of lipid droplets. Moreover, a human subject with a mutation in the human homolog of FSP27, CIDEA, displayed severe lipodystrophy, insulin resistance and diabetes. Further experiments indicated that the closely related protein CIDEA also serves as a lipid droplet protein in both white and brown adipose tissue. In more recent work, we demonstrated that the lipolytic actions of the cytokines TNF- α , IL-1 β and interferon as well as the lipolytic effect of hypoxia are in part mediated by their ability to inhibit expression of FSP27 in adipocytes. Taken together, these and other data suggest the hypothesis that the ability of adipocytes to store and sequester neutral lipid may be highly dependent upon the expression levels of the CIDE proteins. Support for this hypothesis has been obtained from studies from adipose tissues of obese mice compared to lean mice as well as from human subjects, matched for BMI, that show a inverse correlation between lipid droplet protein expression and whole body insulin resistance. Our studies reveal that this novel class of lipid droplet proteins, the CIDE proteins, may play a critical role in regulating the sequestration of neutral lipid within adipocytes and that dysfunctions in their expression or actions can disrupt glucose homeostasis in humans.



Richard J. Deckelbaum

Dr. Richard J. Deckelbaum is the Robert R. Williams Professor of Nutrition, Professor of Pediatrics and Professor of Epidemiology at Columbia University, where he serves as Director of the Institute of Human Nutrition. Dr. Deckelbaum's primary research interests concern human plasma lipoproteins and the cellular effects of dietary fats and free fatty acids on gene expression. Recent studies have emphasized potential basic mechanisms that underlie the ability of omega-3 fatty acids to decrease cardiovascular risk, both coronary artery disease and stroke.

Author of over 300 scientific articles, reviews, and chapters, Dr. Deckelbaum has served on numerous national task forces, as well as review boards for nutrition and clinical research. He has chaired a national conference on Preventive Nutrition: Pediatrics to Geriatrics and served as a member of the United States Department of Agriculture and Health and Human Services Advisory Committee for Dietary Guidelines for the year 2000. A recent honor includes his appointment to the Food and Nutrition Board of the Institute of Medicine, National Academies of Science. Currently, he continues in projects related to health and science as a bridge between different populations in the Mideast, Africa, and Asia.

Omega-3 fatty acids: Preventive and therapeutic molecules for liver, heart, and brain

Richard J. Deckelbaum, MD, Institute of Human Nutrition, Columbia University Medical Center, New York, NY

While omega-3 (n-3) -rich triglycerides have long been available from natural plant and living sources, the past 3 decades have seen substantial advances in their application towards therapeutics and health promotion. Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids are the major bioactive n-3 fatty acids and have distinct roles in cell biology and gene expression, which contribute to key roles in human health. n-3 fatty acids affect growth and development, inflammatory conditions, neurological degeneration, and chronic diseases such as atherosclerosis and cancer. n-3 fatty acids have recently been associated with improvements in TPN related liver disease and non-alcoholic fatty liver disease. What is their role in cardiovascular disease? Higher dietary intakes of n-3 fatty acids are associated with decreased risk of coronary heart disease. Using mouse models our laboratory has studied pathways that contribute to an understanding of these beneficial effects in humans. We have uncovered “novel” mechanisms whereby n-3 fatty acids decrease cholesterol delivery to the arterial wall to protect against atherosclerosis and coronary heart disease. In rodent stroke models acute administration of n-3 fatty acids after cerebral hypoxic-ischemic (HI) injury provides strong mechanistic evidence for a promising therapeutic modality for decreasing brain death after stroke. Our studies indicate that brain injury scores can be decreased up to 5-fold after HI injury and that this is associated with protection against neuronal death and brain edema. n-3 triglyceride emulsion administration leads to increases in brain docosanoids, particularly NPD1, and protection against adverse changes in mitochondrial function, e.g., production of reactive oxygen species (ROS). Thus, increasing chronic dietary n-3 intake will prove protective for certain forms of liver disease and coronary heart disease; and acute administration of n-3 fatty acid is protective against stroke induced brain infarction in animal models - and potentially in humans.



Agnieszka Dobrzyn

Agnieszka Dobrzyn is an associate professor and a head of the Laboratory of Cell Signaling and Metabolic Disorders at the Nencki Institute of Experimental Biology in Warsaw, Poland. She received her PhD in 2001 for work performed in the lab of Jan Gorski at the Medical University of Bialystok, Poland. In 2002, funded by a grant of the American Heart Association she moved to the Biochemistry Department at the University of Wisconsin-Madison to do a postdoc in the lab of James Ntambi. She returned to Poland in 2006 where she received D.Sc. (habilitation). In 2007, she became a group leader at the Nencki Institute. She is a member of the European Molecular Biology Organization (EMBO) Young Investigator Programme since 2008. Last year she was successful in the TEAM program funded with EU Structural Funds. Her research interest is focused on signalling and transcriptional cascades that have far-reaching implications on lipid metabolism and metabolic diseases, i.e. diabetes and obesity-related heart dysfunctions.

Unsaturated Fatty Acids and Regulation of Cardiac Energy Metabolism

Agnieszka Dobrzyn

Nencki Institute of Experimental Biology, Polish Academy of Sciences, 02-093
Warsaw, Poland

Cardiac substrate metabolism plays a fundamental role in the control of the proper function of the heart. Under resting conditions, the heart derives about 70% of its energy from the oxidation of lipids and about 30% of its energy from glycolysis and glucose oxidation. Impaired substrate metabolism contributes to contractile dysfunction and to the progressive left ventricular remodeling characteristic of heart failure. The elevated use of fatty acids (FA) in the heart has been implicated in a number of metabolic, morphological, and mechanical changes and, more recently, in "lipotoxicity". There is evidence that suggests that stearoyl-CoA desaturase (SCD), a rate-limiting enzyme in the biosynthesis of monounsaturated FA, is an important regulating enzyme in the regulation of cardiac substrate utilization and function. The lack of SCD1 expression decreases FA uptake and oxidation and increases glucose transport and oxidation in the heart. Disruption of the SCD1 gene improves the cardiac function in obese leptin-deficient *ob/ob* mice by correcting the systolic and diastolic dysfunction without affecting the plasma triglyceride (TG) and non-esterified fatty acid (NEFA) levels. The improvement is associated with the reduced expression of the genes involved in FA transport and lipid synthesis within the heart, as well as the reduction of the cardiac NEFA, diacylglycerol, TG, and ceramide levels. The rate of FA β -oxidation is significantly lower in the hearts of *ob/ob*;SCD1^{-/-} mice when compared to the *ob/ob* controls. The reduction in myocardial lipid accumulation and inhibition of apoptosis appear to be important factors in the improved left ventricle function in *ob/ob* mice caused by SCD1 deficiency. These data suggest that overexpression of cardiac SCD contributes to dysregulation of lipid and carbohydrate metabolism in the heart. The data also point to endogenous oleic acid as a potential regulator in cardiac substrate utilization. We tested whether energy metabolism of the heart is affected by oleic acid originating from both the endogenous conversion of stearate and the diet. Cardiac SCD1 mRNA and protein are increased in tristearate (TS)-fed rats when compared with chow-fed rats. Overexpression of SCD1 is associated with a significant elevation of oleate and TG levels within the heart. Increased oleate and TG contents are also observed in the hearts of trioleate (TO)-fed rats, although SCD1 protein and mRNA levels are decreased in the myocardium of these rats when compared with TS or chow-fed rats. Feeding of TS or TO results in increased oxidation of mitochondrial FA. Additionally, cardiac glucose uptake is decreased by TS or TO feeding. These results suggest that oleic acid (both dietary and *de novo* synthesized) affects cardiac energy metabolism. Furthermore, our data show that endogenous synthesis of oleate in the heart can compensate for the deficiency of this fatty acid in the diet.



Robert H. Eckel

Dr. Robert H. Eckel is the Charles A Boettcher II Endowed Chair in Atherosclerosis, Professor of Medicine with appointments in the Division of Endocrinology, Metabolism and Diabetes and the Division of Cardiology, and Professor of Physiology and Biophysics at the University of Colorado Denver (UCD) and is Director of the Lipid Clinic at the University of Colorado Hospital. He is Director of the Discovery Translation Program of the recently NIH funded Colorado Clinical Translational Sciences Institute. Dr. Eckel was recently a member of the Scientific Advisory Council of the National Institute of Diabetes, Digestive and Kidney Diseases at the National Institutes of Health (NIH) and was President of the American Heart Association in 2005. Dr. Eckel's NIH funded research has focused on the broad area of preventive cardiology in the areas of the pathogenesis and treatment of lipid disorders and obesity. Studies in animals and humans focus on the impact of nutrition/hormones on lipid and carbohydrate metabolism.

In humans, Dr. Eckel has examined the nutritional/metabolic predictors of weight change, lipid disorders and atherosclerosis. In the laboratory, he uses mice with tissue-specific modification of gene expression to address similar issues in more extensive detail. Overall, his research is targeted to favorably modifying the metabolic profile to reduce the risk for heart disease and stroke.

Lipoprotein Lipase in the Brain Regulates Energy Balance and Body Weight.

Hong Wang and Robert H. Eckel*

University of Colorado Anschutz Medical Campus, Aurora CO 80045 USA

LPL is the rate limiting enzyme for the uptake of fatty acids from the hydrolysis of circulating triglyceride (TG)-rich lipoproteins for lipid storage and/or oxidation in peripheral tissues. LPL is also present throughout the nervous system and LPL mRNA is expressed in neurons in the hippocampus, hypothalamus, pyramidal cortex, and the Purkinje cells of the cerebellum in the brain. We have created a mouse with neuron-specific deletion of LPL (NEXLPL^{-/-}) by crossing loxPLPL mice with mice expressing cre recombinase driven by the regulatory sequences of the neuron-specific NEX transcription factor. By 6 months these mice are obese on a standard chow diet and have reduced metabolic rates mostly attributable to a reduction in physical activity. Of great interest, heterozygous mice (NEXLPL^{+/-}) also become obese between 6 and 12 mo with a period of hyperphagia that precedes marked reductions in metabolic rate. Moreover, the later development of obesity in NEXLPL^{+/-} mice was predicted by an earlier increase in food intake during the hyperphagic period. Homozygous NEXLPL^{-/-} mice also show a period of increased food intake but earlier, i.e. between 12 and 20 wk before the onset of obesity as well. In both NEXLPL^{+/-} and NEXLPL^{-/-} mice, reductions in LPL gene expression resulted in substantial increases in AgRP mRNA in the hypothalamus before the onset of obesity. When chylomicrons containing radiolabeled TG were injected into NEXLPL mice, there was a selective defect in the uptake of TG-derived fatty acids in the hypothalamus. Moreover, when the levels of various fatty acids were assessed in the hypothalamus, only long chain polyunsaturated fatty acids (mostly n-3 PUFAs) were deficient. We propose that LPL-dependent deficiency and metabolism of specific PUFAs in the hypothalamus leads to this dramatic up-regulation of AgRP gene expression and obesity development. Of added interest is that despite the presence of severe obesity and much reduced physical activity at 12 mo, NEXLPL^{-/-} mice have better glucose tolerance than littermate controls, marked brown adipose tissue (BAT) hyperplasia/hypertrophy, and increased BAT UCP-1 gene expression. NEXLPL mice also demonstrate selective and persistent increases in melanocortin-3 receptor gene expression in the hypothalamus. Finally, the Morris Water Maze test has been used to identify a cognitive disturbance in older NEXLPL mice, manifested by reduced learning ability and substantial defects in adaptation to changing the platform position. Overall, this is the first observation that TG-rich lipoproteins and their metabolism regulated by LPL in the brain have physiologic relevance. We hope and intend to uncover mechanisms of how TG-rich lipoprotein sensing and metabolism in the brain impact insulin action, energy balance and body weight regulation.



Robert V. Farese, Jr.

Dr. Farese is a Senior Scientist at the J. Gladstone Institutes and a Professor of Medicine and of Biochemistry & Biophysics at the University of California, San Francisco (UCSF). He studied chemistry at the University of Florida and medicine at Vanderbilt University, the University of Colorado, and UCSF. His research training was in murine genetic models of cholesterol metabolism with Dr. Stephen Young at the J. David Gladstone Institutes, and he has been leading a research group at Gladstone since 1994. Dr. Farese's areas of scientific expertise include the cell biology and physiology of lipid and energy metabolism. His group studies how fats, such as triglycerides, are synthesized and stored in cells, focusing on the enzymes of neutral lipid synthesis and lipid droplets. This work has had therapeutic implications for obesity, diabetes, and atherosclerosis. Recently, his group has also worked on neurodegeneration, providing insights into the basic mechanisms of frontotemporal dementia.

How Cells Fatten Up: Fat Synthesis and Lipid Droplets

Robert V. Farese, Jr*

University of California-San Francisco, Gladstone Institute of Cardiovascular Disease, 1650 Owens Street, San Francisco, CA 94158

Lipids play a crucial and tightly regulated cellular role as the major component of membranes. They are also critical for both cellular and physiological energy homeostasis. Pools of both membrane precursor lipids and energy storage lipids are stored in the lipid droplet, a poorly characterized organelle that has recently been shown to be highly dynamic and involved in many cellular processes. Our lab studies the synthesis of energy storage lipids as well as their storage in and mobilization from lipid droplets. Studies on genetic factors controlling lipid droplet biology, lipid droplet formation, and the physiological functions of lipid droplet storage will be presented.



Christopher Glass

Following undergraduate studies in Biophysics at the University of California, Berkeley, Dr. Glass obtained a PhD in Biology and an MD degree at the University of California, San Diego. He went on to clinical training in Internal Medicine at the Brigham and Women's Hospital of Harvard Medical School before returning to UCSD for clinical subspecialty and research training in Endocrinology and Metabolism. Dr. Glass is currently Professor of Cellular and Molecular Medicine and Professor of Medicine at the University of California San Diego School of Medicine. He has had a long-standing interest in understanding how sequence specific transcription factors, co-activators and co-repressors regulate the development and function of macrophages. His recent work has focused on using genome-wide approaches to understand how lipid-regulated nuclear receptors negatively regulate signaling pathways that mediate innate and adaptive immune responses and underlie the pathogenesis of chronic inflammatory diseases.

Sterol/Oxysterol Regulation of Macrophage Gene Expression

Christopher Glass

Department of Cellular & Molecular Medicine, University of California, San Diego

Inflammation and macrophage foam cells are characteristic features of atherosclerotic lesions, but the mechanisms linking cholesterol accumulation to inflammation are poorly understood. To investigate this relationship, the LIPID MAPS consortium performed global lipidomic and transcriptomic analysis of peritoneal macrophages in WT and LDL receptor-deficient mice fed either a normal or a high fat/high cholesterol diet. These genetic and dietary interventions dramatically altered the macrophage lipidome and unexpectedly resulted in a de-activated, rather than an activated inflammatory phenotype. Several lines of evidence suggest that desmosterol plays a central role in integrating the homeostatic response of peritoneal macrophages to foam cell formation, acting to inhibit SREBP-target genes that drive cholesterol biosynthesis and simultaneously promoting LXR-dependent cholesterol efflux and anti-inflammatory activities. Desmosterol further orchestrates a metabolic adaptation resulting in suppression of most endogenous fatty acid biosynthesis but enhanced production of oleic acid, the primary fatty acid used for cholesterol esterification, and 9Z-palmitoleic acid, a putative lipokine that is demonstrated to exert anti-inflammatory effects. Intriguingly, genome-wide location analysis of LXRs indicates that inflammatory signaling through TLR4 results in the near complete removal of LXRs from its response elements. Together these findings identify integrative functions of desmosterol that suggest new strategies for therapeutic intervention, and imply that exogenous inflammatory mediators contribute to macrophage activation and foam cell formation in atherosclerotic lesions.



Ira J. Goldberg

Dr. Goldberg graduated from MIT, received his medical degree from Harvard Medical School, and served his internship and residency in medicine at New York University-Bellevue Hospital Medical Center. He subsequently completed fellowships in endocrinology and metabolism and atherosclerosis and metabolism, at Mount Sinai School of Medicine in New York City. He was appointed to the faculty of the Department of Medicine at Columbia University in 1983 and is currently Chief of the Division of Preventive Medicine and Nutrition and the Dickinson Richards Professor of Medicine.

Dr. Goldberg has published over 145 articles in peer-reviewed journals. In addition, he has written numerous book chapters, editorials, and reviews. Dr. Goldberg's research has focused on abnormalities of lipoprotein metabolism, macrovascular disease in diabetes, and the role of triglycerides in atherosclerosis. He has received grant support in a number of investigational studies that involve atherogenicity of apolipoprotein B-containing lipoproteins, regulation of plasma triglyceride by lipase enzymes, diabetic macrovascular disease, and lipid uptake and toxicity in the heart.

Dr. Goldberg is the director of the Center for Heart Disease Prevention, Columbia's lipid disorders clinic. He has participated in a number of clinical trials of lipid-lowering medications. In addition, he has consulted on drug development for diabetes and lipid disorders with numerous pharmaceutical and biotech companies.

Among Dr. Goldberg's honors is a MERIT Award from the National Heart, Lung, and Blood Institute. He was chosen to give the 2007, R. Levy Lecture of the NPAM Committee of the American Heart Association and the E. Bierman Lecture on diabetes and heart disease at the 2010 American Diabetes Associated Meeting. In 2008 he was the C. Kilo visiting professor at Washington University. He has previously served as chair of the NIH Metabolism and CADO (cellular aspects of diabetes and obesity) study sections.

Correcting Cardiac Lipotoxicity

Ira J. Goldberg

Columbia University College of Physicians & Surgeons, New York, NY

Excess accumulation of lipids in tissues is thought to be the cause of several human diseases including type 2 diabetes, non-alcoholic liver disease, and some forms of cardiomyopathy. This latter disease has been mimicked in mice by transgenic expression of genes that lead to excess accumulation of lipid within cardiomyocytes. In an attempt to understand the etiology of cardiac lipotoxicity we have used genetic and dietary methods to alleviate lipotoxic cardiomyopathy. These include the following: reduction of lipid uptake in heart PPARalpha overexpressing mice, reduction of ceramide biosynthesis by treatment with myriocin, reduction of ceramide and diacylglycerol (DAG) by transgenic expression of diacylglycerol acyl transferase 1 (DGAT1), and treatment of mice with omega 3 fatty acid-rich diets. In several of the experiments improved heart function and survival occurred without reduced heart triglyceride content or fatty acid oxidation. This occurred with overexpression of DGAT1 and fish oil treatment of cardiomyocyte acylCoA synthetase (ACS1) transgenic mice. In addition, cross of cardiac PPARgamma transgenic mice with PPAR alpha knockout mice improved heart function and survival without a decrease in either ceramide or DAG. These studies provide insights into causes versus associations of lipids and lipid metabolic pathways with disease.



Frank J. Gonzalez

Frank Gonzalez received a Ph.D. in Oncology from the University of Wisconsin, Madison. Following postdoctoral study, he joined the National Cancer Institute where he is Chief of the Laboratory of Metabolism in the Center for Cancer Research. His research is focused on investigating the role of xenobiotic-metabolizing enzymes and xenobiotic receptors in chemical toxicity and carcinogenesis.

Investigating the Roles of Lipids in Disease and Chemical Toxicities using Metabolomics

Frank J. Gonzalez*, Andrew D. Patterson, Kristopher W. Krausz, Caroline H. Johnson, and Jeffrey R. Idle, Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, Bethesda, Maryland 20892, USA

The metabolome, first defined in 1998 as “the set of metabolites synthesized by a biological system”, encompasses all the small metabolites present in a particular biofluid (urine, blood, sebum, cerebral spinal fluid, saliva), cell, or tissue. As the ultimate downstream products of genomic, transcriptomic, and/or proteomic perturbations, changes in metabolite concentration and/or flux can reveal biologically relevant perturbations to the system. Metabolomics can provide novel, relevant, and translatable perspectives of various diseases and yield insights into their mechanisms of pathogenesis and progression. Biomarkers for drug efficacy and toxicity can be uncovered that reveal mechanistic clues to cellular toxicity. There are a number of analytical platforms used for metabolomics including NMR spectroscopy and mass spectrometry coupled to multivariate data analysis. No single platform can capture the whole metabolome due to the different physical properties of metabolites, thus the sample type and chemical constituents to be measured determines which system is optimal. By use of ultra-performance liquid chromatography and gas chromatography as the front ends for orthogonal quadrupole time-of-flight mass spectrometers, cell and tissue extracts, serum, and urine can be probed to discover and develop chemical fingerprints left from various biological processes that result from diseases and chemical insults. The biomarkers found are then characterized and validated to authentic standards by tandem mass spectrometry, and quantitated by triple quadrupole mass spectrometry (multiple reaction monitoring). In some cases, biomarkers can provide mechanistic insights into disease pathogenesis, where an enrichment or depletion of a certain metabolite or a group of metabolites indicates perturbed metabolic processes pointing to alterations at the genomic, epigenomic, transcriptomic, and/or proteomic levels. Indeed, the metabolome might be the most accurate indicator of cellular physiology. In the NCI, metabolomics is being used to identify biomarkers for diseases such as diabetes, alcoholic-induced steatosis and cirrhosis, and cancer, using mouse and monkey models, and samples taken from normal and diseased human patients. Examples will be presented where fluxes in lipid metabolites were discovered from studies of induced diseases in wild-type and genetically-altered mouse model systems, and in experimental drug overdosing. Metabolomics data has revealed altered pathways involving SLC and mitochondrial transporters, and lipid-metabolizing enzymes that contribute to disease pathogenesis.



Daniel S. Greenspan

Daniel Greenspan received his B.A., M.S. and Ph.D. degrees at New York University, with graduate studies that focused on the SV40 large T antigen. He did postdoctoral studies, that included work on on RNA splicing with Sherman Weissman in the Department of Genetics, Yale University School of Medicine. In 1986, he joined the faculty of the Department of Pathology and Laboratory Medicine, and is presently professor and interim chair of the Department of Cell and Regenerative Biology, at the School of Medicine and Public Health, University of Wisconsin-Madison. The Greenspan lab has focused on extracellular modulators of cellular behaviors in development, homeostasis, and disease. Some of this work included roles in isolating and characterizing the loci for the human genetic disorders dystrophic epidermolysis bullosa and classic Ehlers-Danlos syndrome. Much work in the lab currently focuses on extracellular regulatory proteins that control and orchestrate events as diverse as growth factor signaling and formation of the extracellular matrix (ECM), events critical to adult vertebrate homeostasis and to morphogenetic processes. Current studies in the lab also focus on roles of extracellular proteins in adipocyte biology, diabetic symptoms, atherosclerosis, and organ transplant rejection. The latter two studies have included translational studies of human patients.

$\alpha 3(V)$ Collagen Ablation in Mice Induces Diabetes-related Symptoms via Effects on Pancreatic Islets and Peripheral Tissues

Guorui Huang,¹ Gaoxiang Ge,¹ Dingyan Wang,² Bagavathi Gopalakrishnan,¹ Delana H. Butz,^{1,3} Ricki J. Colman,⁴ Andras Nagy,² and Daniel S. Greenspan^{1,3*}

¹Department of Cell and Regenerative Biology, University of Wisconsin, Madison, Wisconsin, USA. ²Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada. ³Program in Molecular and Cellular Pharmacology, ⁴Wisconsin National Primate Research Center, University of Wisconsin, Madison, Wisconsin, USA.

Collagen V, broadly expressed as $\alpha 1(V)_2\alpha 2(V)$ heterotrimers that regulate collagen fibril geometry and strength, also occurs as poorly characterized $\alpha 1(V)\alpha 2(V)\alpha 3(V)$ heterotrimers with limited tissue distribution. We have demonstrated $\alpha 3(V)$ chain expression in white adipose tissue (WAT), skeletal muscle, and pancreatic islets, and show that ablation of the $\alpha 3(V)$ gene *Col5a3* yields mice with deficits in functionality of all three tissues. Such mice are viable, but have WAT abnormalities and are glucose-intolerant, insulin-resistant, and hyperglycemic; with near-diabetic fasting blood glucose levels at one year of age. *Col5a3*^{-/-} mice also have decreased numbers of pancreatic islets, with residual β -cells that have decreased function and increased susceptibility to apoptosis in response to stress and aging. Moreover, *Col5a3*^{-/-} WAT and skeletal muscle are defective in glucose uptake and mobilization of intracellular GLUT4 glucose transporter to the plasma membrane in response to insulin. Our results underscore the emerging view of the importance of extracellular matrix to the microenvironments that inform proper development/functioning of specialized cells, such as adipocytes, β -cells, and skeletal muscle.



Jay D. Horton

Dr. Jay D. Horton is a Professor of Internal Medicine and Molecular Genetics and Robert C. and Veronica Atkins Chair in Obesity and Diabetes Research at UT Southwestern Medical Center. He obtained his B.S. and M.D. degrees from the University of Iowa and completed his internal medicine residency, gastroenterology fellowship, and Howard Hughes post-doctoral fellowship at UT Southwestern Medical Center. Dr. Horton is a former PEW scholar and member of the American Society for Clinical Investigation and Association of American Physicians. In clinical digestive diseases, Dr. Horton has an interest in conditions that lead to hepatic steatosis and obesity. Currently a major focus of the laboratory is to determine how transcriptional regulators of fat metabolism contribute to the development of hepatic steatosis. A second area of investigation centers on determining the function of PCSK9, a protein that is involved in determining plasma LDL cholesterol levels through its ability to post-transcriptionally regulate the expression of LDL receptors in liver.

Molecular Mediators of Hepatic Steatosis

Chai-Wan Kim^{b,1}, Christopher Colbert^{a,1}, Young-Ah Moon^b, Lisa Henry^{a,c}, Maya Palnitkar^{a,c}, William B. McKean^b, Kevin Fitzgerald^e, Johann Deisenhofer^{a,c,2}, Hyock Joo Kwon^a, Jay D. Horton^{b,d,2*}

Department of ^aBiochemistry and ^bMolecular Genetics, ^cHoward Hughes Medical Institute, and Department of ^dInternal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390-9046, ^eAlnylam Pharmaceuticals, 300 Third Street, Cambridge, MA 02142.

Hepatic steatosis that develops in obesity and insulin resistant states is associated with elevated rates of lipogenesis. Lipogenesis in liver is regulated in part by SREBP-1c, a transcription factor that activates all genes involved in fatty acid synthesis. The first committed enzyme in fatty acid synthesis, acetyl-CoA carboxylase (ACC), is also regulated by phosphorylation/dephosphorylation, and an unusual mechanism of protein polymerization. ACC polymerization is induced in vitro by supraphysiological concentrations of citrate (>5 mM), which increases enzymatic activity. Previously, we showed that MIG12, a 22 kDa cytosolic protein of previously unknown function, binds to ACC and lowers the threshold for citrate activation into the physiological range (<1 mM). In vitro, recombinant MIG12 induced recombinant ACC polymerization and increased ACC activity by >50-fold. In vivo, adenoviral overexpression of MIG12 induced ACC polymerization, increased fatty acid synthesis, and produced triglyceride accumulation and fatty liver. A second protein, Spot14 (S14) shares significant homology with MIG12, and is also highly expressed in liver and regulated by SREBP-1c, a transcription factor that activates all genes involved in fatty acid synthesis. The mechanism for S14's ability to modulate lipogenesis has not been elucidated. In a first step in further defining the function and mechanism of S14's action, we determined the crystal structure of S14 to 2.65 Å. In vitro biochemical studies and in vivo studies show that S14 can form a heterodimer with MIG12. Co-expression of S14 and MIG12 in CHO-K1 cells leads to heterodimer formation and reduced ACC polymerization and activity. Conversely, reducing S14 levels in rat primary hepatocytes using siRNA increased ACC polymerization and activity. Therefore, S14 appears to regulate rates of fatty acid synthesis by modulating ACC activity through heterodimer formation with MIG12.



Barbara B. Kahn

Barbara B. Kahn, MD, is Chief of the Division of Endocrinology, Diabetes, and Metabolism at Beth Israel Deaconess Medical Center (BIDMC) and the George Richards Minot Professor of Medicine at Harvard Medical School.

Dr. Kahn is an internationally recognized scientist in the area of obesity and type 2 diabetes. Her lab investigates the molecular mechanisms underlying these conditions, including the regulation of insulin action, food intake, and energy balance. She was one of the first investigators to establish the role of the adipocyte as an endocrine organ. She continues to identify novel adipocyte-associated molecules that regulate systemic insulin sensitivity. She also made the seminal discovery that the AMP-kinase pathway is critical for leptin action on metabolism in peripheral tissues and on regulation of food intake and body weight via hypothalamic neural circuits.

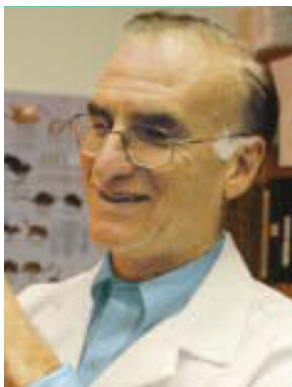
Dr. Kahn received her BA and MD degrees from Stanford University and an MS from the University of California at Berkeley. After completing internal medicine training at the UC Davis Medical Center, she began her career in molecular research at the National Institutes of Health. She joined the Harvard Medical School faculty in 1986. Dr. Kahn has received numerous awards including the Outstanding Scientific Achievement Award and the Mosenthal Award from the American Diabetes Association; the Jacobaeus Prize from the Novo Nordisk Foundation and the Karolinska Institute; the Charles H. Best Award from the University of Toronto; the Gerald D. Aurbach Award from the Endocrine Society and a Fellowship from the Radcliffe Institute for Advanced Studies. Dr. Kahn was elected to the Institute of Medicine of the National Academy of Sciences and is a fellow of the American Association for the Advancement of Science.

Adipose Carbohydrate Response Element Binding Protein, Lipogenesis, and Insulin Sensitivity

Mark A. Herman, Odile D. Peroni, Jorge Villoria, Michael R. Schön, Nada A. Abumrad, Matthias Blüher, Samuel Klein, and Barbara B. Kahn

Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center and Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA; Departments of Surgery and Medicine, University of Leipzig, Leipzig, Germany; Center for Human Nutrition, Washington University School of Medicine, St. Louis, Missouri, USA.

The adipose cell functions as an endocrine organ and as a regulator of substrate availability. It secretes hormones, cytokines and other factors that influence energy balance, glucose homeostasis, insulin sensitivity and vascular biology through actions in the central nervous system and peripheral tissues. In obesity and type 2 diabetes, expression of the Glut4 glucose transporter is down-regulated selectively in adipose tissue and increasing evidence indicates this down-regulation has systemic effects by altering adipocyte biology. Adipose-Glut4 knockout mice are insulin-resistant and have an increased risk of diabetes while adipose-Glut4 overexpressing mice have enhanced glucose tolerance and insulin sensitivity in spite of increased adiposity. To discover novel pathways that regulate glucose homeostasis and adiposity, we used gene set enrichment analyses of gene arrays of adipose tissue from mice with adipose-specific overexpression or knockout of Glut4. In adipose-Glut4 overexpressing mice we found coordinate upregulation of lipogenic genes in adipose tissue and determined that this is driven by Carbohydrate responsive-element binding protein (ChREBP). ChREBP is a glucose-responsive transcription factor that regulates fatty acid synthesis and glycolysis in the liver. Its role in adipose tissue is unknown. We found that in rodents and humans, ChREBP expression in adipose tissue is a dominant regulator of lipogenesis and is associated with enhanced insulin sensitivity even in the presence of obesity. Our data underscore the beneficial role of lipogenesis in adipose tissue for maintenance of glucose homeostasis. The ChREBP driven-lipogenic pathway in adipose tissue may provide novel targets for preventing type 2 diabetes.



C. Ronald Kahn

C. Ronald Kahn, M.D. is the preeminent investigator of insulin signal transduction and mechanisms of altered signaling in disease. He received his education at the University of Louisville. After training in internal medicine at Washington University, he moved to the National Institutes of Health where he rose to Head of the Section on Cellular and Molecular Physiology of NIDDK. In 1981, he became Research Director of the Joslin Diabetes Center, and in 1986 the Mary K. Iacocca Professor of Medicine at Harvard Medical School. From 2000-2007, he served as President and Director of the Joslin Diabetes Center.

Dr. Kahn has received numerous honors and awards, including the highest scientific awards of the American Diabetes Association, Juvenile Diabetes Foundation, European Association for the Study of Diabetes, British Endocrine Society, International Diabetes Federation, and Endocrine Society. In 1999, Dr. Kahn was elected to membership in the National Academy of Sciences and the Institute of Medicine.

Insulin, Diabetes and the Control of Brain Cholesterol Metabolism

C. Ronald Kahn, Ryo Suzuki, and Heather Ferris

Joslin Diabetes Center and Harvard Medical School, Boston, MA 02215

Diabetes mellitus is associated with a variety of cerebral complications, including cognitive dysfunction, depression, and increased risk of Alzheimer's disease. The brain is the most cholesterol-rich organ in the body, most of which comes from in situ synthesis. Brain cholesterol turnover is increased in Alzheimer's and other neurodegenerative diseases. In multiple mouse models of diabetes, there is a global reduction in expression of cholesterol synthetic genes and their major transcriptional regulator, sterol regulatory element binding protein 2 (SREBP-2) in the brain. Quantitative PCR showed a coordinated 30-35% reduction in expression of SREBP-2 and cholesterologenic genes in the hypothalamus and cerebral cortex of STZ-, NOD- and *db/db* diabetic mice. In addition, part of the decrease in cholesterologenic genes was due to a 50% reduction in the sterol sensing protein SCAP, which plays a key role in the processing of SREBP-2. Together, these defects resulted in a ~25% reduction in brain cholesterol synthesis as measured using $^3\text{H}_2\text{O}$ and a parallel decrease in synaptosomal membrane cholesterol content. SREBP-2 in the hypothalamus was also down-regulated in brain-specific insulin receptor knockout (NIRKO) mice. In vitro, insulin directly regulated the expression of SREBP-2 and cholesterol synthetic genes in cultured brain neurons and astrocytes. Reduction of SREBP-2 in cultured neurons using shRNA caused a significant decrease in post-synaptic (PSD95) and synaptic vesicle (VAMP2) markers. Furthermore, injection of the SREBP-2 shRNA lentivirus into the hypothalamus of intact mice resulted in increased feeding behavior and weight gain, and a 34% reduction of circulating norepinephrine levels. Homozygous deletion of the *Scap* gene in the central nervous system created by breeding nestin-Cre transgenic mice with *Scap* floxed mice resulted in perinatal lethality with microcephaly, increased staining for glial fibrillary acidic protein (GFAP), and histological evidence of gliosis. Heterozygous (Het) SCAP KO mice showed a 50% reduction in brain SCAP and a 30% reduction in brain cholesterol synthesis, similar to what was seen in STZ-diabetic mice. Brains of these mice also had increased expression of GFAP, whereas expression of synaptophysin expression was decreased in SCAP Het mice suggesting impaired signaling between neurons. Het mice also showed impaired cognitive function in the novel-object recognition test. Metabolically, SCAP Het KO mice had increased *Agrp* expression with a small increase in nocturnal food consumption; however, this was balanced by an increase in activity and VO_2 . Thus, insulin deficient diabetes results in a reduction in cholesterol synthesis in the brain. This leads to cognitive dysfunction and disordered control of eating which may contribute to the pathology observed in insulin deficient diabetic states.



Ronald M. Krauss

Ronald M. Krauss, M.D., is Senior Scientist and Director of Atherosclerosis Research at Children's Hospital Oakland Research Institute, Adjunct Professor in the Department of Medicine at UCSF and in the Department of Nutritional Sciences at UC Berkeley, and Guest Senior Scientist in the Department of Genome Sciences of Lawrence Berkeley National Laboratory. He received his undergraduate and medical degrees from Harvard University with honors and served his internship and residency on the Harvard Medical Service of Boston City Hospital. He then joined the staff of the National Heart, Lung, and Blood Institute in Bethesda, Maryland, first as Clinical Associate and then as Senior Investigator in the Molecular Disease Branch. Dr. Krauss is board-certified in internal medicine, endocrinology and metabolism, and is a member of the American Society for Clinical Investigation, a Fellow of the American Society of Nutrition and the American Heart Association (AHA), and a Distinguished Fellow of the International Atherosclerosis Society. He is a member of the U.S. National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, founder and past Chair of the AHA Council on Nutrition, Physical Activity, and Metabolism, and a National Spokesperson for the AHA. Dr. Krauss has also served on both the Committee on Dietary Recommended Intakes for Macronutrients and the Committee on Biomarkers of Chronic Disease of the Institute of Medicine of the National Academy of Sciences. He has received numerous awards including the AHA Scientific Councils Distinguished Achievement Award and the Centrum Center For Nutrition Science Award of the American Society for Nutrition, and he is listed in *Who's Who in America* and the *World*. Dr. Krauss is on the editorial boards of a number of journals, and is Associate Editor of *Obesity*. Dr. Krauss has published nearly 400 research articles and reviews on genetic, dietary, and drug effects on plasma lipoproteins and coronary artery disease. In recent years Dr. Krauss' work has focused on interactions of genes with dietary and drug treatments that affect metabolic phenotypes and cardiovascular disease risk.

Blood Lipids and Body Fat - Making the Connection

Patty Siri-Tarino, Nastaran Faghihnia, George A. Brooks, Ronald M. Krauss*

Children's Hospital Oakland Research Institute, Oakland, CA 94609 and
University of California, Berkeley 94720 USA

There is a strong relationship between abdominal adiposity and elevated plasma triglycerides as well as other features of atherogenic dyslipidemia. However, while it is known that weight gain can induce plasma triglyceride elevations, it has not been determined whether individuals with increased plasma triglycerides may also be predisposed to excess adiposity. We tested this possibility in 95 overweight but otherwise healthy men who participated in a weight loss study. They consumed a standardized isoenergetic diet for 3 weeks after which a ~9 kg weight loss was induced by caloric deficit for 9 weeks, followed by 4 weeks of weight stabilization. Higher fasting triglyceride at baseline was shown to predict smaller reductions of total body weight ($p=0.01$) and body fat measured by DEXA ($p=0.006$) per prescribed reduction in energy intake. These correlations remained significant after adjusting for physical activity as assessed by pedometry, and insulin sensitivity as assessed by HOMA-IR. To test whether the association of higher plasma triglyceride with relative resistance to body fat loss could be related to alterations in energy metabolism, we measured resting energy expenditure (REE) and respiratory quotient (RQ) by indirect calorimetry before and after weight loss and stabilization. There was no association of REE with baseline plasma triglyceride concentration, and there was no change in REE with weight loss. In contrast, fasting plasma triglyceride concentrations at baseline were positively correlated with RQ ($r=0.23$, $p=0.02$), such that higher triglyceride levels were associated with reduced whole body fat oxidation. This relationship remained significant after weight loss/weight stabilization ($r=0.25$, $p=0.015$) and was stronger (baseline: $r=0.33$, $p=0.04$, end: $r=0.49$, $p=0.002$) in those individuals ($n=40$) with enlarged waist (>40 in) and elevated triglycerides (>130 mg/dL). Moreover, in the group as a whole, there was a correlation between changes in triglyceride and RQ with weight loss ($p=0.05$). In a separate study of 13 men performing a bout of moderate intensity stationary bicycle exercise, fasting plasma triglyceride was strongly correlated with RQ measured during exercise ($r=0.73$, $p=0.005$). These data are consistent with the hypothesis that higher plasma triglyceride is a marker of reduced whole body fatty acid oxidation, and that this may indicate common metabolic alterations underlying both atherogenic dyslipidemia and a tendency for increased adiposity.



M. Daniel Lane

M. Daniel Lane, Distinguished Service Professor of Biological Chemistry at the Johns Hopkins University School of Medicine, received B.S. and M.S. degrees from Iowa State University and a Ph.D. degree from the University of Illinois. He was a Senior NSF Fellow at the Max-Planck Institute Für Zellchemie in Munich (1962-3). Following faculty positions at Virginia Polytechnic Institute and New York University School of Medicine, he joined the faculty at the Johns Hopkins University School of Medicine and later was DeLamar Professor and Director of the Department of Biological Chemistry (1978 to 1997). In 2002 he received an honorary Doctor's degree from Iowa State University, his alma mater. Dr. Lane was elected to membership in the National Academy of Sciences (1987) and elected as a Fellow of the American Academy of Arts and Sciences (1982) and of the American Society of Nutritional Sciences (1996). He received the Mead Johnson Award from the American Society for Nutritional Sciences (1966) and the William C. Rose Award from the American Society for Biochemistry and Molecular Biology (1981). In 1990-1991 Dr. Lane was President of the American Society of Biochemistry and Molecular Biology and served on numerous editorial boards including the Journal of Biological Chemistry and the Annual Reviews of Biochemistry and was Coeditor of the Encyclopedia of Biological Chemistry. Currently he is Associate Editor for Biochemical and Biophysical Research Communications. He has published over 325 research papers. His early work focused on various enzymatic CO₂ fixation reactions, notably the mechanisms by which the B-vitamin, biotin, functions in enzymes to catalyze carboxylation. His research on acetyl-CoA carboxylase, the key regulatory enzyme of fatty acid synthesis, led him to his present interests on the basic mechanisms of lipogenesis, adipogenesis and obesity and its relationship to Type-2 diabetes.

How Glucose and Fructose Alter Hypothalamic Signaling and Feeding Behavior

M. Daniel Lane

Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

In the brain malonyl-CoA serves the important function of monitoring and modulating energy balance. Because of its central role in the metabolism of higher animals, glucose acts as the principal indicator of global energy status. Specialized neuronal nuclei within the hypothalamus sense blood glucose and signal higher brain centers to adjust feeding behavior and energy expenditure accordingly. As the level of glucose entering the brain rises, food intake is suppressed. Energy status information triggered by glucose is transmitted via hypothalamic signaling intermediaries, i.e. AMPK and malonyl-CoA, to the orexigenic/ anorexigenic neuropeptide system that determines hunger and energy expenditure. The central metabolism of glucose by the glycolytic pathway generates ATP which produces a compensatory decrease in AMP level and AMPK activity. Since acetyl-CoA carboxylase (ACC) is a substrate of AMPK, lowering AMP increases the catalytic activity of ACC and thereby, the level of its reaction product, malonyl-CoA. Malonyl-CoA signals the anorexigenic–orexigenic neuropeptide system to suppress food intake. Unlike glucose, however, centrally metabolized fructose increases food intake. This paradox results because fructose bypasses the rate-limiting step of glycolysis and uses a rapid ATP-requiring reaction that abruptly depletes ATP and provokes a compensatory rise in AMP. Thus, fructose has the opposite effect of glucose on the AMPK/malonyl-CoA signaling system and thereby, feeding behavior. The fact that fructose metabolism by the brain increases food intake and obesity risk raises health concerns in view of the large and increasing per capita consumption of high fructose sweeteners, especially by youth.



Chih-Hao Lee

Chih-Hao Lee received his Ph.D. from University of Minnesota. His thesis work focused on molecular mechanisms of gene regulation. As a postdoctoral fellow at the Salk Institute, he investigated metabolic pathways controlled by lipid-sensing nuclear receptors and explored their therapeutic potentials using mouse disease models. In 2004, he joined the faculty of the Department of Genetics and Complex Diseases, Division of Biological Sciences at Harvard University School of Public Health. His current research focuses on the crosstalk between immune and metabolic mechanisms in the pathogenesis of aging and metabolic diseases.

Metabolic Regulation by Immune Signaling

Prerna Bhargava¹, Kristopher J. Stanya¹, Sihao Liu¹, Changlin Li², Donald A. Harn² and Chih-Hao Lee^{1,*}. ¹Department of Genetics and Complex Diseases, Department of Nutrition, Division of Biological Sciences, Harvard School of Public Health, 665 Huntington Ave, Boston, MA 02115, USA. ²Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA.

Metabolic syndrome is becoming a major medical and economic concern worldwide. Over-nutrition in developing and developed countries is causative to the obesity pandemic as well as metabolic stresses, such as oxidative and endoplasmic reticulum (ER) stress, which lead to metabolic dysfunction. Inflammation has also been recognized as a key contributor of metabolic diseases. Using mouse genetic models, studies have shown that several signaling pathways of the innate immunity responsive to pathogens, such as bacteria and virus, are negative regulators of insulin sensitivity. Interventions that shift the immune profile to an anti-inflammatory state are thought to improve metabolic homeostasis through reducing chronic inflammation associated with metabolic dysfunction. However, whether anti-inflammatory mechanisms have direct roles in metabolic regulation is less understood. We find that immunomodulatory molecules, known to drive immune biasing toward alternative activation (e.g., Th2 cytokines and schistosoma egg derived glycans), are able to directly regulate hepatic glucose production and de novo lipogenesis as well as muscle oxidative metabolism. Data supporting potential underlying mechanisms will be presented.



Aldons J. Lusic

Jake Lusic received his Ph.D. in Biophysics from Oregon State University. He was a postdoctoral fellow with Kenneth Paigen in the Molecular Biology Department, Roswell Park Memorial Institute, Buffalo, NY. Since 1979, he has been on the faculty at the University of California, Los Angeles, where he is presently Professor in the Departments of Microbiology and Medicine. He has a joint appointment in the Department of Human Genetics, where he is also Vice Chairman.

He has served on advisory panels for the National Institutes of Health, the American Heart Association, and various private companies and organizations. Presently, he serves on the Board of Scientific Counselors, NHLBI. Among his awards are the Bristol-Myers Squibb Award for Distinguished Achievement in Research, the American Heart Association Duff Award, and the NAVBO Benditt Award.

His research concerns genetic aspects of cardiovascular and metabolic diseases. During the past ten years, his laboratory has focused on a new approach to complex traits, termed, "systems genetics." This involves the integration of genetic variation, high throughput molecular traits (such as global transcript levels), and clinical traits.

Systems Genetics Approaches to Complex Cardiovascular Traits

Brian Bennett¹, Eleazar Eskin², Charles Farber³, Peter Gargalovic⁴, Anatole Ghazalpour¹, Todd Kirchgessner⁴, Margarete Mehrabian¹, Luz Orozco¹, Brian Parks¹, and Aldons J. Lusis^{*1,5,6}

¹Department of Medicine, UCLA, Los Angeles, CA 90034, USA

²Department of Computer Sciences, UCLA, Los Angeles, CA 90034, USA

³Department of Medicine, Cardiovascular Medicine, University of Virginia, Charlottesville, VA 22908, USA

⁴Bristol-Myers Squibb, New York, NY 10154-0037, USA

⁵Department of Microbiology, Immunology and Molecular Genetics, UCLA, Los Angeles, CA 90034, USA

⁶Department of Human Genetics, UCLA, Los Angeles, CA 90034, USA

We have been interested in genetic studies of complex cardiovascular and metabolic traits in mice. Quantitative trait locus (QTL) analyses have been only modestly successful due in large part to the poor mapping resolution. Moreover, since each mouse from a genetic cross is unique, systems based approaches which, in some cases, require substantial biologic samples, are difficult to apply. Also, it is difficult to examine gene-by-environment interactions. Motivated by these problems, we have attempted to develop a mouse resource consisting of permanent inbred strains that can be used for both mapping and systems based approaches. The resource, which we term the Hybrid Mouse Diversity Panel, consists of over 100 different inbred strains that have been selected for both diversity and suitability for association analysis. A key aspect is to control for population structure which we have achieved through the use of an efficient mixed model algorithm.

We have now used the resource for mapping of expression QTL as well as various metabolic traits. In most cases, the mapping resolution is 10- to 100-fold better than that obtained by linkage analysis. The resource has sufficient power to map loci that explain as little as a few percent of the total trait variance. We are presently attempting to develop a form of meta-analysis in which we combine results from genetic crosses with the diversity panel.

At present, we are focusing on and analyzing the traits of atherosclerosis and responsiveness to a high-fat diet. We have also used the resource to integrate across scales, namely transcriptomic, proteomic, and metabolomic data.



Ormond A. MacDougald

Ormond MacDougald received his undergraduate degree from the University of Guelph in Ontario, Canada, in 1986. From Michigan State University he received his master's degree in 1988 and a doctorate from the Department of Physiology in 1992. He pursued postdoctoral training from 1992-96 in the Department of Biological Chemistry at Johns Hopkins University, where he began his studies on adipocyte biology with M. Daniel Lane, Ph.D. MacDougald joined the University of Michigan faculty in 1996 as an assistant professor of physiology and where he currently holds the John A. Faulkner Collegiate Professorship of Physiology.

Role of Wnt Signaling in Adipocyte Differentiation and Metabolism

Hiroyuki Mori, Tyler C. Prestwich, Michael A. Reid, Isabelle Gerin and Ormond A. MacDougald,* Department of Molecular & Integrative Physiology, University of Michigan, Ann Arbor MI 48105

Preadipocytes secrete several Wnt proteins that act through local mechanisms to inhibit adipogenesis. In addition, endogenous negative regulators of Wnt/beta-catenin signaling, including secreted frizzled-related proteins (sFRP), play regulatory roles. sFRP5 expression is strongly induced during adipocyte differentiation *in vitro* and in various models of obesity. Thus, to test a role for sFRP5 in the regulation of adipose tissue biology, we obtained mice that lack functional sFRP5 (*Sfrp5* *Q27stop*). Though *Sfrp5* *-/-* mice do not have a detectable phenotype when fed a normal chow diet, they have lower body weight and decreased fat mass when fed a high fat diet. Morphological analyses indicate that *Sfrp5* *-/-* mice have proportionally fewer large adipocytes than wild type mice. Effects on food intake, metabolic parameters and activity between genotypes were not observed, although a small decrease in fat absorption was found in *Sfrp5* *-/-* mice. Transplantation of adipose tissue from wild type or *Sfrp5* *-/-* mice into obese *db/db* recipient mice revealed that there were fewer large adipocytes in the gonadal white adipose tissue (G-WAT) of *Sfrp5* *-/-* mice, compared to wild type controls, after development of obesity. To understand why *Sfrp5* *-/-* mice are resistant to high fat diet-induced obesity, we performed gene profiling and found that pathways identified as *Oxidative Phosphorylation* and *Mitochondrial Dysfunction* are increased in adipose tissue from *Sfrp5* *-/-* mice. Indeed, mitochondrial enzyme mRNAs are increased in the G-WAT from *Sfrp5* *-/-* mice, as is oxygen consumption rate of whole adipocytes differentiated from *Sfrp5* *-/-* ear Mesenchymal Stem Cells (eMSCs), and of mitochondria isolated from both eMSC and G-WAT adipocytes of *Sfrp5* *-/-* mice. We are currently further evaluating the relationship between sFRP5 and mitochondria function. Our results support the hypothesis that sFRP5 plays a role in regulating adipocyte growth during development of obesity.



Susanne Mandrup

Susanne Mandrup received her PhD in Biochemistry from Odense University (now University of Southern Denmark) in 1992. She worked as a post doc in Department of Molecular Biology, Odense University 1992-1995 and from 1995-1996 In Prof. M. Daniel Lane's group, Department of Biological Chemistry, Johns Hopkins University, Baltimore. In 1996 she was appointed Assistant Professor and in 1999 Associate Professor at Department of Molecular Biology, Odense University. She has been a Professor at Department of Biochemistry and Molecular Biology, University of Southern Denmark since 2008. She is co-founder and member of the executive board of the Danish PhD school of Molecular Metabolism since 2003, member of the Danish Natural Science Research Council (2005 – 2010) and member of The Medical and Natural Science Committee of the Novo Nordisk Foundation since 2009. She is married to Keld B. Hansen and mother of Anders and Charlotte born 1989 and 1992, respectively.

The research in the Mandrup group focuses on understanding the molecular cross-talk between transcriptional regulation and metabolism in mammalian cells in particular in adipocytes and pancreatic beta-cells. Her group combines genome-wide studies of transcription factor binding, epigenetic marks and chromatin structure with detailed molecular analyses of the cross-talk between transcriptional regulators. The group is particularly interested in the function, regulation and mechanism of action of peroxisome proliferator activated receptors (PPARs) and in the transcriptional network controlling adipocyte differentiation. Finally, the Mandrup laboratory has a long-standing interest in lipid binding proteins and has generated mice with targeted disruption of the acyl-CoA binding protein (ACBP).

Transcriptional Networks of Fat Cell Development

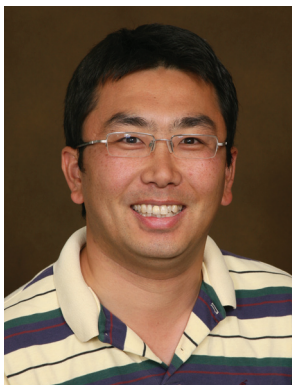
Susanne Mandrup

Department of Biochemistry and Molecular Biology,
University of Southern Denmark, 5230 Odense M, Denmark.

Adipocytes are derived from fibroblastic precursors in the adipose tissue through a tightly regulated differentiation process. The molecular basis for the regulation of adipogenesis has been studied extensively *in vitro* using a variety of preadipocyte cell culture models. In particular, studies of the murine 3T3-L1 preadipocyte cell line have been valuable for gaining insight into the ordered cascade of molecular events required for adipogenesis. The differentiation of 3T3-L1 has been shown to rely on an intricate interplay of activating and inhibitory signals regulating two waves of adipogenic transcription factors. The first wave includes members of the CCAAT/enhancer binding protein (C/EBP) family, C/EBP β , and δ , the glucocorticoid receptor and signaling transducer and activator of transcription 5, which all are activated directly by the adipogenic cocktail used to induce differentiation. These early factors activate the next wave of adipogenic transcription factors, among which peroxisome proliferator-activated receptor (PPAR) γ and C/EBP α are key players.

Recently, we have used DNase I hypersensitive (DHS) site analysis combined with deep sequencing (DHS-seq) to map open chromatin regions at a genome-wide level throughout adipogenesis. Intriguingly, in this first genome-wide map of chromatin remodeling during a differentiation process we found that the major chromatin remodeling events occur within the first four hours of adipocyte differentiation, i.e. at the time when the first wave of transcription factors is activated, but long before the major adipogenic transcription factors are expressed. ChIP-seq profiles of many of the early transcription factors demonstrated that these bind in a cooperative fashion at transcription factor ‘hotspots’, which are enriched in the vicinity of early activated genes.

The transcription factors of the early wave activate expression of major players of the second wave, PPAR γ and C/EBP α . In addition, our data indicate that the early factors participate in remodeling of the chromatin template for subsequent binding of the late factors and late activated genes. As with the early wave of transcription factors, PPAR γ and C/EBP α binding converge on late transcription factor ‘hotspots’, from where they are likely to coordinate the activation of the majority of adipocyte specific genes in a cooperative manner. Consistent with a high degree of cooperativity between PPAR γ and C/EBP α , conservation of binding sites of these two factors between mouse and human adipocytes is highly interdependent, i.e. conservation of shared binding sites is significantly higher than of binding sites where only one of the two factors bind. Thus, our data indicate that adipogenic transcription factors act in a highly cooperative fashion through at least two consecutive waves of transcriptional enhanceosomes established at transcription factor “hotspots”.



Makoto Miyazaki

Makoto Miyazaki received his Ph.D. from Nagoya City University, Japan, in 1999. He then moved to the University of Wisconsin-Madison and worked with James Ntambi for nine years, studying the physiological role of stearyl-CoA desaturase isoforms. In 2008, he joined the faculty of the University of Colorado-Denver as Assistant Professor. His group currently studies the role of bile acid and fatty acid signaling in the regulation of vascular diseases.

Bile Acid Signaling in the Regulation of Atherosclerosis and Vascular Calcification

Makoto Miyazaki*

Division of Renal Diseases and Hypertension, University of Colorado-Denver, Aurora, CO 80045

Cardiovascular disease (CVD) is the major cause of mortality in individuals with chronic kidney disease (CKD). In fact, CKD patients have more than a 20-fold higher risk of developing CVD. More than 80% of men and 50% of women with CKD develop vascular calcification and/or atherosclerosis, leading to death. However, the cellular and molecular mechanisms by which CKD accelerates cardiovascular disease are still poorly understood.

CKD is known to increase circulating bile acids. In addition to their role on the lipid absorption, bile acids are signaling molecules that exert several biological functions mediated through two major bile acid receptors, FXR and TGR5. Our previous study showed that FXR activation inhibited vascular calcification in tissue culture and in animal models using ApoE knockout (KO) mice with 5/6 nephrectomy. We have recently found that FXR deficiency accelerates vascular calcification and atherosclerosis in both ApoE KO mice and LDLR KO mice with 5/6 nephrectomy. In addition, FXR KO mice on FVB background have shorter longevity than mice on other backgrounds—specifically, they die due to cardiac valvular calcification. Our data suggest that FXR is involved in regulation of vascular and cardiac calcification.

In the second part of the lecture, I will present our most recent findings relating to the role of TGR5 in atherosclerosis.



Deborah M. Muoio

Debbie Muoio earned a Ph.D. in Nutritional Biochemistry at the University of North Carolina, Chapel Hill where she worked on fatty acid metabolism and lipid enzymology. She was subsequently awarded a NIH National Research Service Award, jointly sponsored by East Carolina and Duke Universities, to pursue post-doctoral training in the areas of metabolic disease, muscle metabolism and exercise physiology. In 2004 she joined the faculty at Duke University as a member of the Sarah. W. Stedman Nutrition and Metabolism Center, with appointments in the Departments of Medicine and Pharmacology & Cancer Biology. Her laboratory investigates mechanisms of metabolic dysregulation, with emphasis on molecular events that link overnutrition and inactivity to the development of insulin resistance in skeletal muscle. Her program features a translational approach that combines work in animal and cell-based models with human studies, using functional genomics, molecular physiology and mass spectrometry-based metabolomics as tools to understand the interplay between mitochondrial energetics and insulin action. Ongoing studies seek to identify signaling mechanisms that mediate crosstalk between muscle mitochondria and glucose trafficking, with the goal of realizing new therapeutic opportunities for treating metabolic diseases. She receives funding from the NIH and the American Diabetes Association.

Mitochondrial Stress and Metabolic Dysfunction in Skeletal Muscle

Deborah M. Muoio

Sarah W. Stedman Nutrition and Metabolism Center, Duke University Medical Center, Durham NC.

Application of targeted metabolic profiling tools has led to our finding that both diet-induced and genetic forms of insulin resistance are accompanied by high rates of “incomplete” fat oxidation, intramuscular accumulation of acylcarnitines (byproducts of incomplete substrate oxidation), moderate diminution of free carnitine and several TCA cycle intermediates, and impaired switching from fatty acid to carbohydrate substrate during the fasted to fed transition. These metabolic perturbations and the accompanying glucose intolerance are mitigated by genetic, nutritional or pharmacological maneuvers that alleviate mitochondrial fuel load. The role of lipid-induced mitochondrial stress as a potential cause of insulin resistance will be discussed.



Christopher B. Newgard

Christopher B. Newgard, Ph.D. is the Director of the Sarah W. Stedman Nutrition and Metabolism Center and the W. David and Sarah W. Stedman Distinguished Professor of Pharmacology and Cancer Biology at the Duke University Medical Center. Prior to coming to Duke in 2002, Dr. Newgard was the Gifford O. Touchstone Jr. and Randolph G. Touchstone Distinguished Professor, Department of Biochemistry, and Co-Director of the Touchstone Center for Diabetes Research, University of Texas Southwestern Medical Center, Dallas. Dr. Newgard's research focuses on application of an interdisciplinary approach for understanding of diabetes and obesity mechanisms involving gene discovery, metabolic engineering, and comprehensive tools of metabolic analysis ("metabolomics") such as mass spectrometry-based metabolic profiling and NMR-based metabolic flux analysis. Dr. Newgard has authored over 220 peer-reviewed and review articles, and has been the recipient of several awards, including the Kayla Grodsky Award for Outstanding Basic Science Research from the Juvenile Diabetes Research Foundation (1999), the Outstanding Scientific Achievement (Lilly) Award from the American Diabetes Association (2001), a Merit Award from the NIH (2001), the Solomon Berson Prize of the American Physiological Society (2003), and a Freedom to Discover Award in Metabolic Research from Bristol-Meyers Squibb (2006).

See www.stedman.mc.duke.edu for more information.

Metabolomics used for understanding of chronic disease mechanisms

Christopher B. Newgard, Ph.D.

Sarah W. Stedman Nutrition and Metabolism Center &
Department of Pharmacology & Cancer Biology
Duke University Medical Center, Durham, NC

We seek to apply comprehensive metabolic analysis tools (sometimes called “metabolomics”) for understanding of mechanisms underlying chronic human diseases and conditions such as diabetes, obesity, and cardiovascular disease. These tools have been used to investigate the mechanism of glucose-stimulated insulin secretion and mechanisms of its impairment in type 2 diabetes. The tools have also been used to define mechanisms underlying development of peripheral insulin resistance in animals and humans. For example, we have recently identified perturbations of branched chain amino acid (BCAA) catabolism in multiple cohorts of insulin resistant humans compared to normally insulin sensitive controls and have translated these findings to rodent models to demonstrate a contribution of BCAA to development of insulin resistance that is independent of body weight. We have also defined metabolic signatures that predict intervention and disease outcomes in cohorts of obese, insulin resistant subjects and in subjects with high risk of cardiovascular events. Finally, we have been integrating genomic, transcriptomic and metabolomic analysis to identify novel metabolic regulatory pathways in humans and in animal models. These examples will serve to illustrate the potential of comprehensive metabolic profiling methods for providing insights into metabolic disease mechanisms.



James M. Ntambi

James M. Ntambi, Professor of Biochemistry and Steenbock Professor of Nutrition at the University of Wisconsin-Madison USA. Ntambi received his BSc and MSc degrees in Biochemistry and Chemistry from Makerere University Kampala, Uganda and his PhD in Biochemistry and Molecular Biology from the Johns Hopkins University School of Medicine in Baltimore. He did his postdoctoral work at Johns Hopkins University School of Medicine in the laboratory of Dr. M. Daniel Lane where he started his work in lipid metabolism and gene expression. Ntambi has made distinguished contributions to the field of nutritional biochemistry and his pioneering work on the genetic regulation of the stearoyl-CoA desaturase has recently led to many new insights into the importance of this enzyme in metabolism and in disease states such as obesity, diabetes, atherosclerosis and cancer. His pioneering work will help explain the complex aspects of the “metabolic syndrome” and to advance our understanding of nutrient gene interactions. Ntambi is also involved in international research and teaching effort and student and faculty exchange programs between Makerere University Uganda and the University of Wisconsin-Madison.

A Role for Skin Stearoyl-CoA Desaturase-1 in Obesity and Diabetes

James M. Ntambi*

Departments of Biochemistry and of Nutritional Sciences, University of Wisconsin-Madison, Madison, Wisconsin, 53706.

Obesity and diabetes develop as a complex result of genetic, metabolic and environmental factors and are characterized by increased lipogenesis and lipid accumulation in many tissues. A critical regulator of lipogenesis is stearoyl-CoA desaturase (SCD), which catalyzes the synthesis of monounsaturated fatty acids (MUFA), mainly oleoyl- (18:1n7) and palmitoleoyl-CoA (16:1n7). These MUFAs are the major fatty acid substrates for the synthesis of triglycerides, cholesterol esters, phospholipids and wax esters. SCD expression is elevated in human and rodent obese and insulin resistant states, suggesting that excess 18:1n7 or 16:1n7 synthesis may contribute to metabolic disease development. Mice with a global deletion of SCD1 isoform (GKO) are resistant to diet- and genetically-induced obesity, insulin resistance and liver steatosis. This phenotype is complex due to SCD1 deficiency and the associated metabolic changes occurring in many organs simultaneously. Our long-term goal is to determine the tissue-specific contribution of SCD1 to the metabolic and genetic changes associated with obesity and the metabolic syndrome. We have found that deletion of skin SCD1 (SKO mice) recapitulated the skin abnormalities observed in GKO mice and protected SKO mice from high-fat diet-induced obesity, hepatic triglyceride accumulation and glucose intolerance regardless of changes in ambient temperature suggesting that the hypermetabolic phenotype is not simply due to accelerated heat loss to the environment. However, SKO mice maintain normal expression of SCD1 in liver and adipose, indicating that SCD1 deletion from liver and/or adipose is not required for obesity resistance. Microarray analysis of skin gene expression in mice fed a standard rodent diet revealed a robust elevation of skin retinol, retinoic acid and retinoic acid-induced genes in SKO mice. Furthermore, human SEB-1 sebocytes treated with retinol and an SCD inhibitor also display an elevation in retinoic acid-induced genes. These findings indicate that absence of skin SCD1 in the sebaceous glands is sufficient to enhance whole-body energy expenditure and elicit protection from high-fat diet-induced obesity. This also highlights a novel role for skin lipid synthesis in the regulation of energy expenditure, food intake and the development of obesity.



Apostolos Pappas

Apostolos Pappas, Ph.D., earned his Diploma in Chemistry from the Aristotelian University of Thessaloniki in Greece. He continued his studies in the United States and earned a masters degree in Food Science and Nutrition and his doctorate in Biochemistry from Rutgers University Food Science Department. He has received numerous awards and distinctions, including a Fulbright scholarship for his graduate studies. He started his professional career as a research biochemist in the Skin Research Center of Johnson & Johnson and later served as a group leader at Munich Biotech where he worked on cancer research. Thereafter he returned to Johnson & Johnson, where he currently is a Principal Scientist focusing on lipid metabolism research. His areas of interest focus on sebaceous lipid biology, ceramide synthesis in the epidermis and pre-adipocyte differentiation. He has authored multiple scientific publications, patent applications and a diet book. He is currently the editor of the first book on “Nutrition and Skin” (Springer, 2011).

Lipids in Skin Physiology, Health and Disease

Apostolos Pappas

Skin Research Center, A unit of Johnson and Johnson Consumer Companies, Inc.,
199 Grandview Road, Skillman, New Jersey 08558, USA.

Human skin lipids originate from both sebaceous glands and epidermal keratinocyte origin. The sebaceous lipids are a mixture of non-polar lipids, mainly triglycerides, wax esters, squalene, fatty acids and smaller amounts of cholesterol and cholesterol esters. Some of these lipid species are not found in other organs and tissues of the body, and their metabolic pathways, such as $\Delta 6$ desaturation, wax ester synthesis and squalene accumulation are unique to sebaceous lipid biology. Elevated sebaceous lipid synthesis is a major factor in the pathophysiology of acne.

Keratinocyte derived lipids, which contribute to the skin's barrier function, also contain some unique lipid species. Together with essential fatty acids and their metabolites, the epidermal lipids contribute to a healthy skin barrier and impairing their homeostasis results in skin diseases like eczema and atopic dermatitis.

Genetic knockout animal models of lipid synthesis demonstrated major changes in skin physiology and pathology. Impairment of sebaceous lipid pathways results in severe skin and hair phenotypes, including abnormal epidermal differentiation. The notion that these unusual lipids could deter bacteria is gaining recent attention. Additional studies are required for a complete understanding of the roles of skin's surface lipids in skin physiology.



Pere Puigserver

Pere Puigserver joined the faculty of Harvard Medical School as an Assistant Professor in 2006 and promoted to Associate Professor in 2008 in the Department of Cell Biology and Department of Cancer Biology at Dana-Farber Cancer Institute. He received his PhD in Biochemistry from the Universitat de les Illes Balears, Spain in 1992. During his graduate studies he pursued metabolic research at the University of Stockholm. His graduate work was followed by postdoctoral research at the Dana-Farber Cancer Institute and Harvard Medical School. In 2002, he was appointed Assistant Professor at Johns Hopkins University School of Medicine. Pere and his lab at the Dana-Farber Cancer Institute study the basic mechanisms of metabolic control, the process by which living cells respond to regulatory signals and support all the biological specific activities. These pathways are often dysregulated causing metabolic instability in human diseases including age-associated diseases such as diabetes and cancer. Pere has received a number of awards including a New Scholar Award from the Ellison Medical Foundation and a Career Developmental Award from the American Diabetes Association.

A Nutrient Sensor Mechanism for Control of Lipid Oxidation

Pere Puigserver

Department of Cell Biology, Dana Farber Cancer Institute, Center for Life Sciences at Harvard Medical School, Boston, MA

Mammalian cells have evolved a complex regulatory system for controlling flux through fuel utilization pathways in response to nutrient fluctuations. Under energy demanding conditions, for instance, most of the mammalian cells initiate a metabolic response that limits glucose oxidation and switches to fatty acid oxidation. We have recently identified components of the signal transduction and transcriptional pathways involved in this metabolic reprogramming that impinges on the PGC-1 α bioenergetic co-activator. This transcriptional co-activator is able to powerfully coordinate the expression of crucial genes encoding for key enzymes of fatty acid metabolism. The nutrient-mediated acetylation control of PGC-1 α function is tightly correlated with its hyperacetylation state. Here, it will be discussed how the nutrient regulation of PGC-1 α activity controlled by the deacetylase Sirt1 and the acetyltransferase GCN5 is achieved through signaling pathways and components of the transcriptional machinery. The understanding of the underlying basic mechanisms of this metabolic control will lead to new insights into basic cellular energetic processes that are important for the maintenance of metabolic stability. In addition, they will provide the possibility of the development of new treatments for metabolic disorders such as type II diabetes and obesity.



Joyce J. Repa

Joyce Repa received her B.S. (Biochemistry), M.S. (Nutritional Sciences, mentor: Alfred Harper) and Ph.D. (Nutritional Sciences, mentor: Margaret Clagett-Dame) from the University of Wisconsin-Madison. She did her postdoctoral training with David Mangelsdorf at the University of Texas Southwestern Medical Center in Dallas, where her research focused on nuclear orphan receptor biology related to cholesterol and bile acid metabolism.

In 2002, Joyce joined the faculty at UT Southwestern with joint appointments in Physiology and Internal Medicine. Her laboratory is housed in the Division for Hypothalamic Research and is affiliated with the Touchstone Diabetes Center. Her group continues to study the role of nuclear hormone receptors, now in the context of islet biology, neurodegeneration, and intestinal lipid metabolism.

Lipid-sensing Transcription Factors and Beta-cell Biology

Lilja Kjalarsdottir[‡], Jen-Chieh Chuang[‡], and Joyce J. Repa^{*‡,§}

Departments of Physiology[‡] and Internal Medicine[§]

University of Texas Southwestern Medical Center, Dallas, TX, USA, 75390

Diabetes Mellitus occurs when the endocrine pancreas fails to secrete adequate insulin to promote the uptake and utilization of circulating glucose. Over the last several years, an increasing number of nuclear hormone receptors (RXR, PPARs, LXRs, FXR, and CAR) have been implicated in the regulation of serum glucose levels. While these receptors are highly expressed in liver, adipose, and/or muscle and thought to impart their effects by enhancing peripheral insulin sensitivity, there is increasing interest in potential roles for nuclear hormone receptors in the insulin-secreting beta-cell.

We have completed a survey of nuclear hormone receptor expression in the pancreatic islets of mouse and man, and in cell lines commonly used to study beta cell biology¹. High-affinity synthetic ligands for several nuclear hormone receptors have been found to alter glucose-stimulated insulin secretion in adult islets in a receptor-dependent manner. Using a combination of functional assays, immunohistochemistry, and microarray analyses we have identified novel target genes and signaling pathways linking receptor activity with calcium signaling, immune system processes, cell adhesion and intermediary metabolism. These results implicate a subset of nuclear hormone receptors in the regulation of insulin production and secretion to affect glucose homeostasis and the development of Diabetes.

¹Chuang, J.-C., J.-Y. Cha, J. C. Garmey, R. G. Mirmira, and J. J. Repa. Nuclear hormone receptor expression in the endocrine pancreas. *Mol. Endocrinol.* 22: 2353-2363, 2008.



Karen Reue

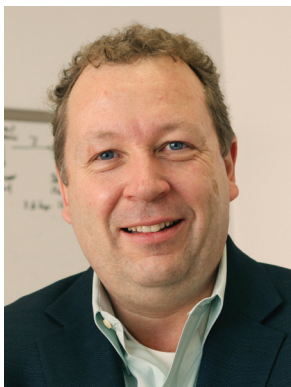
Ever since graduate studies at UCLA with Jake Lusis, I have been fascinated by the power of using naturally occurring mouse variations to identify novel genes. After post-doctoral studies at The Rockefeller University, I returned to UCLA where I continue to utilize spontaneous mutations to isolate genes with important roles in lipid storage and utilization. We have used this strategy to identify the lipin gene family, which has roles in obesity, energy metabolism, and insulin sensitivity, and the Diet1 gene, which is a novel determinant of plasma cholesterol levels and susceptibility to atherosclerosis. We are also using genetically engineered mouse strains to investigate the genetic factors underlying sex differences in lipid metabolism and obesity.

Deciphering the Roles of Lipin Family Members in Tissue Lipid Homeostasis

Karen Reue

Department of Human Genetics, University of California, Los Angeles, CA 90095

The lipin proteins have a role in the maintenance of lipid homeostasis in metabolic tissues through their actions as phosphatidate phosphatase (PAP) enzymes, which catalyze a step in the synthesis of triglycerides. The three lipin genes encode highly related proteins with unique, but overlapping, tissue expression patterns. To elucidate the physiological roles of lipin-1, lipin-2, and lipin-3, we have developed mouse strains with single or double deficiencies in lipin family members. We find that lipin-1 is responsible for PAP activity in adipose tissue and skeletal muscle, with deficiency leading to lipodystrophy and myopathy. Lipin-2 deficient mice exhibit a neurological phenotype with unexpected increases in PAP activity in tissues such as brain and liver. Lipin-3 deficient mice exhibit a modest metabolic phenotype and reduced PAP activity, which is influenced by the presence of other lipin family members. These studies suggest that lipin-1 is a key PAP enzyme *in vivo*, and that lipin-2 and lipin-3 proteins may have secondary roles in the modulation of lipin-1 protein and activity levels. In a broader sense, these results point to biological complexity and individuality in the physiological function of protein family members that cannot be inferred from protein sequence or tissue expression patterns.



Philipp E. Scherer

Dr. Philipp Scherer is currently Professor and Director of the Touchstone Diabetes Center at the University of Texas Southwestern Medical Center in Dallas. Dr. Scherer received his Ph.D. degree from the University of Basel, Switzerland working on mitochondrial biogenesis, followed by post-doctoral time in the Whitehead Institute at the Massachusetts Institute of Technology. In 1997, he joined the faculty of the Albert Einstein College of Medicine where he was a Professor for Cell Biology and Medicine until his move to Dallas in early 2007. Current efforts in his laboratory are focused on the identification and physiological characterization of novel proteins that serve as potential links between the adipocyte, liver, the pancreatic beta cell and the processes of whole body energy homeostasis, inflammation and cardiovascular disease, thereby identifying novel targets for pharmacological intervention and further defining the role of adipose tissue as an endocrine tissue.

Multiple Effects of Xbp1s on Hepatic Lipid Metabolism

Yingfeng Deng and Philipp E. Scherer*

Touchstone Diabetes Center, Departments of Internal Medicine and Cell Biology,
University of Texas Southwestern Medical Center, Dallas, TX, 75390, USA

The switch from the fasted to the fed state is associated with profound metabolic changes from fatty acid oxidation to carbohydrate utilization. To accommodate this switch in metabolic fuel, the liver reduces glucose production and fatty acid oxidation, with a concomitant enhancement in glycogen storage and lipogenesis. The endoplasmic reticulum (ER), a major site for protein and lipid biosynthesis, plays an important regulatory role in this process. However, the relationship between metabolic fuel switching and ER homeostasis remains unexplored. Here, we show that the spliced form of XBP1 (XBP1s), a cellular signal transducer of the unfolded protein response, enhances hepatic insulin action by extending the presence of the activated insulin receptor on the plasma membrane. In this mouse model in which we control the expression levels of Xbp1s by doxycycline, induction of hepatic XBP1s causes hypoglycemia and liver steatosis due to reduced gluconeogenesis and beta-oxidation. Our results highlight XBP1s as a master regulator of postprandial metabolic remodeling in hepatocytes, controlling the switch from lipid to carbohydrate utilization, enhancing insulin activity and thereby conferring additional metabolic flexibility to liver.



Hei Sook Sul

Hei Sook Sul received her Ph.D. from the University of Wisconsin-Madison. She was postdoctoral fellow at the University of California, Davis and Albert Einstein College of Medicine. She joined the Harvard School of Public Health in 1985 as an assistant Professor and moved to University of California, Berkeley in 1994 and is now a professor and holds Doris Calloway Chair in Human Nutrition. In 2006, she received the Osborne and Mendel Award. Hei Sook Sul's research interests are in the area of regulation of adipose tissue development and metabolism.

Sul, Hei Sook
Dept Nutritional Science & Toxicology
University of California
Berkeley, CA 94720
510-642-3978
hsul@berkeley.edu

Regulation and Function of Adipose Lipolysis

The two types of adipose tissue, white adipose tissue (WAT) and brown adipose tissue (BAT), have high level of lipolysis for their function of hydrolyzing stored triacylglycerol (TAG) to release fatty acids (FA) for use by other organs and for thermogenesis, respectively. Thus, lipolysis is the central metabolic process in both types of adipose tissue. We recently identified two enzymes critical for lipolysis, desnutrin/ATGL/PNPLA and AdPLA. Desnutrin is now accepted to be the primary TAG hydrolase in adipocytes and AdPLA (adipose-specific phospholipase A₂) is a main PLA₂ in adipocytes to regulate lipolysis by generating arachidonic acid for synthesis of PGE₂ that by binding to Gai-coupled EP3, decreases cAMP levels to inhibit lipolysis. In addition, we found that desnutrin is regulated by AMPK mediated phosphorylation at S406 to increase its TAG hydrolase activity and thus lipolysis. In mice, overexpressing desnutrin or ablation of AdPLA increase not only lipolysis but also oxidation of fatty acids within adipose tissue. In contrast, ablation of desnutrin in adipose tissue not only decreases lipolysis as expected, but converts BAT to WAT-like phenotype by suppressing BAT-enriched genes, including UCP1 with lower PPAR α binding to its promoter, revealing the requirement of desnutrin-catalyzed lipolysis for maintenance of BAT phenotype.



Alan Tall

Alan Tall is the Tilden-Weger-Bieler Professor of Medicine, head of the Division of Molecular Medicine and director of the Cardiovascular Research Initiative of Columbia University. His major work has been on the regulation and metabolism of plasma high density lipoproteins (HDL). He discovered mutations in the cholesteryl ester transfer protein (CETP) gene that are associated with reduced LDL and increased HDL levels, leading to the development of CETP inhibitors. Recently, Dr Tall has done research on the ATP binding cassette transporters ABCA1 and ABCG1 that promote cholesterol efflux from macrophage foam cells and hematopoietic stem cells to apoA-1 and HDL.

ABC Transporters and ApoE in the Regulation of Hematopoietic Stem Cell Proliferation, Monocytosis and Atherogenesis

Alan Tall

Columbia University Medical Center, New York.

Abstract: Leukocytosis is a risk factor for athero-thrombotic disease in humans, and develops in animal models of atherosclerosis in response to feeding high fat, high cholesterol diets. The ATP binding cassette transporters ABCA1 and ABCG1 promote cholesterol efflux to apoA-1 and HDL, respectively and are targets of LXR transcription factors. Mice lacking ABCA1/G1 develop a dramatic myeloproliferative phenotype with monocytosis and neutrophilia, associated with expansion and proliferation of hematopoietic stem and myeloid progenitor populations. The transporters are highly expressed in HSCs where they act to control proliferative responses to growth factors (IL-3, GM-CSF) by regulating plasma membrane lipid rafts and cell surface expression of the common beta subunit of the IL-3/GM-CSF receptor. In Western type diet fed *ApoE*^{-/-} mice, monocytosis and neutrophilia also develop in association with proliferation and expansion of hematopoietic stem, multipotential progenitor and myeloid progenitor cell populations in the bone marrow and spleen. *ApoE* is highly expressed in long term hematopoietic stem cells and to a lesser extent in progenitor populations, and acts in a cell autonomous fashion to control myeloid proliferation, monocytosis and accumulation of monocytes in atherosclerotic lesions. Treatment of *ApoE*^{-/-} mice with infusions of reconstituted HDL and/or LXR activator resulted in normalization of HSPC proliferation, monocytosis and neutrophilia. Together, the findings suggest that ApoE acts in concert with the ATP binding cassette transporters ABCA1 and ABCG1 to control HSPC and myeloid proliferation, monocytosis and atherogenesis. LXR activators and rHDL infusions exert anti-atherogenic effects by controlling HSPC and myeloid proliferation.



Peter Tontonoz

Peter Tontonoz received his B.A. from Wesleyan University and his M.D. and Ph.D. from Harvard Medical School. His Ph.D. thesis was carried out at the Dana-Farber Cancer Institute and his postdoctoral training at the Salk Institute for Biological Studies. He completed his residency training in Pathology at the University of California, San Diego.

Dr. Tontonoz is currently Professor of Pathology and an Investigator of the Howard Hughes Medical Institute at the University of California, Los Angeles. The focus of his laboratory is the control of gene expression by lipids and the role of nuclear receptors in lipid metabolism. His major research contributions include the delineation of role of PPAR and LXR in adipogenesis and atherosclerosis, and elucidation of mechanisms of crosstalk between metabolism, inflammation and immunity. Dr. Tontonoz is the recipient of the Richard. E. Weitzman Award from the Endocrine Society, the Jeffrey M. Hoeg Award for Basic Science and Clinical Research from the American Heart Association, and a Bristol Myers-Squibb Freedom to Discover Award in Cardiovascular Disease. He serves on the leadership council of the American Society for Clinical Investigation and the editorial boards of *Journal of Clinical Investigation*, *Molecular and Cellular Biology*, and the *Journal of Lipid Research*.

Control of Cholesterol Homeostasis by LXRs

Peter Tontonoz*. Howard Hughes Medical Institute and Department of Pathology and Laboratory Medicine, University of California, Los Angeles, CA 90095

The Liver X Receptors (LXRs) are nuclear receptors that play central roles in the transcriptional control of lipid metabolism. LXRs function as nuclear “cholesterol sensors” that are activated in response to elevated intracellular cholesterol levels in multiple cell types. Once activated, LXRs induce the expression of an array of genes involved in cholesterol absorption, efflux, transport and excretion. They also inhibit cholesterol uptake by inducing the ubiquitination and degradation of the LDL receptor. In addition to their function in lipid metabolism, LXRs modulate immune and inflammatory responses cell of both the innate and acquired immune systems. Synthetic LXR agonists promote cholesterol efflux and inhibit inflammation *in vivo* and inhibit the development of atherosclerosis in animal models. Loss of LXR expression in mice leads to pathologic lipid accumulation, atherosclerosis and the development of autoimmune disease. The ability of LXRs to integrate metabolic and inflammatory signaling makes them potentially attractive targets for intervention in human metabolic disease.



Dennis E. Vance

Dennis E. Vance is a University Professor and Professor of Biochemistry at the University of Alberta. He did his Ph.D. at the University of Pittsburgh supervised by Charles Sweeley. He did postdoctoral training at Harvard University with Konrad Bloch. He was Professor and Chair of the Department of Biochemistry at the University of British Columbia (1982-86) and Associate Dean of Medicine (1978 –81). He moved to the University of Alberta in 1986 to establish a Lipid Research Group. He was Director of the SCOLAR (Stroke, Cardiovascular, Obesity, Lipid, Atherosclerosis Research) Training Program from CIHR and the Heart and Stroke Foundation of Canada. He has served on CIHR committees including Chair of the Metabolism Committee. He is Principal Investigator on two grants from CIHR. His research on mammalian phosphatidylcholine metabolism has been recognized by the Boehringer Mannheim Canada Prize of the Canadian Biochemical Society in 1989, the Heinrich Wieland Prize awarded in 1995 in Munich, Germany, election as Fellow of the Royal Society of Canada (1996), a Canada Research Chair (2002), the Avanti Award from the American Society of Biochemistry and Molecular Biology (2006) and the Kaplan Award from the University of Alberta (2009). He presented the Laurens van Deenen lecture at the 2008 meeting of the International Conference on the Bioscience of Lipids. He was awarded the 2010 Tier I Basic Science Mentoring Award by the Faculty of Medicine at the University of Alberta. He co-authored with Geoff Zubay *Principles of Biochemistry*, an introductory biochemistry textbook and co-edited with Jean Vance five editions of an advanced textbook, *Biochemistry of Lipids, Lipoproteins and Membranes*. The fifth edition was published in March, 2008. He was an Executive Editor of the international journal *BBA* for 9 years. Prof. Vance became Editor in Chief of *BBA* in January, 2007.

Phospholipid Methylation, Diabetes and Obesity

Dennis E. Vance and René Jacobs

Group on Molecular and Cell Biology of Lipids, University of Alberta

Phosphatidylcholine (PC) is synthesized from choline via the CDP-choline pathway. Liver cells can also synthesize PC via the sequential methylation of phosphatidylethanolamine, catalyzed by phosphatidylethanolamine *N*-methyltransferase (PEMT). We investigated whether or not hepatic PC biosynthesis is linked to diet-induced obesity. *Pemt*^{+/+} mice fed a high fat diet for 10 weeks increased in body mass by 60% and displayed insulin resistance whereas *Pemt*^{-/-} mice did not. Compared to *Pemt*^{+/+} mice, *Pemt*^{-/-} mice had increased energy expenditure and maintained normal peripheral insulin sensitivity; however, they developed hepatomegaly and steatosis. In contrast, mice with impaired biosynthesis of PC via the CDP-choline pathway in liver became obese when fed a high fat diet. We, therefore, hypothesized that insufficient choline, rather than decreased hepatic phosphatidylcholine, was responsible for the lack of weight gain in *Pemt*^{-/-} mice despite the presence of 1.3 g choline/kg high-fat diet. Supplementation with an additional 2.7 g choline /kg diet normalized energy metabolism, weight gain and insulin resistance in high fat diet-fed *Pemt*^{-/-} mice. Furthermore, *Pemt*^{+/+} mice that were fed a choline-deficient diet had increased oxygen consumption, improved glucose tolerance and gained less weight. Thus, *de novo* synthesis of choline via PEMT has a previously unappreciated role in regulating whole body energy metabolism.



C. - L. Eric Yen

Eric Yen grew up in the intensely food-oriented culture of Taiwan, fascinated with the relationship between nutrition and health. After graduating from Taipei Medical College with a major in Nutrition and Health Sciences, he entered the graduate program in Nutritional Biochemistry at the University of North Carolina at Chapel Hill in 1995. Under the direction of Dr. Steven Zeisel, he studied how nutrient availability early in life modulates health in adulthood, using apoptosis induced by choline deficiency as a model. From that experience, he was exposed to the fields of phospholipid metabolism and lipid second messengers, including diacylglycerol. Encouraged by his graduate program director Dr. Rosalind Coleman, Eric joined the Gladstone Institutes in San Francisco to work on genes that code for enzymes catalyzing triacylglycerol synthesis in 2000. Under the mentorship of Dr. Bob Farese, Jr., he cloned and identified several human and mouse acyltransferases, including acyl CoA:monoacylglycerol acyltransferase (MGAT) 1 and 2, and he studied the physiological functions of these enzymes, using genetically modified mice. This line of investigation formed the basis for the ongoing studies of the research team Eric established in the Department of Nutritional Sciences at the University of Wisconsin-Madison in 2007. The main goal of the Yen Lab's research is to understand how intestinal lipid processing controls systemic lipid metabolism and energy balance. Current focus is on the role of MGAT2 in regulating nutrient assimilation and energy expenditure.

Intestinal Triacylglycerol Metabolism and Energy Balance

C.-L. Eric Yen* and David W. Nelson

Department of Nutritional Sciences, University of Wisconsin-Madison, WI 53726

Triacylglycerols, the bulk of dietary fat, are condensed metabolic fuels and can be readily stored as body fat. Compared to other energy-yielding nutrients, triacylglycerols exert a lower thermic effect of food (also known as diet-induced thermogenesis), the obligatory energy cost for digestion, absorption, and other metabolic activities needed for the assimilation of nutrients. The absorption of dietary fat involves the re-synthesis of digested triacylglycerol in enterocytes, mainly through a pathway catalyzed by the acyl CoA: monoacylglycerol acyltransferase (MGAT) 2. In mice, MGAT2 is highly expressed only in the intestine. Consistent with a role of MGAT2 in promoting conservation of dietary fat, mice lacking the enzyme are resistant to obesity and other metabolic disorders induced by high-fat feeding. This presentation will discuss our recent findings, supporting the concept that MGAT2 normally reduces diet-induced thermogenesis, enhances metabolic efficiency, and favors positive energy balance, surprisingly, even in the absence of dietary fat.

Poster Presenter Index

Presenter	Poster Title	Abstract Page	Board Number
Zaheer Akhtar	Inhibition of SCD Activity in RAW264.7 Macrophages Promotes Endoplasmic Reticulum Stress Response but Decreases Lipogenesis while Enhancing Cholesterol Trafficking	88	1
Rozalyn M. Anderson	Cellular Aspects of Metabolic Decline in Aging Rhesus Monkey Skeletal Muscle Implicate the Transcriptional Coactivator PGC-1 α	89	2
Sushant Bhatnagar	Positional Cloning of a Type 2 Diabetes Quantitative Trait Locus; Tomosyn-2, a Negative Regulator of Insulin Secretion	90	3
Emilie Boutet	Regulation of Adipocyte Lipolysis by Cytokines and Hypoxia Through FSP27	91	4
Harpreet Brar	G α Signaling Pathways in the Regulation of Pancreatic Beta-Cell Function and Mass	92-93	5
Jessica Curtis	Oxidative Stress and Mitochondrial Dysfunction in Adipocytes	94	6
Laura V. Danai	Suppression of Lipogenesis by The Protein Kinase Map4k4 in Cultured Adipocytes	95	7
Livia S. Eberlin	Desorption Electrospray Ionization Mass Spectrometry Tissue Imaging for Lipid Analysis and Disease State Evaluation	96	8
Christina R. Ferreira	Whole Swine Fetus Imaging of Lipids by Desorption Electrospray Ionization Mass Spectrometry (DESI-MS)	97-98	9
Matthew Flowers	SCD1 is Required for Normal Retinol Metabolism in Skin	99	10
Amanda J. Foltz	Using a <i>Toxoplasma gondii</i> Mutant to Explore the Connections between Inflammation, Reproductive Hormones and Metabolism	100	11
Adilson Guilherme	Enhancement of Lipogenesis by ER-Stress and the Unfolded Protein Response in Cultured Adipocytes	101	12
Hong Guo	Lipocalin 2 Deficiency Alters Peroxisome Proliferator-activated Receptor- γ Activation and Function in Lipid Homeostasis and Energy Expenditure	102	13
Rebecca A. Haeusler	Impaired Generation of 12-Hydroxylated Bile Acids Links Hepatic Insulin Signaling with Dyslipidemia	103	14
Kristina Hellberg	Fatty Acid-Dependent Leukotriene Production in Macrophages	104	15
Mark A. Herman	Adipose Tissue Carbohydrate Responsive-Element Binding Protein Regulates Adipose Tissue Fatty Acid Synthesis and Glucose Homeostasis	105	16
Ann V. Hertzel	Effects of Leukotrienes on Adipocytes	106	17
Shangyu Hong	Nicotinamide N-methyltransferase Regulates PPAR α and LXRA-dependent Pathways	107	18

Presenter	Poster Title	Abstract Page	Board Number
Junghyo Jo	Hypertrophy-driven Adipocyte Death Overwhelms Recruitment Under Prolonged Weight Gain	108	19
Gyo-Nam Kim	Selenate Controls Adipogenesis by Activating TGF- β Signaling	109	20
Alison B. Kohan	Does VLDL or Chylomicron Secretion Contribute to Lowered Plasma Lipids in Apolipoprotein A-IV Knock-out Mice?	110	21
Aparna Lakkaraju	Apolipoprotein E Isoforms and Cholesterol Metabolism in the Retinal Pigment Epithelium: Insight into the Cell Biology of Age-Related Macular Degeneration	111	22
David W. Nelson	Deficiency of the Monoacylglycerol Acyltransferase MGAT2 Increases Energy Expenditure Without High-fat Feeding and Protects Genetically Obese Mice from Excessive Weight Gain	112	23
Kuok Teong Ong	Hepatic ATGL Knockdown Uncouples Insulin Resistance and Glucose Intolerance from Liver TAG Accumulation	113	24
Apostolos Pappas	Sunscreens Block of UV-induced IL-11 Secretion Might Prevent UV-induced Facial Fat Loss	114	25
Chad M. Paton	Muscle Specific Overexpression of SCD1 to Promote Metabolic and Cardiovascular Health	115	26
Ariel D. Quiroga	Loss of Ces1/Es-x In Mice Results In Obesity, Increased Blood Lipid Levels and Decreased Energy Expenditure	116	27
Mark P. Richards	Effect of Obesity on <i>in vitro</i> Hemolysis using Familial Hypercholesterolemic Swine	117	28
Rachel J. Roth Flach	A Novel Role for Ste-20 Like Kinase MAP4K4 in Endothelial Cell Activation	118	29
Lindsay R. Schneider & Brendan J. Floyd	The Role of Sorcs1 in Hormone Transport	119	30
Wan Shen	The Phospholipase C (PLC) Inhibitor U73122 Inhibits Trans-10, cis-12 Conjugated Linoleic Acid (CLA)-Mediated Inflammatory Signaling and Insulin Resistance in Human Adipocytes	120	31
Maggie Strable	Role of Hepatic Monounsaturated Fatty Acid Synthesis in Metabolic Regulation	121	32
Aki Uchida	Increased Intestinal Acyl-CoA: Diacylglycerol Acyltransferase 2 Exacerbates Diet-induced Hepatic Steatosis	122	33
Chen-Yu Wang	Genetics of Nonalcoholic Fatty Liver Disease	123	34
Yan Xie	An Mttp-independent Pathway for Cholesterol Absorption Revealed by LXR Agonist Treatment in Mice	124	35

Inhibition of SCD activity in RAW264.7 macrophages promotes endoplasmic reticulum stress response but decreases lipogenesis while enhancing cholesterol trafficking

Zaheer Akhtar*, Samir Joshi, Xueqing Liu, and James Ntambi, Department of Biochemistry, University of Wisconsin–Madison, Madison WI, 53703, USA

Lipogenesis has been linked to increased endoplasmic reticulum (ER) stress. Loss of stearoyl CoA desaturase (SCD) suppresses lipogenesis, but its role in integrating ER stress and lipogenesis is currently understudied. Here, using RAW264.7 macrophages, we tested the effects of SCD activity on regulating lipogenesis and ER homeostasis. RAW cells treated with thapsigargin and tunicamycin, two compounds commonly used to induce ER stress, showed increased induction of ER stress genes GRP78 and CHOP with decreased expression of lipogenic genes SREBP-1c, ACC, FAS and SCD1. While the expression of these lipogenic genes were suppressed, that of cholesterol efflux gene ABCA1 was increased in response to increasing doses of thapsigargin. Inhibition of SCD activity by A939572, a pharmacological inhibitor targeting the catalytic domain of the enzyme, further enhanced induction of ER stress and suppressed lipogenic gene expression, but increased ABCA1 expression to a higher degree. The above results suggest that SCD may be an important enzyme regulating lipogenesis and ER homeostasis.

Cellular aspects of metabolic decline in aging rhesus monkey skeletal muscle implicate the transcriptional coactivator PGC-1a.

Rozalyn M. Anderson^{1,2}, Matthew W. Conklin³, Trent D. Evans², Hannah J. Barbian⁴, Rachelle L. Pass², Tom D. Pugh³, Susan H. McKiernan⁴, T. Mark Beasley⁵, Kevin W. Elicieri³, Richard Weindruch^{1,2,4}.

¹GRECC, William S. Middleton Memorial Veterans Hospital, Madison WI 53705,

²Department of Medicine, University of Wisconsin, Madison WI 53706,

³Laboratory for Optical and Computational Instrumentation, Department of Pharmacology, University of Wisconsin, Madison WI 53706, ⁴Wisconsin National Primate Research Center, University of Wisconsin, Madison WI 53715,

⁵Department of Biostatistics, University of Alabama, Birmingham AL 35294.

Mitochondrial integrity is a key component of healthy tissue, and in skeletal muscle, mitochondrial dysfunction has been associated with aging, diabetes and metabolic syndrome. We have identified an age-dependent shift in skeletal muscle fiber type distribution in rhesus monkeys *vastus lateralis* (VL) that is suggestive of a shift in metabolism. This shift is coincident with the onset of sarcopenia which occurs at ~16ys of age. Using immunohistochemistry, we find that age negatively impacts the activity of the mitochondrial electron transport system in VL sections from mid-age (15-16y) and old age (28-32y) animals. In addition, the intracellular organization of the most active mitochondria observed in young adults (6-11y) is gradually lost with age. Autofluorescent detection using multiphoton imaging indicates that levels of both NADH and FAD⁺ metabolic cofactors are altered with age in a biphasic manner. Fluorescent decay lifetime analysis, an indicator of local environment of the fluorophores, indicates that cellular localization of the fluorophores is altered. These data suggest that muscle from young, mid-age and old animals are metabolically distinct. Analysis of lipid deposition reveals that intracellular lipid droplet size is increased in sections from mid-aged and old muscle, consistent with a loss of mitochondrial sufficiency in the utilization of lipid fuel sources. These data point to the transcriptional co-activator PGC-1a, a key regulator of fatty acid oxidation and fatty acid metabolism. Although PGC-1a levels do not decline with age, its subcellular localization is disrupted and PGC-1a nuclear accumulation is impaired. These data reveal that a decline in lipid metabolism is coincident with the onset of sarcopenia, and suggests that a dysfunctional cellular response to that shift underlies the aging of skeletal muscle in primates.

Positional Cloning of a Type 2 Diabetes Quantitative Trait Locus; Tomosyn-2, a Negative Regulator of Insulin Secretion

Sushant Bhatnagar*, Angie T. Oler, Mary E. Rabaglia, Donald S. Stapleton, Mark P. Keller, Kathryn L. Schueler, Alan D. Attie

Department of Biochemistry, University of Wisconsin, Madison, WI 53705, USA

Genetic factors contribute approximately 50% towards the risk of type 2 diabetes mellitus (T2DM). To identify the gene(s) conferring diabetes susceptibility, the Attie laboratory took advantage of two mouse strains that differ in their susceptibility towards T2DM. The BTBR T (+) tf (BTBR) *ob/ob* mouse is severely diabetic whereas the C57BL/6 (B6) *ob/ob* is essentially non-diabetic. An F2 intercross derived from these strains was used to map fasting hyperglycemia to a locus on chromosome 16. Consomic mice containing the BTBR chromosome 16 in the B6 *ob/ob* mice (B6.16^{BT} *Leptin*^{ob}) background had reduced fasting plasma insulin and elevated glucose levels similar to BTBR *ob/ob* mice. Further, a reduction in plasma insulin levels in the B6.16^{BT} *Leptin*^{ob} mice appeared to be due to impaired insulin secretion from the pancreatic beta cells. To avoid the metabolic complexities that are attributed to leptin mutation in *Leptin*^{ob} mice, a panel of congenic mouse strains was generated from B6.16^{BT} mice, each containing a small introgressed region from BTBR in a B6 background. This enabled us to narrow the region responsible for the insulin secretion defect to 1.6 Mb. The islets isolated from these mice had reduced second phase insulin secretion and were defective in secreting insulin in response to glucose, arginine, and 8Br-cAMP, suggesting that the gene(s) within the 1.6 Mb is a constitutive inhibitor of insulin secretion. Within this region, syntaxin-binding protein 5-like (Tomosyn-2) is the most plausible candidate. The mRNA abundance of tomosyn-2 was elevated 4-fold in the congenic strains that were defective in insulin secretion. Overexpressing tomosyn-2 in pancreatic beta-cells inhibited glucose (7mM) and 8 Br-cAMP (3mM)-induced insulin secretion. Further, *in-vitro* binding of tomosyn-2 to syntaxin-1A and syntaxin-4 was observed in GST-pull down experiments. Syntaxin-1A and syntaxin-4 are key proteins involved in the formation of the SNARE complex and regulation of insulin secretion. Together, these data suggest that we have indentified a novel regulator of insulin secretion. Understanding the mechanism by which tomosyn-2 regulates insulin secretion will provide insight to the pathophysiology of T2DM.

Regulation of adipocyte lipolysis by cytokines and hypoxia through FSP27

Emilie Boutet*, Srijana Ranjit, Matthieu Prot, Michael P. Czech

Program in Molecular Medicine, University of Massachusetts Medical School,
Worcester, MA 01605, USA

Obesity and type 2 diabetes are associated with chronic inflammation of adipose tissue (AT), which is thought to contribute to insulin resistance through the actions of pro-inflammatory cytokines such as TNF- α , IL-1 β or IFN γ . These same cytokines are known to promote lipolysis in adipocytes, which may impede the ability of these cells to store triglyceride. Cytokines do not seem to regulate lipolysis through modulation of the classical cyclic AMP-dependent pathway, but rather must target other cellular components controlling triglyceride turnover. Fat Specific Protein 27 (FSP27) is a lipid droplet-associated CIDE domain containing protein under the control of PPAR γ that inhibits basal adipocyte lipolysis in cultured and primary adipocytes, thereby enhancing triglyceride accumulation (1, 2). Loss of function of FSP27 in mice or of its human homolog CIDEC in humans causes increased lipolysis and lipodystrophy, which in the latter case is associated with insulin resistance (3, 4). Since TNF- α and other cytokines inhibit PPAR γ expression, we tested the hypothesis that cytokine-stimulated lipolysis might be mediated by downregulation of FSP27. Indeed FSP27 expression in 3T3-L1 adipocytes at both the mRNA and protein levels was markedly inhibited in response to cytokines TNF- α , IL-1 β and IFN γ . When this effect of TNF- α was prevented by simultaneous expression of FSP27 driven by an adenovirus vector, TNF- α induced lipolysis was also blocked. These data indicate that at least part of the cytokine's effect on lipolysis is mediated by its ability to attenuate expression of the lipid droplet protein FSP27.

In the recent years, an emerging role of adipose tissue hypoxia has been implicated in the adipose dysfunction that accompanies obesity. Specifically, hypoxia has been shown to chronically increase basal adipocyte lipolysis (5), but no mechanism has yet been proposed. We therefore tested the hypothesis that downregulation of FSP27 is involved in hypoxia-mediated lipolysis in cultured adipocytes submitted to overnight conditions of 0.5% O₂, 5% CO₂. We discovered that upon exposure to 16 hours of hypoxia, FSP27 was the only lipid droplet protein examined to be highly down regulated at both the mRNA and protein levels. The mechanisms by which hypoxia decreases FSP27 expression are under investigation, although PPAR γ may be involved since it is also highly downregulated by hypoxic conditions. Taken together, our data suggest that regulation of lipid droplet protein FSP27 expression in adipocytes by cytokines and hypoxia may mediate their stimulatory effects on lipolysis.

1. V. Puri *et al.*, *J Biol Chem* **282**, 34213 (Nov 23, 2007).
2. S. Ranjit *et al.*, *J Lipid Res* **52**, 221 (Feb).
3. N. Nishino *et al.*, *J Clin Invest* **118**, 2808 (Aug, 2008).
4. O. Rubio-Cabezas *et al.*, *EMBO Mol Med* **1**, 280 (Aug, 2009).
5. J. Ye, *Int J Obes (Lond)* **33**, 54 (Jan, 2009).

G α_z Signaling Pathways in the Regulation of Pancreatic Beta-Cell Function and Mass

Harpreet Brar*, Elizabeth Klar, and Michelle E. Kimple, Ph.D.

Department of Medicine, Division of Endocrinology, Diabetes, and Metabolism,
University of Wisconsin-Madison; Madison, Wisconsin, 53706; USA

Insulin produced in the beta-cells of the pancreatic islets of Langerhans is necessary to properly regulate blood glucose levels. An insufficient plasma insulin level, due to deficits in both beta-cell function and mass, is an important factor in the pathogenesis of both types 1 and 2 diabetes mellitus. Key components in the regulation of beta cell biologies that impact on insulin production (e.g., insulin secretion, replication, cell survival) are G protein coupled receptors (GPCRs), integral membrane proteins that function in transducing extracellular stimuli through the plasma membrane to membrane-associated guanine nucleotide binding proteins (G proteins), which in turn act on intracellular effectors to regulate second-messenger signaling pathways (Fig. 1). This process, from agonist binding to functional consequence, is termed signal transduction. Our work is focused on determining the role(s) that specific G protein signaling pathways play in regulating pancreatic beta-cell biology. Using an rat beta-cell line, we showed that the alpha subunit of the heterotrimeric G $_z$ protein, G α_z , blocked insulin secretion by transducing signals from an endogenous cellular receptor to decrease levels of the second messenger, cyclic adenosine monophosphate (cAMP). Furthermore, studies with mice genetically deficient for G α_z revealed increases in beta-cell function and mass, likely through a dramatic increase in beta-cell proliferation; thus, protecting mice from developing glucose intolerance when challenged. G α_z -null islets have constitutively increased cAMP production, suggesting that G α_z is a tonic inhibitor of adenylate cyclase, the enzyme responsible for the conversion of ATP to cAMP. These results link signaling through G α_z to both major aspects of the pathogenesis of diabetes: insufficient beta-cell function and mass. In our current studies, we are utilizing various treatments to agonize or antagonize endogenous beta-cell signaling pathways that may be coupled specifically to G α_z . In addition, we are determining whether proteins involved in the G α_z signaling pathway are differentially regulated on the mRNA and/or protein level as a pathogenic response to hyperglycemia. Finally, determining the relevance of the G α_z signaling pathway to human beta-cell dysfunction may reveal whether targeting this pathway may have utility in the treatment of diabetes.

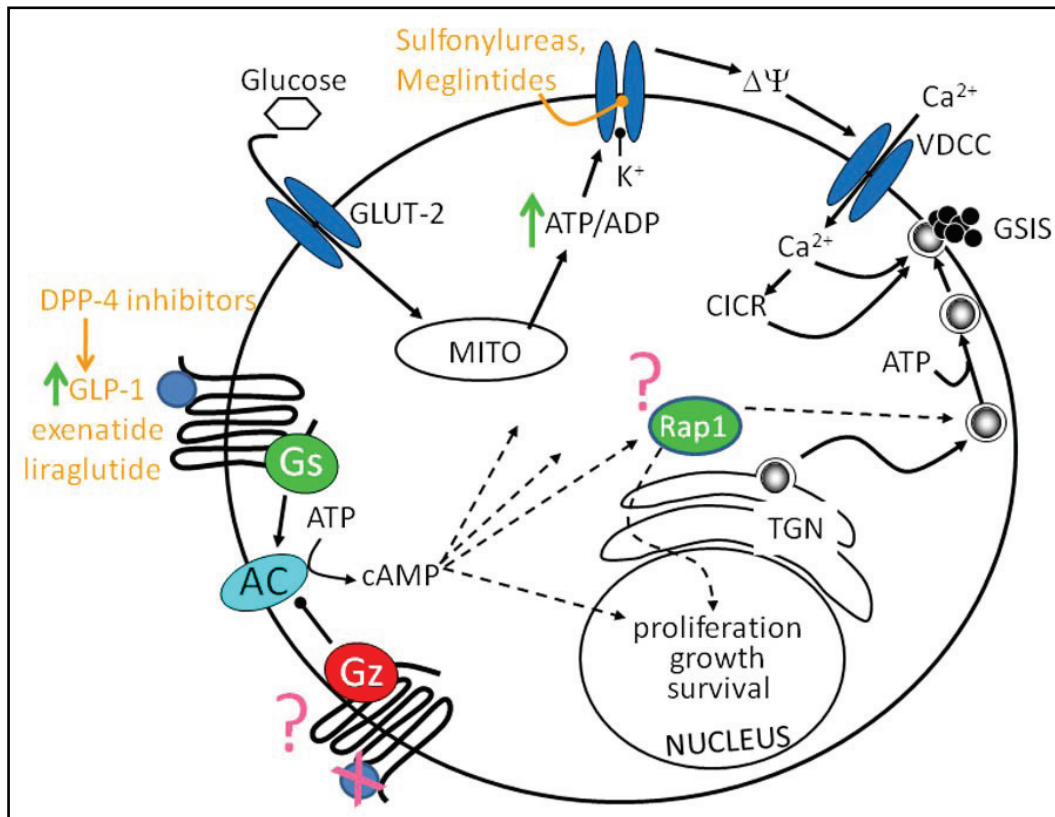


Fig. 1. Drug targets for beta-cell dysfunction and the dual role of cAMP in augmenting the glucose-stimulated insulin secretion (GSIS) response and biologies related to beta-cell mass. The traditional T2DM secretagogue therapies, sulfonyleureas and meglitinides, block the K-ATP-channel, forcing the depolarizing the cell membrane and stimulating calcium-induced calcium release (CICR), the final step in the exocytosis process for insulin granules that have docked and primed with the plasma membrane. Incretin analogs, such as exenatide and liraglutide, act in a different mechanism by increasing cAMP levels, which have been shown to augment many steps in the simulated secretion pathway, both Rap1-dependent and -independent. Because of the effect of cAMP on cell proliferation, growth, and survival, incretin analogs are the only T2DM drugs that have also been suggested to improve beta-cell mass; thus, of potential use as T1DM therapeutics. The role of Gz-coupled receptors in negatively regulating these same signaling pathways is the focus of current research.

Oxidative Stress and Mitochondrial Dysfunction in Adipocytes

Jessica Curtis*, Matt Stone, Wendy Hahn, Jacob Inda, Tim Griffin, David Bernlohr Department of Biochemistry, Molecular Biology & Biophysics, University of Minnesota, Minneapolis, MN, 55455

Carbonylation is the covalent, non-reversible modification of the side chains of cysteine, histidine and lysine residues by lipid peroxidation end products such as 4-hydroxy nonenal or 4-keto nonenal. Detoxification of such reactive lipids by Glutathione S-transferase (GST) A4 is the major antioxidant pathway, however the expression of GSTA4 is specifically down regulated in the obese adipocyte leading to elevation of protein carbonylation. The effects of such adipocyte protein modification in the obese state include increased basal glucose uptake, decreased mitochondrial respiration, increased superoxide production, increased basal lipolysis, decreased TCA cycle flux and diminished fatty acid oxidation.

To identify mitochondrial targets of protein carbonylation in the adipocyte and to address the role of GSTA4 down regulation in the pathogenesis of mitochondrial dysfunction and insulin resistance, mitochondrial proteins were isolated from GSTA4 silenced or over expressing 3T3-L1 adipocytes and respective control cell lines. Protein carbonyl groups were labeled with biotin hydrazide and captured using avidin-affinity chromatography. The isolated carbonylated proteins were trypsin digested and the resultant peptides labeled with iTRAQ isobaric tags and analyzed via LTQ orbitrap mass spectrometry.

Relative to adipocytes over expressing GSTA4, GSTA4 silenced adipocytes displayed elevated carbonylation of several key mitochondrial proteins including the phosphate carrier protein, NADH dehydrogenase 1 alpha subcomplexes 2 and 3, translocase of inner mitochondrial membrane 50, and valyl-tRNA synthetase. These changes in protein carbonylation are associated with diminished mitochondrial membrane potential and attenuated ATP Synthase activity. These results suggest protein carbonylation plays a major initiating role in mitochondrial dysfunction and may be a contributor to the development of insulin resistance.

Supported by NIH-DK084669

Suppression of Lipogenesis by The Protein Kinase Map4k4 in Cultured Adipocytes.

Laura V. Danai*, Adilson Guilherme and Michael P. Czech.

Program in Molecular Medicine, University of Massachusetts Medical School,
Worcester, Massachusetts 01605, USA.

Lipogenesis in adipose tissue is impaired during obesity leading to ectopic triglyceride (TG) deposition and insulin resistance. We have recently identified the yeast Sterile 20 (Ste20) protein kinase, Map4k4, in an RNA interference-based screen of genes that downregulate glucose transporter GLUT4 and lipogenesis in adipocytes [1]. We found that Map4k4 also suppresses the protein kinase mammalian Target of Rapamycin (mTOR) and phosphorylation of its substrate 4EBP1 in cultured adipocytes but not insulin-mediated Akt/PKB activation [2]. Interestingly, mTOR up-regulates lipogenic transcription factor Sterol Regulated Element-Binding Protein (SREBP-1c) in hepatocytes by an unknown mechanism [3]. Previous studies placed Map4k4 upstream of the c-Jun N-terminal Kinase (JNK) signaling pathway since its expression activated JNK[4, 5]; however, several studies using different cell models in our laboratory, 3T3-L1 adipocytes in particular, have not confirmed this [1, 6]. Using adenovirus vector-mediated MAP4K4 over-expression followed by detection of JNK kinase activity by both Western blotting with anti-phosphoJNK antibody and with radiolabeled ³²P-ATP, we found that MAP4K4 over-expression does not affect the activity of endogenous JNK in cultured adipocytes. Furthermore, silencing of JNK does not increase expression of GLUT4 as we reported for MAP4K4 [1]. Using full-genome microarray analysis of MAP4K4-depleted adipocytes, we found increased expression of several genes encoding lipogenic enzymes compared to control cells. RT-qPCR confirmed the increased expression of many of these genes including SREBP-1c and its target genes GLUT4, AcetylCoA Carboxylase (ACC), and Fatty Acid Synthase (FAS). Furthermore, Phosphoenol Pyruvate Carboxykinase (PEPCK-c), a glyceroneogenic protein not known to be regulated by SREBP-1c, also showed increased expression upon MAP4K4 silencing. Most importantly, using metabolic flux studies to measure the incorporation of ¹⁴C-glucose into TG, MAP4K4 gene depletion was found to enhance glucose conversion into glyceride-glycerol. In summary, these studies suggest that: 1) Map4k4 functions as a suppressor of lipogenesis in the adipocyte independent of the JNK signaling pathway; and 2) Map4k4 suppresses lipogenesis by inhibiting the expression of SREBP-1c and its target genes, possibly through the mTOR pathway, as well as inhibiting the expression of glyceroneogenic gene PEPCK-c. Taken together, these data identify Map4k4 as an important regulator of lipogenesis in mouse adipocytes.

Desorption Electrospray Ionization Mass Spectrometry Tissue Imaging for Lipid Analysis and Disease State Evaluation

Livia S. Eberlin*, Allison L. Dill, Christina R. Ferreira and R. Graham Cooks

Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA

Desorption electrospray ionization mass spectrometry (DESI-MS) is one of a recently developed group of ionization techniques in MS in which samples are examined in the ambient environment with minimal pretreatment¹. Using DESI, direct lipid analysis from biological samples such as tissue sections can be performed rapidly and routinely²⁻⁴. Unmodified samples are scanned in the x and y directions while a spray of charged droplets impinges on the sample, extracting lipids into secondary microdroplets which are transferred to a mass spectrometer. Chemical information obtained can be represented as two-dimensional images recording abundances and spatial distributions of specific ions⁵. In a single experiment, DESI-MS allows the characterization and imaging of many lipids including fatty acyls, glycerophospholipids, glycerolipids and sphingolipids. MS imaging of tissue sections has the potential to enable disease diagnosis based on lipid profiles.

DESI-MS imaging experiments have been conducted on human cancerous tissues including bladder, kidney, prostate, testicular and brain cancers. DESI-MS lipid data enabled successful diagnosis of disease referenced against histopathological examination. For brain cancer, different world health organization (WHO) grades of astrocytomas were discriminated by their lipid profiles (Figure 1). Different lipid profiles corresponding to each degree of malignancy II, III, and IV in human astrocytic tumors were observed. Specifically, it was observed that as the malignancy of astrocytomas increases, there is a decreased abundance of two specific classes of lipids, sulfatides and galactoceramides. This simultaneous change in lipid composition with astrocytoma grade observed by DESI-MS analysis agreed with previous reports based on classical lipid extraction methodologies. For bladder, kidney and prostate cancers, statistical methods for classification was used to evaluate DESI lipid data obtained from tissue analysis. For example, in kidney cancer tumor and normal tissues were successfully distinguished for both papillary and clear cell renal cell carcinoma with misclassification rates obtained from the validation set of 14.3% and 7.8%, respectively. These results demonstrate that DESI-MS imaging has the potential to be a diagnostic tool for future cancer detection based on tissue lipid profiles.

Whole Swine Fetus Imaging of Lipids by Desorption Electrospray Ionization Mass Spectrometry (DESI-MS)

Christina R. Ferreira*, Livia S. Eberlin and R. Graham Cooks

Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA

Lipid imaging of tissue sections by desorption electrospray ionization mass spectrometry (DESI-MS) allows 2D positional mapping of a broad range of lipid species such as fatty acyls, glycerophospholipids, glycerolipids and sphingolipids. From the 2D ion images it is also possible to construct 3D models¹, providing information on the molecular features of different organs. The objective of this work is to map the lipid distribution in thin sections (15µm) from whole swine fetuses (54 days of gestation) using DESI-MS imaging. A whole swine fetus (no fixation) was sectioned using a cryotome and sections were mounted onto glass slides and stored at -80°C freezer until analysis. Mass spectrometric images were acquired in the negative ion mode in the m/z range of 100 to 2,000 from different sections representing the whole swine fetus body using 300µm pixel size. Sample preparation is minimal in DESI-MS imaging analysis, since unstained and unmodified sections are used after being dry in air or in a dissecator for few minutes after thawing. Examples of lipid classes commonly detected in the negative ion mode are represented by the species phosphatidylserine (PS 36:1, m/z 788), sulphatide (ST 24:0, m/z 906), and phosphatidylinositol (PI 38:4, m/z 885) (**Figure 1**). Fatty acyls and other small molecules of low molecular weight can also be detected due to the absence of organic matrix interferences at low mass ranges. Since a DESI ion image of a whole fetus section measuring 1.46 X 0.8 in is acquired in two hours with high density of information on lipid structure (each image pixel is represented by a mass spectra containing hundreds of ions), we propose that this technique will become a new biotechnological tool for studies involving lipid metabolic and pathological studies during fetal development in an animal model which is considered important for food production, pharmaceutical studies and in human medicine.

Figure 1 on next page

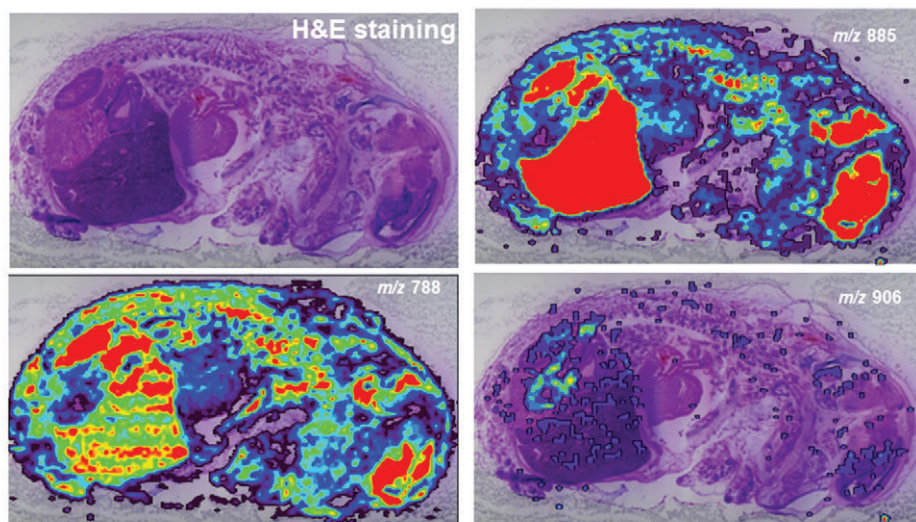


Figure 1. Lipid species detected by DESI-MS in a thin section of a whole swine fetus superimposed to an H&E stained section. Images show lipids of m/z 788, (PS 36:1), m/z 885, PI (38:4) and m/z 906, ST (24:0), representative of the different lipid classes detected.

References

1. Eberlin LS, Ifa DR, Wu C, Cooks RG. Three-dimensional visualization of mouse brain by lipid analysis using ambient ionization mass spectrometry. *Angew Chem Int Ed Engl.* 2010;49(5):873-6.

Continued from previous abstract

SCD1 is Required for Normal Retinol Metabolism in Skin

Matthew Flowers^{*1}, Chad Paton^{1,2}, Sheila O'Byrne⁴, Kevin Schiesser¹, John Dawson³, William Blaner⁴, Christina Kendzierski³, James Ntambi^{1,2}

Departments of ¹Biochemistry, ²Nutritional Sciences and ³Biostatistics, University of Wisconsin, Madison, WI 53706, USA; ⁴Department of Medicine, Columbia University, New York, NY 10032, USA

The synthesis of fatty acids by the skin is necessary for normal sebaceous gland function and maintenance of a barrier between the organism and the environment. These fatty acids are important for the formation of complex lipids, such as triglycerides, sphingolipids, esterified cholesterol, wax esters and retinyl esters. Stearoyl-CoA desaturase-1 (SCD1) is a fatty acid desaturase that converts saturated fatty acids into monounsaturated fatty acids. We have previously shown that mice with a skin-specific deletion of *Scd1* recapitulate the skin phenotype and hypermetabolism observed in mice with a whole-body deletion of *Scd1*. In this study, we first performed a diet-induced obesity experiment at thermoneutral temperature (33°C) and found that skin-specific *Scd1* knockout (SKO) mice still remain resistant to obesity. To elucidate the metabolic changes in the skin that contribute to the obesity resistance and skin phenotype, we performed microarray analysis of skin gene expression in male SKO and control (Lox) mice fed a standard rodent diet. We identified an extraordinary number of differentially expressed genes that support the previously documented histological observations of sebaceous gland hypoplasia, inflammation and epidermal hyperplasia in SKO mice. Additionally, transcript levels were reduced in skin of SKO mice for genes involved in fatty acid synthesis, elongation and desaturation, which may be attributed to decreased abundance of key transcription factors including SREBP1c, ChREBP and LXRα. Conversely, genes involved in cholesterol synthesis were increased, suggesting an imbalance between skin fatty acid and cholesterol synthesis. Unexpectedly, we observed a robust elevation in skin retinol and retinoic acid, as well as increased expression of retinoic acid-induced genes in SKO mice. Excess retinol must be esterified to fatty acids and stored as retinyl esters. Our findings indicate that the availability of monounsaturated fatty acid products of SCD1 is critical for retinol homeostasis. Retinoids have previously been shown to have profound effects of sebaceous gland function. Cultured SEB-1 sebocytes treated with retinol and an SCD inhibitor also display an elevation in retinoic acid-induced genes. These results highlight the importance of monounsaturated fatty acid synthesis for maintaining retinol homeostasis and point to disturbed retinol metabolism as a novel contributor to the *Scd1* deficiency-induced skin phenotype.

Using a *Toxoplasma gondii* Mutant to Explore the Connections between Inflammation, Reproductive Hormones and Metabolism.

Amanda J. Foltz*, Kyle Boldon and Laura J. Knoll
University of Wisconsin-Madison, Department of Medical Microbiology & Immunology, Madison, Wisconsin, 53706, United States

With over two thirds of the adult population currently classified as overweight or obese, obesity has become a serious problem in the United States, yet its development remains poorly understood. Our lab has serendipitously discovered that mice infected with a mutant strain of the parasite *Toxoplasma gondii* become obese, weighing as much as 65 grams (versus 25 gram control mice). This mutant *T. gondii* strain, called N28E2, exhibits a persistence defect in mice: it forms normal brain cysts at the onset of chronic infection, but unlike wild-type cysts, N28E2 cysts do not persist for the lifetime of the host. We have shown by RT-PCR that the lipogenesis genes *ACCI*, *FAS1* and *SCD1* are approximately 100-fold upregulated in the obese, N28E2-infected mice. Preliminary data suggest that these animals consume approximately 30% more chow per day than control mice. Conversely, obese and control mice do not differ in their circulating levels of leptin, a molecule that controls appetite. Additionally, the obese mice appear to exhibit glucose intolerance following glucose challenge. We initially hypothesized that the observed weight gains could be caused by an atypical immune response to the microbe; however, there are no detectable differences in sera levels of IL-6, IL-10, IL-12, TNF- α , IFN- γ or MCP-1 between obese and control mice at any time point examined. In contrast, it appears that levels of the adipokine IL-6 are lower in the brains of N28E2-infected mice than wild-type infected mice. Analyses of the gut microbiotas of obese and control mice showed no differences between the two groups, ruling out the possibility that the gut microbiota is involved in the obesity phenotype. In a subset of the obese animals, we have observed severe damage in an area of the brain called the ventromedial hypothalamus (VMH) that contains a high concentration of estrogen receptor (ER) sites. In this area of the brain, estrogen normally binds to ERs to regulate food intake and energy expenditure, and VMH lesions are known to cause obesity. In support of the involvement of reproductive hormones in our obesity phenotype, only female mice gain weight in response to infection by this microbe. Interestingly, infected male mice mimic castrated males; they present an “emasculated” phenotype characterized by decreased aggression and maintenance of juvenile, glossy black coats. These “emasculated” males appear to have higher estrogen and lower testosterone levels than the uninfected males. We will use this unique system to understand how inflammation and sex hormones can influence metabolism and weight gain.

Enhancement of Lipogenesis by ER-Stress and the Unfolded Protein Response in Cultured Adipocytes.

Adilson Guilherme*, Laura V. Danai and Michael P. Czech.

Program in Molecular Medicine, University of Massachusetts Medical School,
Worcester, Massachusetts 01605, USA.

Adipose tissue plays a central role in mediating the storage of caloric energy in the form of triglycerides (TG), preventing adverse effects associated with the deposition of fatty acids and TG in muscle and liver. The capacity of adipose cells to convert glucose and other substrates to TG and store them is disrupted in insulin resistance induced by obesity. Moreover, decreased expression of lipogenic enzymes involved in TG biosynthesis and storage is observed in obesity. Many of these lipogenic transcriptional factors and enzymes are located in the endoplasmic reticulum (ER) and recent studies in rodent models and humans revealed increased unfolded protein response (UPR) and ER-Stress dysfunction in obese adipose tissue. ER-stress in adipocytes has been linked to insulin resistance and decreased adiponectin and leptin secretion. Here we investigated the impact of ER-stress on lipogenesis, insulin signaling, glucose transport and triglyceride biosynthesis in mature 3T3-L1 adipocytes. We show that induction of ER-stress *in vitro* by tunicamycin treatment can lead to the enhancement of [¹⁴C]-glucose conversion into TG in adipocytes. Treatment of adipocytes with tunicamycin markedly induced the expression of several ER-stress markers, such as the spliced X-box binding protein (sXBP1), glucose-regulated protein 78 (GRP-78) and CAAT/enhancer binding protein homologous protein-10 (CHOP10). Furthermore, tunicamycin enhanced the expression levels of AP-1 transcriptional factors, cJUN, FOS, and FRA-1, consistent with the notion that ER-stress activates the JNK/AP-1 signaling pathway in adipocytes. In contrast, the expression levels of two key transcriptional factors in adipocyte glucose and lipid metabolism, PPAR γ and C/EBP α , were markedly down regulated upon ER-stress. Also, insulin signaling to Akt phosphorylation was attenuated. Surprisingly, treatment of adipocytes with tunicamycin enhanced [¹⁴C]-glucose conversion into TG, triglyceride-glycerol and triglyceride fatty acid. Under this ER-stress, no effect on insulin-stimulated [³H]-deoxyglucose uptake nor GLUT1 and GLUT4 levels was observed. Overall, these results reveal the unexpected finding that while ER-Stress strongly represses PPAR γ and C/EBP α and attenuates insulin signaling to Akt, it paradoxically increases TG biosynthesis in adipocytes. These results further suggest that one or more pathways downstream of ER-stress are able to override effects of the decreases in PPAR γ and C/EBP α to enhance lipogenesis pathways in mouse adipocytes.

Lipocalin 2 Deficiency Alters Peroxisome Proliferator-activated Receptor-gamma Activation and Function in Lipid Homeostasis and Energy Expenditure.

D Jin, H Guo*, SY Bu, Y Zhang, J Hannaford, DG Mashek, X Chen.

Department of Food Science and Nutrition, University of Minnesota-Twin Cities, St. Paul, Minnesota, 55108, USA.

Abstract

We have previously identified lipocalin 2 (Lcn2) as a cytokine playing a critical role in the regulation of body fat mass, lipid metabolism, and insulin resistance. Lcn2 deficiency reduces PPAR γ gene expression in adipocytes. In this study, we investigated the role of Lcn2 in PPAR γ activation and function via assessing the insulin sensitization and fatty acid (FA) homeostasis of PPAR γ agonist in high-fat diet (HFD)-induced obesity in Lcn2(-/-) mice. We found that rosiglitazone (Rosi) significantly improved insulin sensitivity in Lcn2(-/-) mice as effectively as in wild-type (WT) mice; unfed-state levels of blood glucose, free FAs, and triglycerides (TGs) were significantly reduced after a 25-d treatment of Rosi in Lcn2(-/-) mice. However, Rosi action on fat deposition and FA homeostasis was altered; Rosi-induced body weight and subcutaneous fat gain and liver lipid accumulation were markedly lessened in Lcn2(-/-) mice. The results of in vivo metabolic labeling showed that Rosi markedly reduced de novo lipogenesis in adipose tissue of Lcn2(-/-) mice. In brown adipose tissue (BAT), the expression of the genes functioning in TG hydrolysis and mitochondrial oxidation was up-regulated more in Lcn2(-/-) than in WT mice. Most strikingly, Rosi stimulated significantly higher levels of uncoupling protein-1 expression in BAT, and completely rescued cold intolerance in Lcn2(-/-) mice. We demonstrate that Lcn2 is a critical selective modulator of PPAR γ activation and function in lipid homeostasis and energy expenditure.

Impaired Generation of 12-Hydroxylated Bile Acids Links Hepatic Insulin Signaling with Dyslipidemia

Rebecca A. Haeusler^{1*}, Matthew Pratt-Hyatt², Carrie L. Welch¹, Curtis D. Klaassen,² and Domenico Accili¹

¹Department of Medicine, Columbia University, New York, NY 10032

²Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS 66160

The association of insulin-resistant (type 2) diabetes with elevated plasma triglyceride and very low-density lipoproteins, and intrahepatic lipid accumulation represents a pathophysiological enigma and an unmet therapeutic challenge. Recently it has come to light that defective lipid metabolism in type 2 diabetes may be due to a detrimental admixture of insulin resistance and insulin sensitivity in selective hepatic signaling pathways. FoxO1 is a transcription factor that is tightly regulated by insulin, and it has an established role in regulating the glucose production branch of hepatic insulin signaling. Mice lacking FoxO1 in liver (L-FoxO1) are insulin-sensitive and display low glucose production. Here we show that, surprisingly, when challenged with a western diet, L-FoxO1 mice have considerable defects of lipid metabolism including steatosis and, when crossed with *Ldlr*^{-/-} mice, high serum lipids. Using gene expression and metabolomic analyses, we identified alterations in bile acid composition in L-FoxO1 mice, with a deficiency in production of 12-hydroxylated bile acids. As bile acids are known to affect triglyceride and cholesterol homeostasis, we hypothesized that the lipid metabolic abnormalities were due to impaired feedback inhibition of lipid metabolism by bile acids. Supplementing L-FoxO1 mice with the major product of 12-hydroxylation, cholic acid, or a synthetic agonist of the bile acid-responsive nuclear receptor FXR, rescued their hyperlipidemia. This work reveals a novel mechanism by which insulin regulates triglyceride and cholesterol metabolism through its ability to modulate bile acid composition.

Fatty Acid-Dependent Leukotriene Production in Macrophages

Kristina Hellberg*, Eric K. Long, Ann V. Hertz, Rocio Foncea, David A. Bernlohr, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota, 55455, USA

Obesity and insulin resistance is associated with chronic low-grade inflammation in adipose tissue. Insulin resistance leads to dysfunctional lipolysis and the fatty acids released from adipocytes in the obese state promote secretion of pro-inflammatory molecules such as cytokines. In response to fatty acids macrophages not only secrete protein cytokines but also pro-inflammatory lipid molecules such as leukotrienes (LT). Furthermore, secreted leukotrienes bind to leukotriene receptors on both adipocytes and macrophages promoting adipose tissue inflammation by recruiting macrophages and affecting signaling pathways. In vitro, fatty acid binding proteins stabilize LTA_4 towards hydrolysis suggesting that they may play a role in mediating inflammatory leukotriene production. To address this, the role of fatty acids and fatty acid binding proteins on leukotriene synthesis was evaluated as well as the downstream effect of such molecules on macrophage cytokine secretion.

Using LC-MS/MS analysis the production of leukotrienes and other eicosanoids has been quantified in primary peritoneal macrophages from C57Bl/6J mice treated with fatty acids and in adipose tissue of low fat or high fat fed C57Bl/6J mice. Primary macrophages secreted increased levels of LTC_4 when stimulated with palmitate but not n-6 or n-3 fatty acids. Pre-incubation of the macrophages with HTS01037, a small molecule inhibitor of fatty acid binding proteins abolished LTC_4 production and increased the level of 5,6-diHETE. Adipose tissue of mice on a high fat diet contain increased levels of LTE_4 , PGE_2 , PGD_2 , 5(S)-HETE and 15(S)-HETE compared to low fat fed mice. To assess the downstream effects of leukotrienes, Raw264.7 macrophages were stimulated with leukotrienes and secretion of monocyte chemoattractant protein 1 was evaluated. LTD_4 stimulation increased secretion of this proinflammatory cytokine 3-fold while LTE_4 stimulated MCP-1 secretion 2-fold and LTC_4 was ineffective.

Together, these results indicate that adipocyte-derived fatty acids stimulate pro-inflammatory leukotriene synthesis in macrophages in a fatty acid binding protein-mediated manner. Stimulation of macrophages with leukotrienes elevates cytokine secretion suggesting that leukotrienes released from macrophages in the obese state can promote inflammation in adipose tissue.

Adipose Tissue Carbohydrate Responsive-Element Binding Protein Regulates Adipose Tissue Fatty Acid Synthesis and Glucose Homeostasis

Mark A. Herman*; Odile D. Peroni; Jorge Villoria; Michael R. Schön; Nada A. Abumrad, Matthias Blüher; Samuel Klein; and Barbara B. Kahn
Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center and Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA; Departments of Surgery and Medicine, University of Leipzig, Leipzig, Germany; Center for Human Nutrition, Washington University School of Medicine, St. Louis, Missouri, USA.

Insulin resistance is a major pathogenic factor underlying the increased prevalence of type 2 diabetes, cardiovascular disease, and the associated early mortality. In insulin resistant states, expression of the GLUT4 glucose transporter is down-regulated in adipocytes, but not in muscle. Genetic ablation of GLUT4 selectively in adipose tissue (AG4KO) causes whole-body insulin resistance. Conversely, transgenic mice overexpressing GLUT4 selectively in adipocytes (AG4OX) have enhanced glucose tolerance and fasting hypoglycemia. We aimed to determine the molecular mechanisms by which adipocytes sense and respond to changes in availability of glucose and other nutrients. Carbohydrate responsive-element binding protein (ChREBP) is a glucose-responsive transcription factor that regulates fatty acid synthesis and glycolysis in the liver. Its role in adipose tissue is unknown. ChREBP expression is increased 2-fold in adipose tissue of AG4OX and decreased by 50% in AG4KO while expression of SREBP1c, and other lipogenic transcription factors, are unaltered. High-fat feeding down-regulates adipose-ChREBP expression in WT and AG4OX, resulting in reduced adipose tissue fatty acid synthesis and attenuation of the enhanced glucose tolerance in AG4OX. To determine whether ChREBP is essential for the improved glucose homeostasis in AG4OX, we crossed AG4OX with ChREBP KO mice. Ablating ChREBP in AG4OX normalized the increased adiposity, adipose tissue de novo lipogenesis, glucose tolerance, and the fasting hypoglycemia. In 123 non-diabetic human subjects with normal glucose tolerance and widely ranging body mass index, ChREBP expression in adipose tissue correlates with insulin sensitivity. Moreover, among obese humans, adipose-ChREBP expression correlates with insulin sensitivity indicating that increased adipose tissue-ChREBP activity may protect against obesity-associated insulin resistance. Our data identify adipose tissue-ChREBP as an important regulator of insulin sensitivity and support the potential beneficial role of lipogenesis in adipose tissue on glucose homeostasis. This pathway in adipose tissue provides novel targets for preventing and treating obesity-related metabolic dysfunction and type 2 diabetes.

Effects of Leukotrienes on Adipocytes.

Ann V. Hertzel*, Rocio Foncea, Kristina Hellberg, Eric K. Long and David A. Bernlohr. Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN 55455, USA.

Obesity leads to the development of a chronic inflammatory state in adipose tissue, with multiple cell types secreting factors that initiate and perpetuate this phenotype. For adipocytes, the primary factor(s) implicated in this pathology are lipolytic fatty acids, whereas for macrophages, inflammatory cytokines have been shown to be involved. Our hypothesis expands this and focuses on inflammatory lipids, specifically leukotrienes, made from macrophages (and perhaps other cells) in a fatty acid-dependent manner. In support of this, adipose tissue from obese mice have increased levels of leukotriene E₄ and leukotriene B₄ as compared to adipose tissue from lean mice.

Primary adipocytes express receptors known to bind leukotrienes: cysLTR1, cysLTR2 and BLT1. However, cultured 3T3L1 adipocytes express very little to no cysLTR1, cysLTR2 or BLT1 receptors. Interestingly, conditioned media from LPS treated Raw264.7 macrophages dramatically upregulated the expression of cysLTR1, cysLTR2 and BLT1 in 3T3L1 adipocytes. The factor required for this upregulation is >3,000 MW and is heat sensitive. Proinflammatory cytokines were not effective in upregulating the cysLTRs in 3T3L1 adipocytes. To develop a cultured adipocyte model that more closely resembles primary adipocytes, cysLTR1 was overexpressed in 3T3L1 cells. Similarly, the overexpression of cysLTR2 is currently being developed. Leukotriene C₄ impaired insulin-stimulated AKT phosphorylation in cysLTR1 overexpressing adipocytes. Our results of the presence of leukotrienes in adipose tissue, expression of leukotriene receptors in adipocytes, and altered signaling suggest a physiological role for leukotrienes affecting adipocyte function(s).

Horrillo, et al., 2010. 5-Lipoxygenase Activating Protein Signals Adipose Tissue Inflammation and Lipid Dysfunction in Experimental Obesity. *Journal of Immunology* 184:3978-87.

Nicotinamide N-methyltransferase Regulates PPARα and LXRA-dependent

Pathways

Shangyu Hong*, Deepthi Prasad, Eleftheria Maratos-Flier and Pavlos Pissios

Endocrinology, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA

Nicotinamide N-methyltransferase (NNMT) is a liver enzyme mediating the clearance of nicotinamide and related compounds. Nicotinamide, one of the two forms of vitamin B3 is a precursor for nicotinamide adenine dinucleotide (NAD). NAD is a redox cofactor in multiple reactions in intermediary metabolism and a substrate for NAD-dependent enzymes such as sirtuins. For this reason we tested the effect of NNMT knockdown on the activity of two important nuclear receptors regulating lipid metabolism, PPARα and LXRA, whose activity is modulated by sirtuins.

We tested this hypothesis by virally-mediated knockdown of NNMT in vitro and in vivo. Adenoviral knockdown of NNMT in mouse primary hepatocytes decreased the expression of several LXRA target genes involved in cholesterol metabolism (ABCA1, CYP7A) and in fatty acid synthesis (FAS, SCD1, SREBP1c). Consistent with the gene expression data, acetate lipogenesis was significantly decreased by NNMT knockdown. The basal expression of several known PPARα target genes involved in fatty acid oxidation (Acox1, CD36, CPT1a, FGF21) was also decreased by NNMT knockdown. In addition, the activation of PPARα and LXRA target genes in response to selected activators WY-14643 and T-0901317 respectively, was also significantly impaired by NNMT knockdown.

Consistent with the in vitro results, NNMT knockdown in vivo decreased the expression of genes regulating lipogenesis and cholesterol metabolism (SREBP1c, SCD1, LXRA, ABCB11) and fatty acid oxidation (FGF21, CD36, Cyp4A10). NNMT knockdown also increased serum free fatty acids, serum cholesterol and liver cholesterol in vivo suggesting impaired function of these nuclear receptors

Our results identify NNMT as a novel regulator of PPARα and LXRA signaling and hepatic lipid metabolism.

Hypertrophy-driven adipocyte death overwhelms recruitment under prolonged weight gain

Junghyo Jo^{1*}, Juen Guo¹, Teresa Liu², Shawn Mullen², Kevin D. Hall¹, Samuel W. Cushman² and Vipul Periwal¹

¹Laboratory of Biological Modeling and ²Diabetes Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

Fat pads dynamically regulate energy storage capacity under energy excess and deficit. This remodeling process is not completely understood, with controversies regarding differences between fat depots and plasticity of adipose cell number. We examined changes of mouse adipose cell-size distributions in epididymal, inguinal, retroperitoneal, and mesenteric fat under both weight gain and loss. With mathematical modeling, we specifically analyzed the recruitment, growth/shrinkage, and loss of adipose cells, including the size dependence of these processes. We found a qualitatively universal adipose tissue remodeling process in all four fat depots: 1), There is continuous recruitment of new cells under weight gain; 2), the growth and shrinkage of larger cells (diameter >50 μm) is proportional to cell surface area; and 3), cell loss occurs under prolonged weight gain, with larger cells more susceptible. The mathematical model gives a predictive integrative picture of adipose tissue remodeling in obesity.

Selenate Controls Adipogenesis by Activating TGF- β Signaling

Gyo-Nam Kim*, Choon Young Kim, Julie Wiacek, and Kee-Hong Kim

Department of Food Science, Purdue University, West Lafayette, IN 47907, USA

Obesity is a major health problem and it also closely associated with metabolic disorders. Adipogenesis was defined as a differentiation process of preadipocyte into the adipocytes. Thus, to control of this process can be one of the most important strategies to prevent obesity. Selenium has been reported to possess potent antioxidant and pro-apoptotic properties. Moreover, its role in cancer prevention has been well-documented. However, the precise biological role of selenium for prevention of obesity remains unknown. Here we showed the potential role of selenium and its molecular mechanism underlying regulation of adipogenesis of 3T3-L1 cells. Selenium exists in various forms. Among selenate, selenite, and methylseleninic acid (MSA), we found that selenate showed no effect on the viability of 3T3-L1 preadipocytes. Both selenite and MSA exerted cytotoxicity in 3T3-L1 cells. We also found that selenate dose-dependently inhibited adipogenesis, accompanied by reduced level of adipogenic marker genes, such as peroxisome proliferator-activated receptor-gamma (PPAR γ) and fatty acid synthase (FAS). This anti-adipogenic function of selenate was limited to the early phase of adipogenesis. Interestingly, selenate pretreated preadipocytes also displayed impaired adipogenesis, suggesting a role of selenate in fate determination of preadipocytes. However, other forms of selenium at low concentration enough to be safe for 3T3-L1 cells had no effect on adipogenesis. To obtain further mechanistic insight into selenate's anti-adipogenic function, we then tested the effects of various inhibitors such as SB431542 (a TGF- β receptor inhibitor), SP600125 (a JNK inhibitor), LY294002 (a PI3K inhibitor), U0126 (an ERK inhibitor), on adipogenesis of selenate-treated preadipocytes. Among these inhibitors, SB431542 and U0126 completely restored selenate-inhibited adipogenesis. Moreover, selenate treated 3T3-L1 cells exhibited a dose-dependent expression of genes involved in TGF β signaling such as TGF β 1 and fibronectin. Selenate was able to induce expression of TGF β downstream genes in preadipocytes to a similar extent of recombinant TGF β protein. These results implicate that the inhibitory action of selenate is strongly linked to the TGF β 1 activation both in undifferentiated preadipocytes and the early phase of adipogenesis. Taken together, our results reveal a novel function of selenate in adipogenesis. This preventive and inhibitory role in adipogenesis appears to be specific to selenate, which is generally considered to have the least cancer preventive function among other forms of selenium. Since TGF β is a multipotent cytokine affecting on a wide range of cellular processes, including inhibition of adipogenesis, our study also elucidates selenate as a new inorganic dietary activator of TGF β signaling.

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

Alison B. Kohan*, Fei Wang, Xiaoming Li, and Patrick Tso

Department of Pathology and Laboratory Medicine, University of Cincinnati, Cincinnati OH, 45237

Many functions have been attributed to apoA-IV, an intestinally derived apolipoprotein secreted in response to fat in the diet, including its role as an anti-oxidant, 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 here that apoA-IV plays a role in mediating triglyceride and cholesterol homeostasis in plasma. Loss of apoA-IV causes a significant decrease in plasma lipid levels in mice maintained on chow diet, confirming the findings of Breslow et al (1997). Further, apoA-IV KO mice are resistant to high fat diet-induced increases in plasma TG and cholesterol. The lowered plasma lipids in apoA-IV knockout mice are not due to differences in body weight, since apoA-IV KO and WT animals have comparable body weights. Additionally, apoA-IV KO mice have a total fat mass to body weight ratio that is comparable to WT 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 KO mice do not have altered triglyceride-rich lipoprotein secretion from the liver. These findings suggest 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 triglycerides or cholesterol from the intestine into lymph. Whether apoA-IV affects chylomicron metabolism is the focus of ongoing experiments.

Apolipoprotein E Isoforms and Cholesterol Metabolism in the Retinal Pigment Epithelium: Insight into the Cell Biology of Age-Related Macular Degeneration

Aparna Lakkaraju* and Jin Xu, Department of Ophthalmology & Visual Sciences, University of Wisconsin-Madison, Madison, WI, 53706, USA.

Increasing evidence suggests that age-related macular degeneration (AMD), atherosclerosis and Alzheimer's disease share a common etiological pathway. Both atherosclerosis and Alzheimer's disease are characterized by chronic inflammation caused, in part, by disturbances in cholesterol homeostasis. Cholesterol is an abundant component of drusen, lipid-protein aggregates that accumulate beneath the retinal pigment epithelium (RPE), similar to cholesterol accumulation in the vascular intima in atherosclerosis. Drusen and lipofuscin, autofluorescent lipid-protein aggregates which accumulate within RPE lysosomes, are hallmarks of AMD. We have previously shown that lipofuscin interferes with cholesterol metabolism and trafficking in RPE cells (Lakkaraju *et al*, 2007).

Epidemiological studies indicate that allelic variants in cholesterol transporters ABCA1 and apolipoprotein E (ApoE) and elevated C-reactive protein and homocysteine levels are associated with AMD. ApoE occurs in three isoforms in humans: ApoE2, E3 and E4. The E4 allele is a risk factor for Alzheimer's disease and the E2 allele is protective. Interestingly, the converse is true for AMD: ApoE2 is a risk factor and ApoE4 is protective. However, mice expressing human ApoE4 on a high fat diet exhibit many features of AMD. Interpretation of these seemingly conflicting data is hampered by the lack of information about isoform-specific effects of ApoE on cholesterol uptake, recycling and metabolism in the RPE. To address this issue, we have begun to investigate the intracellular trafficking and function of ApoE2, E3 & E4 in RPE cells.

Immunofluorescence staining and confocal microscopy showed that endogenous ApoE is present throughout the cytoplasm in vesicular structures with a small fraction present in late endosomes and the Golgi complex. Live imaging of GFP-tagged ApoE2, E3 or E4 in RPE cells using spinning disk confocal microscopy showed vesicles and tubules undergoing vectorial transport with mean velocities of 3 $\mu\text{m}/\text{second}$. ApoE4 vesicles were larger and more numerous compared to ApoE2 or ApoE3 vesicles. Treating RPE cells with liver X receptor (LXR) agonists or loading the cells with lipofuscin increased ApoE expression. Analysis of cholesterol distribution and measurement of cholesterol content in cells expressing different ApoE isoforms are ongoing. Understanding the role of ApoE2/3/4 in the RPE and retina is important because ApoE isoforms influence response to statins, which may be beneficial in AMD. ApoE2, E3 and E4 also exhibit differential lipoprotein binding preferences (VLDL vs LDL vs HDL), which could influence the delivery of dietary carotenoids to the retina and RPE.

Reference: Lakkaraju, A., Finnemann, S.C. and Rodriguez-Boulan, E. (2007) The lipofuscin fluorophore A2E perturbs cholesterol metabolism in retinal pigment epithelial cells. *Proc Natl Acad Sci U S A*, **104**, 11026-11031.

Support: Research to Prevent Blindness Career Development Award, American Health Assistance Foundation grant M2009093, Karl Kirchgeßner Foundation Vision Research Grant, UWMF

Deficiency of the monoacylglycerol acyltransferase MGAT2 increases energy expenditure without high-fat feeding and protects genetically obese mice from excessive weight gain

David W. Nelson^{*}, Yu Gao, Nicole M. Spencer, Taylor Banh, and C.-L. Eric Yen

Department of Nutritional Sciences, University of Wisconsin–Madison, WI 53706, USA

Acyl CoA:monoacylglycerol acyltransferase (MGAT) 2 is thought to be crucial for dietary fat absorption. Indeed, mice lacking the enzyme (*Mogat2*^{−/−}) are resistant to obesity and other metabolic disorders induced by high-fat feeding (1). However, these mice absorb normal quantities of fat. To explore if a high level of dietary fat is an essential part of the underlying mechanism(s), we examined metabolic responses of *Mogat2*^{−/−} mice to diets containing varying levels of fat. *Mogat2*^{−/−} mice exhibited 10–15% increases in energy expenditure compared to wildtype littermate controls; while high levels of dietary fat exacerbated the effect, this phenotype was expressed even on a fat-free diet. When deprived of food, *Mogat2*^{−/−} mice expend energy and lose weight like wildtype controls. To determine if MGAT2 deficiency prevents excess weight gain in the absence of high-fat feeding, we conducted two studies. In the first study, wildtype and *Mogat2*^{−/−} mice were fed a calorie-dense diet rich in refined carbohydrate. While wildtype mice increased their body weight a significant 14.5% after 2 months, *Mogat2*^{−/−} mice maintained their baseline body weights. In the second study, *Mogat2*^{−/−} mice were crossed with genetically obese Agouti mice. MGAT2 deficiency increased energy expenditure and prevented these mice from adult-onset obesity seen in Agouti mice (35.3±0.7 g versus 48.3±1.1 g; 40-week-old males). Our results suggest that MGAT2 modulates energy expenditure through multiple mechanisms, including one independent of dietary fat. Inhibiting MGAT2 may prove to be useful in combating obesity and related metabolic disorders resulting from excessive calorie intake.

Literature Cited

1. Yen, C. L., Cheong, M. L., Grueter, C., Zhou, P., Moriwaki, J., Wong, J. S., Hubbard, B., Marmor, S., and Farese, R. V., Jr. (2009) Deficiency of the intestinal enzyme acyl CoA:monoacylglycerol acyltransferase-2 protects mice from metabolic disorders induced by high-fat feeding. *Nat Med* **15**, 442–446 (PMCID: PMC2786494)

Hepatic ATGL Knockdown Uncouples Insulin Resistance and Glucose Intolerance from Liver TAG Accumulation

Kuok Teong Ong*, Mara T. Mashek, So Young Bu and Douglas G. Mashek
Department of Food Science and Nutrition, University of Minnesota, St. Paul,
MN, 55108

Hepatic adipose triglyceride lipase (ATGL) catalyzes the first step in triglyceride (TAG) hydrolysis. We have shown that mice with suppressed hepatic ATGL expression accumulate TAG due to attenuated TAG hydrolysis. Since hepatic steatosis is tightly associated with insulin resistance, we investigated whether hepatic ATGL mediates glucose tolerance and hepatic insulin action. Mice treated with adenovirus harboring shRNA towards ATGL exhibited lower fasting serum glucose when fed either chow or high fat (HF) diets for 7 d following administration of the adenovirus. Additionally, ATGL knockdown completely blocked the increase in serum insulin following 7 d of the HF diet despite higher hepatic TAG content. Oral glucose tolerance test (OGTT) demonstrated that ATGL shRNA-treated mice were more responsive to an oral glucose load than control mice suggesting that decreased ATGL expression confers enhanced glucose tolerance. Although ATGL shRNA-treated mice had steatotic livers, acute insulin administration had no effect on phosphorylation of insulin signaling mediators including Akt and IRS-1 compared to control mice. To further examine the role of hepatic ATGL in insulin resistance and glucose tolerance, we also evaluated the effects of hepatic ATGL deficiency on mice that underwent a 12-week HF diet regimen. The increase of both serum glucose and insulin levels by the HF diet was completely blocked in ATGL knockdown mice. Moreover, ATGL knockdown prevented glucose intolerance induced by the HF diet; OGTT revealed that ATGL knockdown mice fed the HF diet had a similar AUC to that of control mice fed the chow diet. Interestingly, ATGL knockdown also dissociated impairments in hepatic insulin signaling from the elevated TAG content. Taken together, these data suggest that ATGL knockdown enhances glucose tolerance and uncouples hepatic insulin action from TAG accumulation.

Sunscreens block of UV-induced IL-11 secretion might prevent

UV-induced facial fat loss

Wen-Hwa Li, Apostolos Pappas*, Li Zhang, and Druie Cavender.

Skin Research Center, A unit of Johnson and Johnson Consumer Companies, Inc.,

199 Grandview Road, Skillman, New Jersey 08558, USA.

Interleukin-11 (IL-11) is produced by fibroblasts when exposed to IL-1, TGF- β or phorbol esters. The differentiation of preadipocytes in vitro is inhibited by exposure to IL-11. We have hypothesized that solar radiation induced IL-11 secretion from dermal fibroblasts would inhibit preadipocyte differentiation and therefore contributes to the loss of subcutaneous fat in sun-exposed skin. Human epidermal-dermal equivalents exposed to a physiological dose of radiation (5 J/cm²) from a solar simulator had a marked increase in IL-11 expression and secretion. When equivalents were treated with a sunscreen before solar irradiation, the production of IL-11 by the dermal fibroblasts was blocked. The differentiation of human facial preadipocytes was prevented by treatment of conditioned medium from equivalents exposed to solar radiation. However, preadipocytes incubated with conditioned medium from sunscreen-treated equivalents, but not placebo, continued to differentiate normally. These results suggest that IL-11 is involved in the loss of subcutaneous fat and photoaging. Sunscreens could inhibit this process which may slow photoaging-induced changes in facial contouring.

Muscle Specific Overexpression of SCD1 to Promote Metabolic and Cardiovascular Health.

Chad M. Paton^{1,2}, Matthew T. Flowers², James M. Ntambi².

¹Division of Animal & Nutritional Sciences, West Virginia University, Morgantown, WV 26506.

²Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, 53706.

Email: chad.paton@mail.wvu.edu

Purpose: Stearoyl-CoA desaturase (SCD1) converts saturated fatty acids into monounsaturated fatty acids. A number of recent reports have suggested that SCD1 expression decreases in liver with exercise training, while others have suggested it increases in skeletal muscle. Using muscle knockout and transgenic models, we sought to determine the role of SCD-1 expression in contributing to exercise induced remodeling of muscle metabolism.

Methods and Results: Using transgenic mouse models, we sought to determine the role of SCD-1 expression in contributing to muscle glucose and lipid metabolism. Male wild type (WT) and SCD1 actin-transgenic (SCD1 Tg) mice were studied for acute exercise capacity using swim and grip time to exhaustion. Exercise training was conducted by providing access to a free wheel for 4 weeks. Tissues were collected for protein, mRNA, and lipid analysis as well as body weights. Glucose tolerance tests (GTT) were conducted following IP injection of 1g/Kg glucose. SCD1 Tg mice displayed higher SCD1 protein in both white and red muscle. The increase in SCD1 expression dramatically affected white gastroc lipid composition to increase triglyceride (TG) content (52.3±15 vs. 111.4±24 mg/μg protein WT vs. SCD1 Tg). The fatty acid composition of the muscle revealed a striking increase in PUFA content of TG. Untrained SCD1 Tg mice also displayed significantly increased exercise capacity with over two-fold increases in grip and swim time to exhaustion and free wheel running distance. Lastly, overexpression of SCD1 in muscle decreased fasting plasma glucose, increased glucose tolerance, fatty acid oxidation, and PPARδ target gene expression.

Conclusion: The present model lends further support to the notion that increasing metabolic demand using physical activity is an effective means to assess the impact of SCD1 on muscle metabolism. The result of elevated SCD1 expression appears to contribute to exercise capacity by promoting oleate synthesis and increasing the *de novo* lipogenic capacity of muscle. Continuing studies will be conducted to clearly delineate the contribution of *de novo* oleate synthesis in enhancing muscle lipid metabolism and metabolic flux.

Loss of *Ces1/Es-x* In Mice Results In Obesity, Increased Blood Lipid Levels and Decreased Energy Expenditure

Ariel D. Quiroga^{*1,3}, Lena Li^{1,3}, Spencer D. Proctor^{4,3}, Harald C. Köfeler⁵ and Richard Lehner^{1,2,3}

¹Department of Pediatrics, ²Department of Cell Biology, ³Group on Molecular and Cell Biology of Lipids, ⁴Metabolic and Cardiovascular Diseases Laboratory, University of Alberta, Edmonton, AB T6G 2S2, Canada; ⁵Medical University Graz, Graz, Austria.

Mouse carboxylesterase1/esterase-x (*Ces1/Es-x*) is a close homolog of carboxylesterase3/triacylglycerol (TG) hydrolase (*Ces3/TGH*). Its over-expression in McArdle-RH7777 (McArdle) cells led to decreased TG levels and to increased β -oxidation when incubated with oleic acid (Ko et al., 2009). We proposed that mice lacking the *Ces1/Es-x* gene would have altered lipid metabolism. *Ces1/Es-x*^{-/-} mice on a chow diet presented increased body weight, increased adiposity and enlarged adipocytes despite the same level of food intake as wild-type mice. The *Ces1/Es-x* knockout mice accumulated more lipids in plasma and liver, and had increased chylomicron and VLDL secretion with delayed lipoprotein clearance. Hepatocytes from *Ces1/Es-x*^{-/-} mice showed increased lipid biosynthesis, due to activation of the sterol regulatory binding protein-1c (SREBP-1c) and its target genes. *Ces1/Es-x* deficient mice also accumulated polyunsaturated fatty acid (PUFA) in TG. On the other hand, McArdle cells stably expressing *Ces1/Es-x* cDNA, presented with lower amounts of TG enriched in long chain fatty acids. These experiments suggested that *Ces1/Es-x* might be hydrolyzing long chain fatty acid-enriched TG, presumably, PUFA-enriched TG. In line with this potential mechanism, incubation of *Ces1/Es-x*^{-/-} hepatocytes with PUFA decreased lipid synthesis to the same levels obtained in wild-type hepatocytes. In conclusion, *Ces1/Es-x* may regulate lipoprotein production/secretion and therefore fat accumulation. We found that *Ces1/Es-x* protein expression undergoes nutritional regulation and its activity seems to play a key role in modulating the expression of enzymes that control lipid biosynthesis. These results make *Ces1/Es-x* a striking pharmacological candidate for preventing hepatic steatosis and obesity.

Ko, K.W., Erickson, B., and Lehner, R. (2009). *Es-x/Ces1* prevents triacylglycerol accumulation in McArdle-RH7777 hepatocytes. *Biochimica et biophysica acta* 1791, 1133-1143.

Effect of Obesity on in vitro Hemolysis using Familial Hypercholesterolemic Swine

Mark P. Richards*, Andrea J. Smiltneek, Dhanansayan Shanmuganayagam, Christian G. Krueger, Thomas D. Crenshaw, and Jess D. Reed, Animal Sciences, University of Wisconsin-Madison, Madison, WI 53706, USA.

A link between hemolysis and atherosclerotic vascular disease (chronic risk) has been described. In addition, hemolysis is believed to increase the incidence of thrombosis (acute risk). Excess free hemoglobin depletes plasma nitric oxide, leading to platelet activation and impaired fibrinolysis. The liberation of hemoglobin from erythrocytes leads to release of heme that can oxidize various biomolecules including low-density lipoproteins and nitric oxide that play a mechanistic role in the development of atherosclerosis and thrombosis. Eighteen familial hypercholesterolemic piglets (females) were separated into 3 treatment groups (n=6): an ad libitum group, 80% of ad libitum, and 60% of ad libitum. Hemolysis values were determined at 5, 6, 7, 8, and 9 months of age. Weights of pigs at 9 months were 85-108 kg (ad lib), 64-81 kg (80% diet) and 41-50 kg (60% diet). Hemolysis of whole blood was induced with varying concentrations of hydrogen peroxide (0.1 to 500 mM). All samples were adjusted to 0.5% erythrocytes (v/v) during incubation. Hemolysis score was determined based on the concentration of hydrogen peroxide required to induce a threshold level of hemolysis using 406 nm absorbance values in the supernatant after centrifugation. The scores ranged from 0-10 with higher score indicating higher hemolysis. Hemolysis scores were significantly higher in the ad lib group compared to the 60% group at some but not all of the time points. Hemolysis scores at each time point were also combined to generate a single hemolysis value for each pig over the time course of the study. The hemolysis values were 1.7-6.5 (ad lib), 1.7-6.0 (80% diet) and 1.4-3.2 (60% diet). The ad lib group was significantly higher than the 60% group. These results suggest that obesity increases the incidence of hemolysis in an animal model of atherosclerosis. The large pig to pig variation within group suggests that factors other than body composition and adiposity dictate susceptibility to hemolysis.

A Novel Role for Ste-20 Like Kinase MAP4K4 in Endothelial Cell Activation

Rachel J. Roth Flach* and Michael P. Czech

Program in Molecular Medicine, University of Massachusetts Medical School,
Worcester, MA 01605 USA

Adipose tissue dysfunction is a key component in the development of insulin resistance and type II diabetes(1). In obesity, increased levels of cytokines such as tumor necrosis factor α (TNF- α) from adipocytes and/or adipose resident macrophages may cause adipose tissue endothelial cells comprising the vasculature to express cell surface adhesion molecules that increase leukocyte infiltration into adipose tissue(2). Consistent with this concept, soluble forms of endothelial derived leukocyte adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin are increased in obese and diabetic humans(3). Therefore, determining the contribution of adipose tissue endothelial cell activation to adipose tissue dysfunction in obesity may provide insight into how these events may contribute to insulin resistance and type II diabetes.

In an siRNA screen to find protein kinases that regulate lipogenesis, our laboratory identified the ste-20 like kinase mitogen activated protein kinase kinase kinase 4 (MAP4K4)(4). We found that MAP4K4 suppresses lipogenesis in adipocytes by decreasing PPAR γ expression through an mTOR-dependent pathway(5), and is a positive mediator of systemic inflammation in macrophages(6). Furthermore, multiple studies have demonstrated that MAP4K4 is positively regulated by TNF α and plays an essential role in TNF α -mediated dysfunction in skeletal muscle and pancreas(7-9). However, a role for MAP4K4 in endothelial cell activation has not yet been addressed. We have found that MAP4K4 is expressed at relatively high levels in human and mouse endothelial cells, and silencing of MAP4K4 in these cells attenuates the induction of leukocyte adhesion molecules ICAM-1, VCAM-1, and E-selectin by over 30% at both the RNA and protein level in response to TNF α stimulation. In addition, knockdown of MAP4K4 in human endothelial cells results in a 40% decrease in adhesion of human monocytes to a monolayer of endothelial cells that have been activated by TNF α . These data implicate MAP4K4 as a novel positive regulator of endothelial cell activation and suggest that endothelial MAP4K4 signaling in response to cytokines and other factors may contribute to leukocyte recruitment and inflammation in adipose tissue and potentially to adipose tissue dysfunction.

The Role of Sorcs1 in Hormone Transport

Lindsay R. Schneider*¹, Brendan J. Floyd*², Angie Oler² and Alan Attie²

¹Department of Cellular and Molecular Pathology, ²Department of Biochemistry,
University of Wisconsin-Madison, Madison, WI, 53706, USA

Dysfunction in several hormone pathways, including leptin, insulin and glucagon contributes to the pathogenesis of diabetes. Leptin is released by adipocytes in response to feeding and suppresses food intake. Defective hormone transport across cellular and endothelial membranes prevents the hormones from reaching target tissues, decreasing the effectiveness of signaling pathways.

Recent human genetic studies show association of SNPS in *SORCS1* with type 2 diabetes and diabetes and hemoglobin A1c in type 1 diabetics. We recently generated whole-body and tissue-specific *Sorcs1* knockout (KO) mice. Female whole-body *Sorcs1*^{-/-} mice consume more food, display twice the fat mass, and yet weigh 9% less than their controls. In contrast to wild type mice, *Sorcs1*^{-/-} mice do not suppress food intake in response to intraperitoneal leptin injection (5 mg/kg). Surprisingly, neuronal *Sorcs1*^{-/-} mice (*Nes-Cre*) displayed the normal reduction in food intake in response to i.p. leptin. Additionally, the whole-body *Sorcs1*^{-/-} mice have elevated C-peptide to insulin ratios and decreased plasma insulin or glucagon 30 minutes after injection of those hormones, suggesting faster insulin and glucagon turnover kinetics.

We hypothesize that Sorcs1 is involved in leptin transport across the blood-brain barrier and perhaps also, leptin and insulin transport in the kidney proximal tubules. These results explain how genetic variation at *Sorcs1* can contribute to obesity and diabetes.

The Phospholipase C (PLC) Inhibitor U73122 Inhibits Trans-10, cis-12 Conjugated Linoleic Acid (CLA)-Mediated Inflammatory Signaling and Insulin Resistance in Human Adipocytes

Wan Shen*, K. Martinez, C-C. Chuang, A. Overman, M. McIntosh, Department of Nutrition, University of North Carolina at Greensboro, Greensboro, NC, 27402.

Our lab has shown that CLA increases intracellular calcium, inflammation, and insulin resistance in human primary adipocytes, which is consistent with the actions of diacylglycerol and inositol triphosphate from PLC-dependent cell signaling. Thus, we hypothesized that PLC is an upstream activator of these cellular responses to CLA. To test this hypothesis, cultures were pretreated with U73122, a universal PLC inhibitor, followed by CLA treatment for 18-48 h. U73122 prevented CLA-induced gene expression of Ca^{2+} /calmodulin-dependent protein kinase- β , cyclooxygenase-2, monocyte chemoattractant protein-1, interleukin (IL)-6, and IL8, as well as IL-6 and IL8 secretion. Consistent with these data, U73122 attenuated CLA-mediated activation of extracellular signal-related kinase, c-Jun N-terminal kinase, and c-Jun, an activator protein-1 transcription factor. U73122 also attenuated CLA-suppression of 1) the mRNA levels of peroxisome proliferator-activated receptor (PPAR)- γ , insulin-stimulated glucose transporter-4, acetyl-CoA carboxylase-1, and stearoyl-CoA desaturase-1, 2) PPAR- γ protein level, and 3) insulin-stimulated glucose uptake. Taken together, these data suggest that PLC plays an important role in CLA-mediated activation inflammation and insulin resistance. Supported by NIH DK-ODS 5R01 DK063070-07, NCARS 06771.

Role of Hepatic Monounsaturated Fatty Acid Synthesis in Metabolic Regulation

Maggie Strable¹, Matthew Flowers², Xueqing Liu², James Ntambi^{1,2}

¹Department of Nutritional Sciences, ²Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706, USA

Stearoyl-CoA desaturase 1 (SCD1) catalyzes the *de novo* synthesis of monounsaturated fatty acids (MUFA) from saturated fatty acids. Global SCD1 deletion in mice (GKO) results in reduced lipogenesis, increased fatty acid oxidation, and protects from obesity, insulin resistance, and hepatic steatosis. Impaired endogenous MUFA synthesis coupled to feeding a high-sucrose very low-fat diet (VLF; Harlan TD03045; 2.5% kcal fat) causes liver dysfunction and hypoglycemia. Hepatic gene expression microarray analyses revealed that GKO mice fed the VLF diet for 10 days develop severe hepatic endoplasmic reticulum (ER) stress and inflammatory responses. However, GKO exhibit a complex phenotype and it is difficult to determine tissue-specific roles of SCD1 in metabolic regulation in this model, as SCD1 is deleted simultaneously from all tissues. To address this issue, we have created liver-specific SCD1-deficient (LKO) and liver-specific human SCD5 transgenic mouse models. LKO recapitulated the impaired VLF diet-induced gluconeogenesis and lipogenesis. LKO mice also developed the VLF-induced hepatic ER stress and inflammation as observed by increased phosphorylation of eIF2 α , as well as dramatically elevated expression levels of CHOP, spliced XBP-1, Grp78, ATF3, ASNS, TNF α , ICAM-1, and CD68, among others. Additionally, gluconeogenesis remains impaired in VLF-fed LKO mice. VLF supplementation with oleate, but not stearate, rescues these phenotypes with normalization of gluconeogenesis, lipogenesis, and liver dysfunction.

To establish the role of hepatic oleate in metabolic regulation and to determine if it is sufficient to restore the impaired lipogenic program and normalize the ER stress and inflammation in SCD1-deficient mice, we introduced the liver-specific human SCD5 transgene into GKO mice (hSCD5/GKO). Hepatic oleate synthesis prevented VLF-induced weight loss and resulted in increased white adipose tissue weights. Hepatic SREBP-1 maturation is increased in the hSCD5/GKO mice while increased expression of several lipogenic genes also occurs. Furthermore, plasma glucose levels are increased as compared to GKO mice. Expression of hSCD5 normalized ER stress and inflammation mRNA levels in GKO mice. Overall, these results suggest that hepatic MUFA availability is not only involved in the regulation of lipogenesis but is also important for gluconeogenesis and ER homeostasis.

Supported by NIH.

Increased intestinal acyl-CoA:diacylglycerol acyltransferase 2 exacerbates diet-induced hepatic steatosis

Aki Uchida^{1,2}, Mary C. Whitsitt², Trisha Eustaquio^{3,5}, James F. Leary^{3,4,5}, and Kimberly K. Buhman^{1,2}

¹Interdisciplinary Life Science Program, ²Department of Food and Nutrition, ³Weldon School of Biomedical Engineering, ⁴Department of Basic Medical Sciences and ⁵Birck Nanotechnology Center
Purdue University, West Lafayette, IN

Acyl-CoA:diacylglycerol acyltransferase 2 (DGAT2) is one of two DGAT enzymes that catalyze the final and committed step in triacylglycerol (TG) synthesis in the absorptive cells of the intestine, enterocytes. DGAT2 mRNA is higher in intestinal mucosa of mice challenged acutely and chronically by a high fat diet (HFD). To determine the effect of increased intestinal DGAT2 mRNA levels on delivery of dietary fat, we generated and characterized mice with intestine specific over-expression of DGAT2 (DGAT2^{Int}). The postprandial triglyceridemic response was determined in chow and HFD fed mice by measuring blood TG concentration before and after an oral oil bolus. We found a higher AUC for DGAT2^{Int} compared to wild-type (WT) mice fed chow and similar chylomicron size under these conditions. However, a similar postprandial triglyceridemic response was found in DGAT2^{Int} and WT mice fed a HFD. TG secretion from the intestine was determined by inhibiting clearance of TG from blood and measuring blood TG concentrations before and after an oral oil bolus. We found a higher TG secretion rate in DGAT2^{Int} compared to WT mice fed chow or HFD. In addition, we found no difference in TG accumulation in enterocytes of DGAT2^{Int} and WT mice fed a HFD. DGAT2^{Int} and WT mice had similar growth curves, food intake, and fecal fat when fed a HFD. Interestingly, DGAT2^{Int} mice had significantly higher fasting blood TG concentration and liver TG levels compared to WT mice fed a HFD. These outcomes suggest that altering dietary fat absorption by increased intestinal DGAT2 mRNA exacerbates diet-induced hepatic steatosis.

Funded by an AHA Scientist Development Grant to KKB and an AHA Predoctoral Fellowship to AU.

Genetics of Nonalcoholic Fatty Liver Disease

Chen-Yu Wang

Department of Biochemistry

University of Wisconsin Madison

Nonalcoholic fatty liver disease (NAFLD), which is characterized by the accumulation of triglyceride (TG) in hepatocytes, is the most common cause of liver dysfunction and affects ~25% of the US population. NAFLD is associated with several metabolic diseases, including obesity, insulin resistance, type 2 diabetes, dyslipidemia, and cardiovascular diseases. Susceptibility to NAFLD is affected by genetic factors. I have mapped several gene loci influencing the content of TG in liver tissue in a cross between C57BL/6 (B6) and BTBR mouse strains made genetically obese with the Lepob/ob mutation. Two quantitative trait loci (QTL), on chromosome 5, and 17 respectively, were identified, where the largest effect is on chromosome 17. The goal of my studies is to identify the gene(s) underlying the chromosome 17 QTL and the downstream pathway leading to fatty liver. The interval-specific congenic strain strategy is being used to positionally clone the major regulator(s) on chromosome 17 for TG accumulation in NAFLD. While generating the congenic strains, I also investigated the genes on chromosome 17 from 0 to 60 Mb and identified a plausible candidate regulator for NAFLD, Tsc2. Tsc2 encodes tuberin and functions as a GAP (GTPase-activating protein) toward Rheb to regulate the phosphorylation and activation of mammalian target of rapamycin (mTOR), which is involved in many physiological processes, including cell growth, proliferation and metabolism. We show that Tsc2^{+/-} mice have increased SREBP1c expression in an insulin-independent manner.

An Mttp-independent Pathway for Cholesterol Absorption Revealed by LXR Agonist Treatment in Mice

Yan Xie*, Susan Kennedy, Rohini Sidhu, Jianyang Luo, Daniel S. Ory, Nicholas O. Davidson
Washington University School of Medicine, St. Louis, MO63017, USA.

Intestinal cholesterol (Ch) absorption is a multistep process that involves coordinated interactions of the recycling transporter, Npc1L1, assembly and maturation (via Mttp) of intestinal chylomicrons (CM) as well as the regulated expression of the basolateral efflux pump, Abca1. The relative importance of these distinct processes, however, is poorly understood. Liver X receptors (LXRs) are important regulators of cholesterol (Ch) metabolism, as shown by studies where the LXR agonist T0901317 (T) raised HDL cholesterol in wild type but not Npc1l1^{-/-} or intestine-specific Abca1 knockout mice. In addition, LXR agonist treatment induces hepatic lipogenesis in WT but not Npc1l1^{-/-} mice, suggesting that intestinal cholesterol absorption is required for the effects of LXR agonists on hepatic lipid metabolism. We reexamined the pathways involved in intestinal Ch mobilization and the integrated regulation of hepatic lipid metabolism in response to LXR agonist treatment using intestine-specific microsomal triglyceride transfer protein knockout mice (Mttp-IKO) which exhibit an almost complete block (<3%) in Ch absorption. Mttp-IKO and WT mice were treated with T for 7 days. Serum HDL cholesterol levels increased in both groups, although the absolute levels were higher in control T mice. T treatment increased fecal neutral sterol excretion in WT (from 6.2±0.8 to 25.2±2.7 mg/day/100g BW), but paradoxically reduced fecal neutral sterol excretion in Mttp-IKO mice (from 81.0±12.7 to 52.2±2.5 mg/day/100g BW). In parallel, Ch absorption decreased in WT mice (37.7±2.8 to 11.7±2.2 %, p<0.01) but increased in the T treated Mttp-IKO group (from 3.9 ± 0.9 to 8.8 ± 2.9%, p<0.05). The findings suggest that LXR agonists decrease cholesterol absorption in WT mice but induce a paradoxical increase in cholesterol absorption in the absence of Mttp. Intestinal Ch and oxysterol content increased in Mttp-IKO mice and intestinal Npc1L1 expression was reduced >80% in Mttp-IKO mice with or without T compound. Intestinal Abca1 expression was similar in both genotypes at baseline; T treatment induced a similar, 7-fold increase in both genotypes. We then went on to examine the regulation of hepatic lipid metabolism in response to LXR agonist treatment. Hepatic triglyceride content increased with T in both WT (71.3±10.3 vs 260.4±18.5, p<0.01) and Mttp-IKO (39.5±6.3 vs 246.7±21.7, p<0.01). Biliary Ch secretion was increased by T in WT (0.02 ± 0.003 vs 0.07 ± 0.03 μmol/min/Kg BW, p< 0.01), but T treatment induced no further induction in Mttp-IKO mice (0.11 ±0.02 vs 0.09 ± 0.01 μmol/min/Kg BW). In line with these findings, hepatic lipogenic (Fas, Srebp-1c, Scd1, Acc) and biliary cholesterol transporter (Abcg5/8) mRNA expression was comparable in T treated WT and Mttp-IKO mice. These findings suggest that the augmented hepatic lipogenesis observed with LXR agonist treatment is independent of CM secretion and does not require either Npc1l1 expression or physiological amounts of cholesterol absorption. In addition, our findings demonstrate a surprising and novel pathway for intestinal cholesterol absorption that is LXR dependent, yet independent of CM assembly and does not require intestinal apoB secretion.

Symposium Participant Addresses

Caroline Alexander
UW-Madison
McArdle Laboratory
MadisonWI 53706 USA
alexander@oncology.wisc.edu

Rozalyn Anderson
UW-Madison
2870 University Avenue
MadisonWI 53705 USA
cmrevels@medicine.wisc.edu

Fariba Assadi-Porter
UW-Madison
433 Babcock Drive
MadisonWI 53706 USA
fariba@nmrfam.wisc.edu

Alan Attie
UW-Madison
433 Babcock Drive
MadisonWI 53706 USA
dschroeder@biochem.wisc.edu

Mieke Baan
UW-Madison
3314 Thorp Street
MadisonWI 53714 USA
baan@svm.vetmed.wisc.edu

Taylor Banh
UW-Madison
548 West Main Street
MadisonWI 53703 USA
banh@wisc.edu

David Bernlohr
University of Minnesota-Twin Cities
321 Church Street SE
MinneapolisMN 55455 USA
bernl001@umn.edu

Sushant Bhatnagar
UW-Madison
433 Babcock Drive
MadisonWI 53706 USA
dschroeder@biochem.wisc.edu

Sudha Biddinger
Children's Hospital Boston
300 Longwood Avenue
BostonMA 02130 USA
sudha.biddinger@childrens.harvard.edu

Emilie Boutet
University of Massachusetts
373 Plantation Street
WorcesterMA 01605 USA
Emilie.Boutet@umassmed.edu

Harpreet Brar
UW-Madison
580 Biochemistry Addition
MadisonWI 53706 USA
hbrar@wisc.edu

Dawn Brasaemle
Rutgers University
96 Lipman Drive
New BrunswickNJ 08901 USA
Brasaemle@aesop.rutgers.edu

David Brockman
University of Minnesota
1334 Eckles Avenue
St. PaulMN 55108 USA
broc0084@umn.edu

Kimberly Buhman
Purdue University
700 West State Street
West LafayetteIN 47907 USA
kbuhman@purdue.edu

Guoqing Cao
Eli Lilly Company
Lilly Corporate Center
IndianapolisIN 46285 USA
cao_guoqing@lilly.com

Joshua Carson
UW-Madison
3107 Stevens Street
MadisonWI 53705 USA
jcarson2@wisc.edu

Chih-Yu Chen
Purdue University
745 Agriculture Mall Drive
West LafayetteIN 47907 USA
chen22@purdue.edu

Xiaoli Chen
University of Minnesota
1334 Eckles Avenue
St. PaulMN 55108 USA
xlchen@umn.edu

Brad Clark
UW-Madison
433 Babcock Drive
MadisonWI 53706 USA
bradclark@wisc.edu

Rosalind Coleman
University of North Carolina
Michael Hooker Research Center
Chapel HillNC 27599 USA
rcoleman@unc.edu

Mark Cook
UW-Madison
1675 Observatory Drive
MadisonWI 53706 USA
mcook@wisc.edu

Vincent Cryns
Northwestern University
Lurie 4-113
ChicagoIL 60611 USA
v-cryns@northwestern.edu

Jessica Curtis
University of Minnesota
321 Church Street SE
MinneapolisMN 55455 USA
curti165@umn.edu

Michael Czech
University of Massachusetts Medical
School
373 Plantation Street
WorcesterMA 01545 USA
Michael.Czech@umassmed.edu

Laura Danai
University of Massachusetts Medical
School
373 Plantation Street
WorcesterMA 01605 USA
Laura.Danai@umassmed.edu

Dawn Davis
UW-Madison
H4/526 CSC, MC5148
MadisonWI 53792 USA
dbd@medicine.wisc.edu

Hector DeLuca
UW-Madison
433 Babcock Drive
MadisonWI 53578 USA
deluca@biochem.wisc.edu

Symposium Participant Addresses

Richard Deckelbaum

Columbia University Medical Center
Institute of Human Nutrition
New York NY 10032 USA
rjd20@columbia.edu

Agnieszka Dobrzyn

Nencki Institute of Experimental Biology
3 Pasteur Street
Warsaw PL 02-093 Poland
adobrzyn@nencki.gov.pl

Pawel Dobrzyn

Nencki Institute of Experimental Biology
3 Pasteur Street
Warsaw PL 02-093 Poland
pdobrzyn@nencki.gov.pl

Livia Eberlin

Purdue University
106 South 17th Street
Lafayette IN 47905 USA
leberlin@purdue.edu

Robert Eckel

University of Colorado
MS 8106,
Aurora CO 80045 USA
robert.eckel@ucdenver.edu

Merik Erikci

Harvard University
1575 Tremont Street
Roxbury Crossing MA 02120 USA
merikci@fas.harvard.edu

Robert Farese

J. David Gladstone Institutes
1650 Owens Street
San Francisco CA 94158 USA
bfarese@gladstone.ucsf.edu

Christina Ferreira

Purdue University
2243 US 52 Highway
West Lafayette IN 47906 USA
cferrei@purdue.edu

Heather Ferris

Joslin Diabetes Center
1 Joslin Place
Boston MA 02215 USA
heather.ferris@joslin.harvard.edu

Milton Fischer

Central Wisconsin Center
317 Knutson Drive
Madison WI 53704 USA
miltonfischer@dhs.wi.gov

Matthew Flowers

UW-Madison
433 Babcock Drive
Madison WI 53706 USA
mtflowers@wisc.edu

Brendan Floyd

UW-Madison
2156 Allen Blvd.
Middleton WI 53562 USA
dsewell2@wisc.edu

Amanda Foltz

UW-Madison
1550 Linden Drive
Madison WI 53705 USA
afoltz@wisc.edu

Danielle Fontaine

UW-Madison
Dept. of Endocrinology, H4/547 CSC
Madison WI 53792 USA
dfontaine@wisc.edu

Christopher Glass

University of California-San Diego
9500 Gilman Drive
La Jolla CA 92093-0651 USA
ckg@ucsd.edu

Ira Goldberg

Columbia University
630 West 168th Street
New York NY 10032 USA
ijg3@columbia.edu

Frank Gonzalez

National Cancer Institute
Building 37, Room 3106
Bethesda MD 20892 USA
gonzalef@mail.nih.gov

Daniel Greenspan

UW-Madison
5675 MSC
Madison WI 53706 USA
dsgreens@wisc.edu

Paul Grimsrud

UW-Madison
Department of Biochemistry
Madison WI 53706 USA
pgrimsrud@wisc.edu

Adilson Guilherme

University of Massachusetts Medical
School
373 Plantation Street
Worcester MA 01605 USA
adilson.guilherme@umassmed.edu

Jenny Gumperz

UW-Madison
Microbial Sciences Building, Room 4157
Madison WI 53706 USA
jegumperz@wisc.edu

Hong Guo

University of Minnesota
225 FSCN
St. Paul MN 55112 USA
guoxx186@umn.edu

Rebecca Haeusler

Columbia University
1150 St. Nicholas Avenue
New York NY 10032 USA
rah2130@columbia.edu

Chuanming Hao

Fudan University
Huashan Hospital
Shanghai 200040 China
Chuanminghao@fudan.edu.cn

Kristina Hellberg

University of Minnesota
BMBB, Rm 6-155 Jackson Hall, 1214
Minneapolis MN 55455 USA
hellb127@umn.edu

Mark Herman

Harvard Medical School
300 Brookline Avenue
Boston MA 02115 USA
mherman1@bidmc.harvard.edu

Ann Hertzfel

University of Minnesota
321 Church Street SE
Minneapolis MN 55455 USA
vogel006@umn.edu

Symposium Participant Addresses

Shangyu Hong

Beth Israel Deaconess Medical Center
3 Blackfan circle
BostonMA 02115 USA
shong1@bidmc.harvard.edu

Jay Horton

UT Southwestern Medical Center
5323 Harry Hines Blvd.
DallasTX 75390-9046 USA
jay.horton@utsouthwestern.edu

Ann Jerkins

NIH
6707 Democracy Blvd
BethesdaMD 20892 USA
hasseg@mail.nih.gov

Junghyo Jo

National Institutes of Health
12A South Drive
BethesdaMD 20892/5621 USA
jojunghyo@mail.nih.gov

Barbara Kahn

Harvard Medical School
330 Brookline Avenue
BostonMA 02215 USA
bkahn@bidmc.harvard.edu

C. Ronald Kahn

Harvard Medical School
64 Valentine Park
West NewtonMA 02465 USA
c.ronald.kahn@joslin.harvard.edu

Arvinder Kapur

UW-Madison
600 Highland Avenue
MadisonWI 53792 USA
akaur@wisc.edu

Choon Young Kim

Purdue University
374 Elston Road
LafayetteIN 47909 USA
kim410@purdue.edu

Gyo-Nam Kim

Purdue University
Phillip E. Nelson Hall of Food Science,
Room 2268
West LafayetteIN 47907 USA
kim1132@purdue.edu

Kee-Hong Kim

Purdue University
745 Agriculture Mall Drive
West LafayetteIN 47907 USA
kim618@purdue.edu

Michelle Kimple

UW-Madison
902 Columbia Road
MadisonWI 53705 USA
mkimple@medicine.wisc.edu

Elizabeth Klar

UW-Madison
580 Biochemistry Addition
MadisonWI 53706 USA
lizzyklar@yahoo.com

Rolf Kletzien

Metabolic Solutions Development Com-
pany
161 East Michigan Avenue
KalamazooMI 49007 USA
rkletzien@msdrx.com

Alison Kohan

University of Cincinnati
2180 East Galbraith Road
CincinnatiOH 45237 USA
alison.kohan@uc.edu

Ronald Krauss

Children's Hospital Oakland Research
Institute
5700 Martin Luther King Jr. Way
OaklandCA 94609 USA
rkrauss@chori.org

Changhua Lai

UW-Madison
1675 Observatory Drive
MadisonWI 53703 USA
clai22@wisc.edu

Aparna Lakkaraju

UW-Madison
1300 University Avenue
MadisonWI 53706 USA
lakkaraju@wisc.edu

Daniel Lane

Johns Hopkins School of Medicine
717 Maiden Choice Lane
CayonsvilleMD 21228 USA
dlane@jhmi.edu

Chih-Hao Lee

Harvard University
665 Huntington Avenue
BostonMA 02115 USA
clee@hsph.harvard.edu

Kelly Lowe

Merck
105 Orono Orchard Road
OronoMN 55356 USA
kelly_lowe@merck.com

Aldons Lusis

UCLA
650 Charles E. Young Drive South
Los AngelesCA 90095 USA
jlusis@mednet.ucla.edu

Ormond MacDougald

University of Michigan
Brehm Diabetes Center
Ann ArborMI 48105 USA
macdougald@umich.edu

Susanne Mandrup

University of Southern Denmark
Campusvej 55
Odense MFyn 5230 Denmark
s.mandrup@bmb.sdu.dk

Oni Mapp

Carnegie Institution of Washington
3520 San Martin Drive
BaltimoreMD 21218 USA
mapp@ciwemb.edu

Thomas Martin

UW-Madison
433 Babcock Drive
MadisonWI 53706 USA
tmmartin@wisc.edu

Douglas Mashek

University of Minnesota
1334 Eckles Avenue
St. PaulMN 55108 USA
dmashek@umn.edu

Makoto Miyazaki

University of Colorado-Denver
12700 East 19th Avenue
AuroraCO 80045 USA
Makoto.Miyazaki@ucdenver.edu

Symposium Participant Addresses

Shinobu Miyazaki
University of Colorado-Denver
12700 East 19th Avenue
DenverCO 80045 USA
Shinobu.Miyazaki-Anzai@ucdenver.edu

Deborah Muoio
Duke University
106 Innisbrook Ct
CaryNC 27513 USA
muoio@duke.edu

David Nelson
UW-Madison
1034 Waban Hill
MadisonWI 53711 USA
nelson@biochem.wisc.edu

David Nelson
UW-Madison
1415 Linden Drive
MadisonWI 53706 USA
nelson@nutrisci.wisc.edu

Christopher Newgard
Duke University
4321 Medical Park Drive
DurhamNC 27704 USA
newga002@mc.duke.edu

James Ntambi
UW-Madison
433 Babcock Drive
MadisonWI 53706 USA
ntambi@biochem.wisc.edu

Angie Oler
UW-Madison
3409 Kingston Drive
MadisonWI 53713 USA
dsewell2@wisc.edu

Kuok Teong Ong
1334 Eckles Avenue
St. PaulMN 55108 USA
ongxx043@umn.edu

Jessica Otis
UW-Madison
2015 Linden Drive
MadisonWI 53706 USA
jotis@wisc.edu

Dave Pagliarini
UW-Madison
3535 Strawberry Loop
MiddletonWI 53562 USA
pagliarini@wisc.edu

Apostolos Pappas
J&J
199 Grandview Road
SkillmanNJ 08558 USA
APappas@its.jnj.com

Renee Pasker
UW-Madison
580 Biochemistry Addition
MadisonWI 53706 USA
pasker@wisc.edu

Manish Patankar
UW-Madison
600 Highland Avenue
MadisonWI 53792-6188 USA
patankar@wisc.edu

Chad Paton
West Virginia University
1040 Ag. Sciences Bldg
MorgantownWV 26506 USA
chad.paton@mail.wvu.edu

Pere Puigserver
Dana-Farber Cancer Institute/Harvard
Medical School
44 Binney Street
BostonMA 02115 USA
pere_puigserver@dfci.harvard.edu

Ariel Quiroga
University of Alberta
328 Heritage Medical Research Centre
EdmontonAB T6G 2S2 Canada
quiroga@ualberta.ca

Joyce Repa
UT Southwestern Medical Center
5323 Harry Hines Blvd.
DallasTX 75390-9077 USA
joyce.repa@utsouthwestern.edu

Karen Reue
UCLA
David Geffen School of Medicine at UCLA
Los AngelesCA 90095 USA
reuek@ucla.edu

Mark Richards
UW-Madison
1805 Linden Drive
MadisonWI 53706 USA
mprichards@ansci.wisc.edu

Rachel Roth Flach
University of Massachusetts
7 Fyrbeck Avenue
ShrewsburyMA 01545 USA
rachel.roth@umassmed.edu

Philipp Scherer
UT Southwestern Medical Center
5323 Harry Hines Blvd.
DallasTX 75390-8549 USA
philipp.scherer@utsouthwestern.edu

Lindsay Schneider
UW-Madison
401 North Eau Claire Avenue
MadisonWI 53705 USA
dsewell2@wisc.edu

Anath Shalev
University of Alabama at Birmingham
1825 University Blvd.
BirminghamAL 35294 USA
shalev@uab.edu

Wan Shen
University of North Carolina-Greensboro
318 Stone Building
GreensboroNC 27402-6170 USA
w_shen@uncg.edu

Mufaddal Soni
UW-Madison
433 Babcock Drive
MadisonWI 53726 USA
msoni@wisc.edu

Espen Spangenburg
University of Maryland
School of Public Health
College ParkMD 20742 USA
espen@umd.edu

Nicole Spencer
UW-Madison
820 Williamson Street
MadisonWI 53703 USA
nmsspencer@wisc.edu

Symposium Participant Addresses

Maggie Strable

UW-Madison
433 Babcock Drive
MadisonWI 53706 USA
strable@wisc.edu

Yewseok Suh

UW-Madison
945 Eagle Heights
MadisonWI 53705 USA
yewseok@gmail.com

Hei Sook Sul

University of California-Berkeley
219 Morgan Hall
BerkeleyCA 94720 USA
hsul@berkeley.edu

Alan Tall

Columbia University Medical Center
630 West 168th Street
New YorkNY 10032 USA
art1@columbia.edu

Peter Tontonoz

University of California
675 Charles Young Drive
Los AngelesCA 90095-1662 USA
ptontonoz@mednet.ucla.edu

Nathan Truchan

UW-Madison
580 Biochemistry Addition
MadisonWI 53706 USA
truchan@wisc.edu

Aki Uchida

Purdue University
700 West State Street
West LafayetteIN 47907 USA
auchida@purdue.edu

Dennis Vance

University of Alberta
Dept. Biochemistry
EdmontonAB T6G2S2 Canada
dennis.vance@ualberta.ca

Chen-yu Wang

UW-Madison
433 Babcock Drive
MadisonWI 53706 USA
dschroeder@biochem.wisc.edu

Hao Wei

University of Washington
815 Mercer Street
SeattleWA 98109 USA
haowei@uw.edu

Mary Whitsitt

Purdue University
700 West State Street
West LafayetteIN 47907 USA
mwhitsit@purdue.edu

Yan Xie

Washington University
Campus Box 8124, CSRB Room 920
St. LouisMO 63110 USA
yxie@wustl.edu

Yongna Xing

UW-Madison
1400 University Avenue
MadisonWI 53706 USA
xing@oncology.wisc.edu

Eric Yen

UW-Madison
1415 Linden Drive
MadisonWI 53726 USA
yen@nutrisci.wisc.edu

Paul Yen

Duke-NUS Graduate Medical School
8 College Road
SingaporeSIN 018987 Singapore
paul.yen@duke-nus.edu.sg

Uzma Zakai

UW-Madison
1101 University Avenue
MadisonWI 53706 USA
zakaiu@chem.wisc.edu

Yuanyuan Zhang

University of Minnesota
1334 Eckles Avenue
Saint PaulMN 55108 USA
zhang682@umn.edu

Ming Zhao

University of Minnesota
1990 Knapp Avenue
St. PaulMN 55108 USA
zhao0374@umn.edu