Michigan Metabolomics and Obesity Center *(MMOC)*
Michigan Nutrition Obesity Research Center *(MNORC)*
2011 SYMPOSIUM

Wednesday, October 19, 2011
8:00 am - 4:30 pm Forum Hall, Palmer Commons

7:30 a.m. **Continental Breakfast**

8:00- 8:15 **Charles Burant, MD, PhD**
Professor of Internal Medicine
University of Michigan
Malcolm Low, MD, PhD
Professor of Molecular & Integrative Physiology
University of Michigan
"Introductions"

8:15-9:15 **David Wasserman, PhD**
AM Lyle Professor of Molecular Physiology and Biophysics
Director, Vanderbilt Mouse Metabolic Phenotyping Center
Vanderbilt University School of Medicine
"Extramyocellular Determinants of Muscle Insulin Resistance"

9:15-10:15 **Jay Heinecke, MD**
Professor of Medicine
Karasinski Chair in Metabolic Research
University of Washington
"When Good Cholesterol Goes Bad"

10:15-10:30 **Break**

10:30-11:30 **Michael Schwartz, MD**
The Robert H. Williams Endowed Chair in Medicine, Professor and Director, Diabetes and Obesity Center of Excellence
University of Washington
"New Insights into Obesity Pathogenesis: A Hypothalamic Perspective"

11:30-12:15 **Cristen Willer, PhD**
Assistant Professor
Department of Internal Medicine
Department of Human Genetics
Center for Computational Medicine and Bioinformatics
Center for Statistical Genetics
University of Michigan
"Identifying Loci for Obesity in the Post-GWAS Era"

12:15-1:45 **Scientific Poster Session/Lunch**
Great Lakes North Central

1:45-2:30 **Carey Lumeng, MD, PhD**
Assistant Professor, Pediatrics and Communicable Diseases, Molecular & Integrative Physiology and Pulmonary Medicine
University of Michigan
"Adipose Tissue and the Inflammatory Response to Obesity"

2:30-3:30 **Neil Rowland, PhD**
Professor, Chair, Department of Psychology
University of Florida
"Foraging, Economics and Obesity"

3:30-4:30 **Laurence Tecott, MD, PhD**
Maurice Eliaser, Jr. MD and Marjorie Meyer Eliaser Chair in Molecular Biology and Genetics in Psychiatry
University of California, San Francisco
"Quantitative Assessment in Obesigenic Lifestyles in the Small and Furry"

TARGET AUDIENCE

- Physicians, nurses, and allied health professionals with an interest in obesity, diabetes, and metabolism
- Researchers interested in basic and translational research in metabolic diseases
- Graduate and undergraduate students in health related fields of study

OBJECTIVES

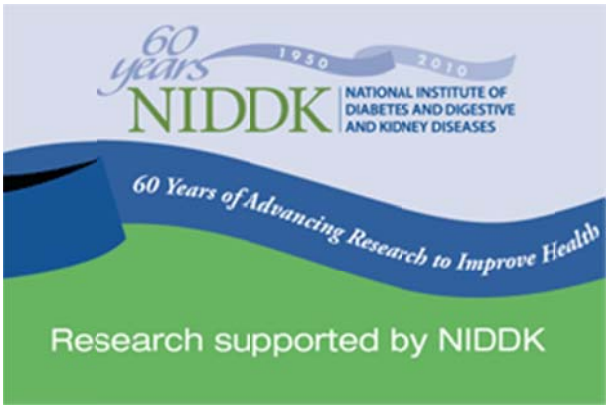
Symposium participants will enhance their performance and the outcomes of their patients through better understanding of:

- The latest science in the area of metabolism, obesity, and diabetes
- The role of metabolomics in the pathophysiology of disease
- Causes and treatments of obesity and the ability to implement in their practice
- Technology in the preclinical assessment of obesity and nutrition-related diseases

CME CREDITS

The University of Michigan Medical School is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians. The University of Michigan Medical School designates this live activity for a maximum of 6.5 *AMA PRA Category 1 Credit(s)*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Our grateful appreciation to the financial supporters of the 2011 MMOC symposium



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Michigan Diabetes Research and Training Center

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where scientists create cures

University of Michigan Health System

David Wasserman, PhD

AM Lyle Professor of Molecular Physiology and Biophysics
Director, Vanderbilt Mouse Metabolic Phenotyping Center
Vanderbilt University School of Medicine

David H. Wasserman obtained his PhD in Physiology from the University of Toronto in 1985 and joined the faculty at Vanderbilt in 1987. In 2001 he became the Founding Director of the Vanderbilt Mouse Metabolic Phenotyping Center and in 2006 he was appointed the Director of the Vanderbilt Metabolic Physiology Shared Resource. He is a member of the Executive Committee of the Vanderbilt Diabetes Research Center. In 2007 he was named the Ron Santo Chair in Diabetes Research and in 2010 he was named the Annie Mary Lyle Professor of Molecular Physiology and Diabetes.

The research that Dr. Wasserman and his laboratory undertake has been instrumental in defining the regulation of metabolic control systems in health, insulin resistance, and diabetes. The Wasserman laboratory uses isotopic methods for tracing glucose and other metabolic substrates to define the functional role of hormones, nerves, and specific transporters and enzymes in flux control. Their work has been important in defining the robust control of the liver metabolic network by the endocrine pancreas. The Wasserman laboratory has also made seminal contributions to control of muscle glucose metabolism by defining the contributions of insulin-dependent and –independent control mechanisms during exercise and insulin stimulation. Dr. Wasserman and his colleagues have revised the conventional view that muscle glucose uptake is controlled by the rate-limiting step paradigm, by demonstrating that control is distributed between multiple regulatory sites. Dr. Wasserman has published over a 160 papers and more than 25 book chapters. He has received include the Henry Pickering Bowditch Award (1997) and Solomon A. Berson Award from the American Physiology Society (2008), the C.R. Park Award for Excellence in Research from Vanderbilt University (2010), and an NIH M.E.R.I.T Award (2008).

Jay Heinecke, MD

Professor of Medicine
Karasinski Chair in Metabolic Research
University of Washington

Jay Heinecke, MD is Professor of Medicine, Director of the Mass Spectrometry Resource, Mentor in the Molecular and Cell Biology Graduate Program, and Karasinski Chair in Metabolic Research in the Department of Medicine, University of Washington.

Research in the Heinecke laboratory focuses on understanding the role of macrophages in the pathogenesis of atherosclerosis, obesity, and insulin resistance. Major efforts focus on using a proteomics approach to identify specific proteins targeted for oxidative modifications and building a systems biology view of the macrophage. Current studies include: (1) Investigating oxidative pathways that regulate the cardioprotective activities of high-density lipoprotein (HDL), which are of central importance in atherosclerosis; (2) Use of animals with genetically engineered deficiencies to understand the role of oxidants and macrophages in the pathogenesis

of vascular disease and obesity; (3) Identifying macrophage protein networks involved in immune function and atherogenesis; and (4) Translational studies exploring the links between the HDL proteome, the macrophage proteome, and susceptibility to cardiovascular disease.

Michael Schwartz, MD
The Robert H. Williams Endowed Chair in Medicine, Professor and Director, Diabetes and Obesity Center of Excellence
University of Washington

Dr. Schwartz is Robert H. Williams Endowed Chair, Professor of Medicine in the Division of Metabolism, Endocrinology and Nutrition at the University of Washington and Director of the UW Medicine Diabetes & Obesity Center of Excellence. His research investigates brain mechanisms governing energy balance and glucose metabolism and how obesity and diabetes result from impairment of these brain systems. He has published more than 200 articles and book chapters related to these topics and his research has been continuously funded by the NIH since joining the faculty of UW 18 years ago. Dr. Schwartz is a member of the Association of American Physicians, the Western Association of Physicians and the American Society for Clinical Investigation, is the recipient of the 2007 Williams-Rachmiel Levine Award for Outstanding Mentorship from the Western Society for Clinical Investigation, the 2006 Naomi Berrie Award for Outstanding Achievement in Diabetes Research from Columbia University among other awards, and is a member of the editorial boards of the *Journal of Clinical Investigation, Endocrinology, American Journal of Physiology, Endocrine Reviews,* and *Frontiers in Neuroendocrinology.*

Cristen Willer, PhD
Assistant Professor
Department of Internal Medicine
Department of Human Genetics
Center for Computational Medicine and Bioinformatics
Center for Statistical Genetics
University of Michigan

Dr. Willer studied for a PhD in molecular genetics at Oxford University in the UK, studying the epidemiological and genetic basis of multiple sclerosis at the Wellcome Trust Center for Human Genetics. She then continued her training during a postdoctoral fellowship at the University of Michigan in the Department of Biostatistics, using large-scale association studies to identify dozens of novel genes associated with lipid levels and obesity.

Since her appointment as Assistant Professor in the Division of Cardiovascular Medicine, Dr. Willer's research group focuses on the analysis of high-throughput genetic and sequencing data to understand the genetic basis of cardiovascular and metabolic diseases. We have identified several hundred new genetic regions associated with lipid levels and obesity using whole-genome association studies and are now moving towards fine-mapping these loci to identify functional genetic variants. We are also performing state-of-the-art whole exome and whole

genome sequencing studies to identify rare genetic variants with potentially large effects on disease risk.

Carey Lumeng, MD, PhD
Assistant Professor, Pediatrics and Communicable Diseases, Molecular & Integrative Physiology and Pulmonary Medicine
University of Michigan

Dr. Lumeng is an Assistant Professor in the Department of Pediatrics Molecular and Integrative Physiology at the University of Michigan Medical School. He completed the M.D. Ph.D. Program at the University of Michigan with a Ph.D. in Human Genetics mentored by Jeffery Chamberlain Ph.D. After residency in the Boston Combined Pediatric Residency Program, he completed a Pediatric Pulmonology Fellowship at the University of Michigan. During this training, he did post-doctoral research in the laboratory of Alan Saltiel Ph.D. in the Life Sciences Institute and was named a Biological Sciences Scholar at the University of Michigan.

Research in the Lumeng Lab seeks to understand the mechanisms by which obesity influences disease with a focus on the association between obesity and inflammation. The lab investigates the biology of adipose tissue macrophages (ATMs) which serve as a link between the innate immune system and metabolic control. Projects involve mouse models of obesity and focus on the interactions between macrophages and lymphocytes in fat, the role of macrophages in adipose tissue development, and the mechanisms by which macrophages are activated with obesity. He was awarded an MNORC Pilot and Feasibility Grant in 2011 to initiate a translational study of inflammation in childhood obesity.

Neil Rowland, PhD
Professor, Chair, Department of Psychology
University of Florida

Dr. Rowland received his Ph.D. in 1974 from London University, with research performed under Dr. Stylianos Nicolaidis at the College de France in Paris. He then did postdoctoral work at the University of Pittsburgh before joining the faculty at the University of Florida in 1981. His research, using rodents, has focused on the relationship between neurobiological signaling and ingestive behavior and has resulted in almost 300 refereed publications. For the past several years, one of the focus areas of his research has been the effect of effort on food-related decision-making, and implications for body weight and obesity.

Laurence Tecott, MD, PhD

Maurice Eliaser, Jr. MD and Marjorie Meyer Eliaser Chair in Molecular Biology and Genetics in Psychiatry
University of California, San Francisco

Laurence Tecott, MD, PhD, holds an Endowed Professorship in the Department of Psychiatry at the University of California, San Francisco, where he also serves as the Associate Director for the UCSF Center for Neurobiology and Psychiatry. He received his B.A. with Distinction in Biology and Psychology from Swarthmore College and his M.D. at the University of California, San Francisco. He subsequently trained at Stanford University, receiving a Ph.D. in Neurosciences for the development of novel technology for the amplification of messenger RNA. Dr. Tecott then completed his medical internship at Yale University before training in Psychiatry at UCSF. As a psychiatric resident and thereafter, Dr. Tecott pioneered the application of mouse molecular genetic technology to behavioral regulation and obesity research. His laboratory focuses on mouse molecular genetic approaches to the roles of central serotonin systems in neuropsychological processes relevant to psychiatric diseases and obesity.

Toward this end, the Tecott laboratory has developed a novel “behavioral informatics” approach, applying computational methods for pattern recognition in high resolution behavioral datasets collected in animals’ home cages. This permits comprehensive examination of the coordinated regulation of diverse behaviors exhibited spontaneously by caged mice; “mouse lifestyles”. Automated data collection devices and analytical algorithms reveal highly organized patterns of interactions among diverse behaviors and provide “behavioral fingerprints” highly sensitive to diet, environment and genetic endowment. The development of modeling approaches for these rich behavioral datasets will provide tools for querying aggregate data contained within a “mouse lifestyle database”, revealing into the impact of genes, drugs and environment on feeding behavior and energy balance.

Disclosure of Relevant Financial Relationships with Commercial Companies

The Accreditation Council for Continuing Medical Education requires that the planners and presenters of continuing medical education activities disclose financial relationships with commercial companies whose products or services are discussed in educational presentations.

The following planners/speakers have no financial relationships with companies whose products are addressed in their planning/presentations.

Charles Burant, MD, PhD
David Wasserman, PhD
Jay Heinecke, MD
Michael Schwartz, MD
Cristen Willer, PhD
Carey Lumeng, MD, PhD
Neil Rowland, PhD

The following planners/speakers have financial relationships with companies whose products are addressed in their planning/presentations.

Laurence Tecott, MD, PhD	Ethologics (stock shareholder, self-managed)

Abstract #1

A SINGLE SESSION OF EXERCISE IMPROVES INSULIN SENSITIVITY IN OBESE ADULTS: EFFECTS OF EXERCISE INTENSITY

Sean A. Newsom, Allison C. Everett, and Jeffrey F. Horowitz
Substrate Metabolism Laboratory, School of Kinesiology, University of Michigan, Ann Arbor, MI

A single session of aerobic exercise can improve insulin sensitivity for hours and even into the next day. However, the impact of exercise intensity on insulin sensitivity is not completely understood. **PURPOSE:** Determine the effect of mild (50% VO_2peak) and moderate (65% VO_2peak) exercise intensity on meal tolerance measured 1 hour after exercise and insulin sensitivity measured the next day in obese adults. **METHODS:** Six sedentary obese adults (M/F: 2/4; BMI: 37±2 kg/m^2; age: 28±1 yrs; VO_2peak: 20.5±1.3 mL/kg/min) were admitted to the hospital for a 2-day trial on three separate occasions. On two occasions, subjects expended 350kcals during an exercise session in the afternoon of the first day. These two exercise trials were identical except for the intensity of exercise performed (50% VO_2peak [EX50] and 65% VO_2peak [EX65]). Subjects also completed a control trial [CON] in which they remained sedentary. We assessed meal tolerance starting 1h after exercise by measuring plasma glucose and insulin concentrations during the 2h period after they ate a standardized mixed meal. The next morning we measured insulin sensitivity via hyperinsulinemic-euglycemic clamp (insulin infusion rate: 100μU/m^2/min). **RESULTS:** Meal tolerance was improved during both EX50 and EX65 compared with CON as demonstrated by the trend for lower plasma glucose area under the curve (AUC) after the meal (745±32, 751±32, and 804±41 mM*min; P=0.07, P=0.05 vs CON). Plasma insulin AUC after the meal was not different among trials. The next morning, insulin sensitivity was markedly increased during EX50 compared with CON (13.5±3.1 vs. 9.6±2.2 mg/kgFFM/min; P=0.03). Interestingly, although insulin sensitivity tended to be greater during EX65 (11.5±2.8 mg/kgFFM/min) compared with CON, this increase did not reach statistical significance (P=0.17). **CONCLUSIONS:** A single session of exercise in obese adults improved meal glucose tolerance in the few hours after exercise and insulin sensitivity measured the next day. The improvement in meal tolerance was not affected by exercise intensity. However, mild intensity exercise (50% VO_2peak) may provide a more potent stimulus to enhance insulin sensitivity the next day compared with the same energy expenditure during exercise at a moderate intensity (65% VO_2peak).

Supported by NIH-NIDDK Grant #R01DK077966

Abstract #2

DECREASED HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN A COHORT OF 6TH GRADE CHILDREN: ASSOCIATION WITH CARDIOVASCULAR RISK FACTORS AND LIFESTYLE BEHAVIORS

Shannon Flynn, Roopa Gurm, Jean DuRussel-Weston, Susan Aaronson, Lindsey Gakenheimer, Joe Smolarski, Daniel Simhaee, Nicole Corriveau, Cathy Fitzgerald, Taylor Eagle, Ravi Rao, Kim A. Eagle, Caren Goldberg, Elizabeth A. Jackson

Background: High density lipoprotein cholesterol (HDL-C) levels are inversely associated with coronary heart disease risk in adults due to anti-inflammatory, anti-atherogenic, and anti-thrombotic mechanisms. Atherogenesis starts early in life, but the significance of low HDL-C in children and adolescents has scarcely been explored. We determined the prevalence of low HDL-C (≤ 40mg/dL) in a population of 6th-grade students to assess associations between low HDL-C and cardiovascular risk factors and lifestyle behaviors.

Methods: Data from 1104 participants in Project Healthy Schools, a school-based intervention program in southeast Michigan, were collected, including lipid and glucose levels, body mass index (BMI), blood pressure, heart rate and a standardized questionnaire assessing dietary, exercise and sedentary habits. Chi-squared analyses and unpaired t-tests used to compare the two cohorts.

Results:

Variable	N	HDL-C ≤40mg/dL	HDL-C>40mg/dL	P Value
N(%)	1104	177(16.03)	927(83.97)	
Female (%)	1096	103(58.9)	458(49.7)	0.027
BMI (kg/m^2)-mean	1099	22.93±5.05	19.54±3.70	0.001
BMI>85th percentile (%)	854	82(62.12)	205(28.4)	0.001
Systolic blood pressure (mmHg)-mean	1101	110.88±11.47	107.98±11.16	0.002
Diastolic blood pressure (mmHg)- mean	1101	66.01±8.90	63.67±7.74	0.001
Resting heart rate (beats/min)-mean	1101	84.34±11.30	80.22±10.81	0.001
Recovery heart rate (beats/min)-mean	1040	110.72±18.13	103.39±17.38	0.001
Triglycerides (mg/dL)-mean	1064	175.01±102	111.88±67.16	0.001
Low density lipoprotein cholesterol (mg/dL)-mean	1000	93.53±27.32	87.90±24.72	0.009
# days strenuous exercise/week-mean	1025	4.02±2.13	4.53±2.01	0.003
# days moderate exercise/week-mean	1029	2.99±2.39	3.41±2.29	0.032

Of the 177 students in the low HDL-C cohort, 90 (50.8%) had at least two additional criteria for metabolic syndrome in adolescents, as defined by the National Cholesterol Education Program.

Conclusions: We found a high prevalence of low HDL-C in our population, which tended to cluster with inactive, overweight, less fit girls with high triglycerides and higher blood pressure. Our findings suggest that there may be a large population of youth already at risk for adverse health events; thus lipid screening in children and adolescents may have significant value.

Abstract #3

HEART RATE RECOVERY: AN INDICATOR OF FITNESS AMONG MIDDLE SCHOOL CHILDREN

Daniel Simhaee, Roopa Gurm, Elizabeth A. Jackson, Susan Aaronson, Jean DuRussel-Weston, Catherine Fitzgerald, Shannon Flynn, Zachary Geiger, Nicole Corriveau, Julia Winfield, Caren Goldberg, Kim A. Eagle, University of Michigan, Ann Arbor, MI

Background: Heart Rate Recovery (HRR) has been used in adults to evaluate cardiovascular fitness and is a strong predictor of morbidity and mortality. We sought to determine if HRR is associated with body mass index (BMI), blood pressure, lipid levels, and lifestyle behaviors such as physical activity among middle school children.

Methods: Data from 1276 participants in Project Healthy Schools, a school-based intervention program in southeast Michigan, were collected. In addition to demographic characteristics, data on physiologic factors were collected including lipid and glucose levels, BMI, blood pressure, and heart rate. Standardized questionnaires were used to collect information on behaviors including diet, physical activity and sedentary behaviors. HRR was determined by measurement of heart rate after a 3 minute step test. Using quartiles of HRR as a marker of fitness, associations with demographic, physiologic and behavioral factors were explored using Chi-squared and t-tests.

Results: Compared to children in the lowest quartile of HRR (i.e. most fit), those in the upper quartile of HRR (i.e. less fit) had higher mean LDL cholesterol (93.0 mg/dL vs. 86.7 mg/dL; P=0.02), lower mean HDL cholesterol (50.9 mg/dL vs. 55.9 mg/dL; P<0.001), and higher mean triglycerides (132.4 mg/dL vs. 111.74 mg/dL; P=0.004). Children in the upper 95% of BMI had higher mean HRR compared to those in the normal BMI range (5 to 85%) (116.6 kg/m2 vs. 100.3 kg/m2). Children in the upper quartile of HRR reported fewer days of strenuous to moderate exercise per week compared to children in the lowest quartile of HRR (4.8 vs. 4.1; P<0.001 for moderate exercise, and 3.6 vs. 3.0; P=0.001, for strenuous exercise).

Conclusions: We observed that longer HRR times were associated with a less favorable lipid profile and higher BMI, suggesting HRR can identify middle school children at risk for obesity and increased cardiovascular risk. Longer HRR was also associated with decreased physical activity suggesting HRR may be a clinical useful tool to measure fitness in children.

Abstract #4

UNDERSTANDIN G CHILDHOOD OBESITY IN AMERICA: LINKAGES BETWEEN HOUSEHOLD INCOME, COMMUNITY RESOURCES, AND CHILDREN'S BEHAVIORS

Taylor F. Eagle, Anne Sheetz, MPH, Roopa Gurm, MS, Alan C. Woodward, MD, Eva Kline-Rogers, MS, RN, Robert Leibowitz, Jean DuRussel-Weston, RN, MPH, LaVaughn Palma-Davis, MA, Susan Aaronson, MA, RD, Catherine M. Fitzgerald, MA, RD, Lindsey R. Mitchell, MPH, Bruce Rogers BS, Patricia Bruenger BA, CCRC, Katherine A. Skala, MPH, CHES, Caren Goldberg, MD, Elizabeth A. Jackson, MD, MPH, Steven R. Erickson, PharmD, and Kim A. Eagle, MD.

Background

Childhood obesity is our nation's most pressing health problem. Understanding its causes is critical to the creation of strategies to improve our children's health.

Study Questions

What is the association between childhood obesity and household income? How does household income effect childhood behaviors that promote childhood obesity?

Methods

We assessed BMI in 109, 634 children screened in Massachusetts public schools in 2009. We identified the percentage of children who were overweight/obese and compared this to the percentage of children in each community who reside in low income homes.

We compared activity patterns, and diet in a cohort of 999 sixth graders residing in four Michigan communities which differed in annual household income. We compared these behavioral/patterns using one-way analysis of variance.

Results

In Massachusetts, overall percent of overweight/obese by community varied from 9.6% to 42.8. As a community's average household income dropped, the percent of children overweight/obese rose, becoming 35-45% when more than 20% of households were low income (figure).

Abstract #4

In Michigan sixth graders, as mean household income goes down, fried food consumption per day doubles from 0.23 to 0.54 ($p<0.002$), and daily TV/video time triples from 0.55 hours to 2.00 hours ($p<0.001$) while vegetable consumption and participation in moderate or vigorous exercise goes down.

Conclusions

The incidence of overweight/obese children rises in communities with lower household income. Children residing in lower income communities exhibit poorer dietary and physical activity behaviors, which affects the incidence of obesity. Reducing childhood obesity in America will require education for each child and family and community-wide collaboratives offering better nutritional choices, and access to recreational facilities and programs.

Abstract #5

Andrea S. Cornford, Ariel L. Barkan, Jeffrey F. Horowitz, FACSM
University of Michigan, Ann Arbor, MI

We previously found that overeating for only a few days markedly suppressed the secretion of growth hormone (GH), which is known to be an important lipolytic agent. **PURPOSE:** The primary aim of this study was to determine the role of this reduction in GH concentration on lipolytic rate throughout 2 weeks of overeating. **METHODS:** A total of 17 healthy, non-obese adults were admitted to the hospital for 2 wks, during which time they ate ~4000 kcals/day (70 kcals/kg fat free mass/day; 50% carbohydrate, 35% fat, 15% protein). Seven of these subjects (6 men, 1 woman; BMI: 23±1kg/m^2) received exogenous GH treatment (GHT) administered in 4 daily injections to mimic physiologic GH secretion throughout the 2 wks of overeating. The remaining 10 subjects (7 men, 3 women; BMI: 24±1kg/m^2) did not receive GH (CONTROL). Plasma GH concentration was measured for 24h (Q20") before overeating (Baseline), and again at 3 days and 2 weeks of overeating. We also measured lipolytic rate (rate of appearance (Ra) of $^2H_5$-glycerol in plasma - expressed relative to body fat mass) and plasma insulin concentration. **RESULTS:** As reported previously, 24h average GH concentration was reduced ~60% by 3 days of overeating in our CONTROL subjects (1.4±0.2 vs. 0.5±0.2 ng/ml for Baseline and day 3; $p<0.05$) and GH remained low at 2 weeks of overeating (0.4±0.1 ng/ml, $p<0.05$). GHT prevented the fall in plasma GH concentration, maintaining plasma GH concentration at Baseline levels (1.3±0.2 ng/ml). After 3 days of overeating, lipolytic rate was suppressed ~30% below Baseline levels in CONTROL ($p<0.05$) and lipolysis remained low through 2 weeks of overeating ($p<0.05$). Interestingly, preventing the fall in GH concentration during overeating in GHT did not attenuate this suppression in lipolysis. Preventing the suppression in GH during GHT did augment plasma insulin concentration ($p<0.05$ vs. CONTROL). Therefore, the anti-lipolytic effects of this elevated plasma insulin concentration may counterbalance a GH-induced increase in lipolysis. **CONCLUSION:** Overeating suppressed plasma GH concentration with an accompanying reduction in lipolytic rate. However, preventing the fall in plasma GH concentration with overeating did not attenuate the reduction in lipolysis, likely a consequence of a greater plasma insulin concentration.

Supported by NIH Grant #R01DK71955.

Abstract #6

MMOC Abstract
R. Nelson

Rapid Alterations in Plasma and Muscle Lipid Profiles in Response to a High Saturated Fat Diet

The purpose of this study was to determine the effect of short-term exposure to diets high in either saturated or unsaturated fat on lipid profiles in plasma and skeletal muscle. Thirteen overweight-to-obese women (BMI: 29±1 kg/m^2; age: 34±2 y) were randomly assigned to a 4 wk diet high in proportion of either saturated fat (SAT; ~60% saturated fat) or unsaturated fat (UNSAT; ~80% unsaturated fat). Diets were designed to maintain body weight with identical macronutrient content (~50% carbohydrate, 35% fat, 15% protein) differing only by type of fat ingested. Fasting plasma samples were collected weekly for assessment of plasma fatty acid (FA) profile (using standard chromatography techniques) and cholesterol concentrations (Total-C, LDL-C, and HDL-C). Muscle samples were obtained before and after the 4 wk interventions to measure FA profile within the intramyocellular triglyceride pool (IMTG). Compared with pre-diet values, only 1 wk of SAT was sufficient to significantly increase the proportion of saturated FA (%SFA) in plasma (37±2% vs. 41±2%; P<0.01). This increase was maintained throughout the 4 wk treatment (42±2%; P=0.01). Accompanying this increase in %SFA, SAT also increased Total-C (143±6 vs. 161±7 mg/dL), due to a significant increase in LDL-C (84±7 vs. 104±8 mg/dL) (both P<0.05). In contrast, UNSAT significantly reduced %SFA in plasma (36±1% vs. 33±2%; P<0.01), and was accompanied by reductions in Total-C (164±11 vs. 141±9 mg/dL) and LDL-C (96±7 vs. 74±10 mg/dL) compared with pre-diet values (both P<0.05). Plasma HDL-C was not affected by either diet. In muscle, SAT significantly increased %SFA (32±1% vs. 37±1%; P<0.01) within the IMTG pool. Conversely, the reduction in %SFA in IMTG during UNSAT did not reach statistical significance (33±2% vs. 31±3%; P=0.13). In conclusion, significant changes in plasma lipid profile were evident as quickly as only 1 week on a high saturated fat diet. Additionally, changes in the FA profile within muscle lipids also occurred relatively quickly in response to a diet high in saturated fat, and generally reflected the changes in plasma FA.

This study was supported by the Robert C. and Veronica Atkins Foundation

Nelson RK[1], Li M[1], Hinko A[1], Burant CF[2], and JF Horowitz[1]
[1]School of Kinesiology, University of Michigan, Ann Arbor, MI and [2]School of Medicine, University of Michigan, Ann Arbor, MI

Abstract #7

BIOENERGETIC METABOLITES IN THE DIABETIC PERIPHERAL NERVOUS SYSTEM

Lucy M. Hinder, Carolyn L. Buller, Subramaniam Pennathur, Eva L. Feldman

University of Michigan, Ann Arbor, MI, USA

Diabetic neuropathy (DN) occurs in approximately 60% of diabetic patients and is characterized by progressive loss of peripheral axons, resulting in pain and eventual loss of sensation. Although there has been substantial research in to bioenergetic causes of diabetes, fundamental biological abnormalities that are most critical to the progression of DN remain unclear. Modern systems metabolite approaches were used to gain preliminary insight into the bioenergetic abnormalities in BLKS-db/db type 2 diabetic mouse sciatic nerve (SCN). Targeted mass-spectrometry-based metabolic profiling demonstrated reduced acyl-CoA, carnitine and acyl-carnitine species, along with decreases in glycolytic and TCA cycle intermediates, in 24-week db/db (diabetic) compared with db/+ (control) SCN. The diabetic alterations in glucose and fatty acid bioenergetics in peripheral nerve axons differ from those in classically insulin-responsive organs, such as liver and muscle. Such long-standing changes likely contribute to the pathogenesis and progression of DN. Further identification of the bioenergetic pathways critical for tissue-specific progression will lead to identification of pathways that will be useful in the diagnosis and therapeutic management of patients.

Abstract #8

REPRODUCIBILITY OF THE MEASUREMENT OF SWEET TASTE PREFERENCES

Keiko Asao, Wendy Luo, William H. Herman

Background: Developing interventions to prevent and treat obesity is a medical and public health imperative. Taste is a major determinant of food intake and the methods to measure taste preferences need to be established.
Objective: This study aimed to establish the short-term reproducibility of sweet taste preference measurements using 5-level and 11-level sucrose concentrations in healthy adult volunteers.
Design: We defined sweet taste preference as the mean preferred sucrose concentration from the two series of two-alternative, forced-choice staircase procedures 10 minutes apart in a single day. We repeated the same procedure at the second visit three to seven days later. Approximately half of study subjects were measured using 11-level sucrose concentrations in addition to 5-levels concentrations.
Results: Twenty-six adults (thirteen men and thirteen women, age 33.2 ± 12.2 years) completed the measurements. The median number of pairs presented for each series was three (25 and 75 percentiles: 3, 4) for the 5-level test, and an additional two (25 and 75 percentiles: 2, 2) for the 11-level test. The intraclass correlations between the measurements at the two visits were 0.78 (95% confidence interval [C.I.]: 0.57 to 0.90) for the 5-level test and 0.57 (95% C.I.: 0.07 to 0.84) for the 11-level test.
Conclusions: This study showed high short-term reproducibility for measuring sweet taste preferences. This method may be a useful tool for assessing sweet taste preferences and the risks resulting from them.

Abstract #9

CELLULAR PHENOTYPE OF HETEROZYGOUS MUTATIONS IN *SH2B1* THAT ARE ASSOCIATED WITH SEVERE OBESITY, REDUCED FINAL HEIGHT AND MALADAPTIVE BEHAVIOR

Michael E. Doche[1]*, Elena G. Bochukova[3]*, Hsiao-Wen Su[1], Laura Pearce[3], Joel M. Cline[1], Liangyou Rui[1], Christin Carter-Su[1,2#], and I. Sadaf Farooqi[3#]

[1] Department of Molecular and Integrative Physiology and [2]Cellular and Molecular Biology Graduate Program, Univ of Michigan Medical School, Ann Arbor, MI; [3]University of Cambridge Metabolic Research Laboratories, Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, UK. *, # These authors contributed equally to this work.

SH2B1 is an adaptor protein and signaling molecule for many hormones and cytokines including growth hormone (GH), insulin and leptin. Targeted deletion of *SH2B1* in mice results in increased food intake, severe obesity, insulin resistance, as well as aggressive behavior. Human *SH2B1* is located on chromosome region 16p11.2 and deletions on 16p11.2 are associated with learning difficulties, behavioral abnormalities and obesity. In this study we sequenced *SH2B1* from 300 patients with severe early-onset obesity from the Genetics of Obesity Study (GOOS) consortium and identified four heterozygous mutations in *SH2B1* in five unrelated probands: a frameshift mutation F344LfsX20, which leads to a truncated protein product, and point mutations P90H (2 patients), P322S and T175N, which were absent from 300 control chromosomes. We performed co-segregation studies that showed that the mutations were inherited from overweight/obese parents and some mutation carriers also had reduced final height as adults. Additionally, we found that all of the probands had a history of behavioral abnormalities which included delayed speech and language development in early childhood, aggressive behavior, social isolation and, in some instances, criminal behavior as adults. Assays in which SH2B1β has been previously shown to be important were used to assess the ramifications of the mutations. We examined the effect of the mutations on SH2B1β expression, sub-cellular localization, and ability to enhance NGF-induced neuronal differentiation, cycling through the nucleus, GH-induced macrophage motility, JAK2 activation, leptin signaling, and insulin signaling. The truncation mutant expresses poorly, mislocalizes and cannot mediate activation of signaling pathways, while the point mutants express and localize normally, and enhance JAK2, leptin and insulin signaling to the same extent as WT. However, all mutants significantly impaired the ability of SH2B1β to enhance NGF-induced neuronal differentiation, cycle through the nucleus, and enhance GH-induced macrophage motility. In summary, we have identified loss of function mutations in *SH2B1* in patients with severe obesity, insulin resistance and reduced final height who also exhibit a spectrum of maladaptive behaviors, leading us to conclude that *SH2B1* plays a critical role in the control of human body weight and may be implicated in some aspects of behavior. The inability of mutant SH2B1 to enhance neuronal differentiation and cellular migration suggests that these actions of SH2B1 are likely to contribute to improper neuronal development and the human phenotype.

Abstract #10

PHOSPHORYLATION OF THE ADAPTER PROTEIN SH2B1β REGULATES ITS ABILITY TO ENHANCE GROWTH HORMONE (GH)-DEPENDENT MACROPHAGE MOTILITY. Hsiao-wen Su and Christin Carter-Su. Dept of Integrative & Molecular Physiology, Univ of Mich Med School, Ann Arbor, MI (cartersu@umich.edu)

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SH2B1β is an adapter protein recently implicated as a human disease gene associated with obesity, insulin resistance, short stature and a spectrum of maladaptive behaviors. In response to ligand binding, SH2B1β is recruited to a variety of receptor-associated tyrosine kinases, including the GH receptor-associated tyrosine kinase JAK2. SH2B1β has also been shown to promote GH-dependent actin cytoskeleton remodeling. In this study, we examined the role of SH2B1β in GH regulation of cell migration in RAW264.7 macrophages. We found that GH acts as a chemoattractant to induce RAW cell migration using a transwell migration assay. SH2B1β overexpression enhanced GH-dependent motility of RAW cells while SH2B1 knockdown using shRNA inhibited migration. Phosphospecific antibodies revealed that GH promotes the phosphorylation of SH2B1β on the previously identified JAK2 substrates Tyr439 and Tyr494. Mutation of these two tyrosines to phenylalanine substantially decreased both basal and GH-stimulated macrophage migration but had no effect on the ability of SH2B1β to localize to the plasma membrane (assessed using confocal imaging of GFP-SH2B1β in live cells). Previously, we showed that SH2B1β localizes to the plasma membrane via a polybasic nuclear localization sequence (NLS). We also provided evidence that phosphorylation of Ser161 and/or Ser165 proximal to this NLS releases SH2B1β from the plasma membrane, presumably by decreasing the electrostatic interactions between SH2B1β's polybasic NLS and negatively charged phospholipids in the plasma membrane. Mutating basic residues (K_{146} PKLKKR to K_{146}PALAAA) in the NLS or creating the phosphomimetic SH2B1β(S165E), both of which release SH2B1β from the plasma membrane, enhanced macrophage motility without altering their ability to be phosphorylated on Tyr439 and Tyr494 by JAK2. Conversely, mutating Ser161 and Ser165 to alanine to prevent their phosphorylation and increase binding of SH2B1β to the plasma membrane inhibited both basal and GH-stimulated macrophage migration. Taken together, our results suggest that SH2B1β is required for GH-dependent macrophage migration. Further, phosphorylation of Tyr439 and Tyr494 in SH2B1β by JAK2 appears to be required, perhaps by enabling recruitment of key signaling proteins to the GH receptor/JAK2/SH2B1β complex. Phosphorylation of serines and the accompanying release of Sh2B1β from the plasma membrane also appear to be required. This ability of the cell to regulate the function and subcellular localization of SH2B1β via phosphorylation provides a mechanism by which the cell can prioritize which of the many possible responses involving SH2B1β are utilized at any given time.

Abstract #11

ROLE OF CIRCADIAN PROTEIN E4BP4 IN THE REGULATION OF LIVER AMPK AND GLUCOSE METABOLISM

Katie Buelow[1], Kimberly Carlson[2], Gogol Guha[1], Tanay Kulkarni[1], Lei Yin[1], Xin Tong[1]
[1] Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI 48109;[2]University of Texas Medical School at Houston, Houston, TX 77030 Corresponding Author: leiyin@umich.edu

Many physiological processes in the human body exhibit a 24-hour cyclic nature, known as circadian rhythms. Circadian rhythms are generated by the 24-hour light/dark cycle of night and day, the feeding/fasting patterns of an organism, and transcription/translation feedback loops. Recently, much attention has been given to circadian control of metabolic processes. One circadian output protein linked to metabolic activity is the transcriptional repressor, E4BP4. Our preliminary data indicates a role of E4BP4 in regulation of AMPK, which is involved in glucose metabolism. We hypothesize that manipulating levels of E4BP4 in the liver will alter glucose metabolism via the AMPK pathway. To determine whether lack of E4BP4 expression results in metabolic alteration in mice, we performed metabolic phenotyping analysis in both *E4bp4* wildtype (WT) and knockout (KO) mice that were fed normal chow (NC) or high-fat diet (HFD) for 7 weeks. The KO mice were protected from HFD-induced weight gain and hyperglycemia during the course of HFD feeding. Moreover, the KO mice showed a better glucose tolerance and insulin sensitivity under both NC and HFD conditions, measured by insulin tolerance tests and glucose tolerance tests. Thus, loss of *E4bp4* expression provides protection from HFD-induced weight gain, glucose intolerance, and insulin insensitivity. HFD has also been shown to increase hepatic gluconeogenesis and elevate fasting glucose. To examine whether E4BP4 affects the hepatic gluconeogenesis process upon HFD feeding, we measured the mRNA levels of *G6pase* and *Pepck*, two rate-limiting enzymes for *de novo* gluconeogenesis. The expression of *G6pase*, but not *Pepck*, was significantly down-regulated in the liver of KO mice. Consistent with its expression level, G6Pase enzymatic activity was also significantly decreased in the KO mice, suggesting a defect in gluconeogenesis. To test whether the AMPK pathway could contribute to the glucose phenotype observed in the KO mice, we measured the expression of AMPK subunits and AMPK phosphorylation (AMPK-P) in the liver. The levels of AMPK-P, measured by ELISA, were significantly elevated in KO mice and the expression of AMPK-gamma-1 subunit was induced in the KO animals, indicating that E4BP4 acts as a repressor of the AMPK pathway. The E4BP4-dependent regulation of AMPK pathway highlights an important component of how glucose homeostasis is maintained in response to circadian and nutritional cues.

Abstract #12

Differences in nutrient absorption and metabolism following Roux-en-Y and adjustable gastric banding bariatric surgery.

Charles Evans[1], Tanu Soni[1], Jie Cao[1], Gerardo Febres[2], Judith Korner[2], Charles Burant[1]

Background: Maintenance of weight loss and improvement in insulin resistance are greater following Roux-en-Y (RY) gastric bypass compared with adjustable gastric banding (GB). To understand the role of metabolite dynamics in these differences, we used directed and undirected metabolomics to profile plasma during a mixed meal challenge (MMTT) using Optifast®.

Methods: Changes in nutrient metabolism was assessed in 8 lean individuals (BMI=21.8±1.5), 15 obese individuals before (BMI=53.3±10.6) and after (BMI=38.0±5.9) RY, and 11 obese individuals before (BMI=43.5±10.1) and after (BMI=37.9±11.5) GB using LC/MS and GC/MS.

Results: HOMA-IR improved in in RY (6.3±3.9 to 2.5±1.4) and in GB (5.6±2.2 to 3.3±1.1). A consistent alteration in the absorption and disposal of glucose and amino acids was seen post-RY, with a sharper and higher rise in most amino acids compared to the profiles in lean controls or following GB without changes in area under the curve. Very short chain acylcarnitines (C2-C4) levels were increased significantly during MMTT following RY compared to GB, indicating increased glucose and branched chain amino acid catabolism (BCAA). Consistent with this was a concomitant increase in plasma citrate and malate levels and the BCAA metabolites 2-ketovaline, 2-oxoisocaproic acid and 3-hydroxy-3-methyl-glutaric acid. Preliminary analysis of plasma by unbiased metabolomics showed significant differences between lean, RY and GB in both fasting and post MMTT samples.

Conclusions: The results suggest that obesity-related changes in BCAA are due to alterations in catabolism. Dynamics of nutrient absorption and metabolism may contribute to the differences in insulin resistance and long term weight loss in RY and GB.

Abstract #13

Oxidative Stress and Altered Arginine Methylation contribute to Accelerated Atherosclerosis in an Animal Model of Chronic Kidney Disease

Lixia Zeng, Jaeman Byun, and Subramaniam Pennathur, Division of Nephrology, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109

Atherosclerotic cardiovascular disease is the leading cause of death in patients with chronic kidney disease (CKD). However, the molecular mechanisms underlying this increased risk remain poorly understood. We developed a pathophysiologically relevant animal model of CKD-accelerated atherosclerosis and investigated the role of oxidative stress pathways and altered arginine methylation by liquid chromatography tandem mass spectrometry (LC/MS) in this process. Male LDL receptor deficient ($LDLr^{-/-}$) mice at age 6 weeks were subjected to sham (Control) or 5/6 nephrectomy (CKD) surgery. Subsequently, the animals were maintained in high fat diet for 24 weeks. As anticipated, the CKD mice had significantly higher plasma creatinine, lower hematocrit, decreased body weight and higher mortality. Interestingly, the CKD mice did not have significant blood pressure changes compared with controls. Quantification of lesion area revealed that CKD mice had significantly elevated aortic plaque area fraction, necrotic core, fibrosis as well as greater luminal narrowing consistent with accelerated atherosclerosis. Additionally, cholesterol content and macrophage infiltration were elevated in the aortic lesions of the CKD animals. LC/MS analysis of oxidation markers (nitrotyrosine and dityrosine) showed marked elevation in the aortic lesions of the CKD animals consistent with enhanced oxidative stress. Targeted metabolomic analysis of arginine methylation pathways in plasma was performed by isotope dilution LC/MS including asymmetric dimethyl arginine (ADMA), symmetric dimethyl arginine (SDMA), N-mono-methylarginine (NMMA), homoarginine, arginine and its catabolites (citrulline and ornithine). No significant changes were noted for NMMA, homoarginine and ornithine between groups. Although elevated plasma levels of ADMA and SDMA were found in the CKD mice, only higher ADMA level correlated with aortic lesion area. These findings strongly implicate that oxidative stress and selectively altered arginine methylation contribute to CKD accelerated atherosclerosis.

Abstract #14

SRA/SRAP regulates fat formation and adipocyte gene expression in mice

Shannon Liu[1], Ruichuan Xu[1], Hongzhi Miao[2], Jingcheng Yu[1], Thomas, L. Saunders[3], Ormond A. MacDougald[1,4], Ronald J. Koenig[1] and Bin Xu[1]

[1]Department of Internal Medicine, Division of Metabolism, Endocrinology and Diabetes,
[2]Department of Pediatrics., [3]Department of Internal Medicine, Transgenic Animal Model Core,
[4]Department of Molecular & Integrative Physiology,
University of Michigan Medical Center, Ann Arbor, Michigan 48109, USA

Obesity develops when energy intake exceeds energy expenditure, and recruits excess mature adipocytes in white adipose tissue. Understanding the transcriptional control and cell biology underlying the conversion of preadipocytes to adipocytes, the developmental origins of adipose tissue and the exact pathways and intermediates between the embryonic stem cell and mature adipocyte are critical to control obesity. White adipose tissue (WAT) is specialized for the storage of chemical energy as triglycerides, whereas brown adipose tissue (BAT) dissipates chemical energy in the form of heat and functions as a defense against obesity. We have recently found novel functions of the non-coding RNA, Steroid Receptor RNA Activator (SRA) in adipocyte biology. The *Sra1* gene expresses two functional molecules, a non-coding SRA RNA (SRA), and as a coding protein by SRA mRNA, SRA protein (SRAP). We have shown that SRA associates with PPARγ and coactivates PPARγ-dependent reporter gene expression. Overexpression of SRA in ST2 mesenchymal precursor cells promotes their differentiation into adipocytes. Conversely, knockdown of endogenous SRA inhibits 3T3-L1 preadipocyte differentiation. Microarray analysis reveals hundreds of SRA-responsive genes in adipocytes. Some functions of SRA may involve mechanisms other than coactivation of PPARγ. For example, SRA promotes preadipocyte proliferation, and increases phosphorylation of Akt and insulin-stimulated glucose uptake in adipocytes. Strikingly, our recent *in vivo* experiments discovered that mice with SRA knockdown (SRAKD) have complete loss of white adipose tissue. These mice have growth defects, fatty liver and are hypoglycemic. To further assess the consequences of SRA loss-of-function, we have also generated the first mouse model with SRA global knockout (SRAKO). These SRAKO mice exhibit very unique phenotypes, in which SRAKO females have defects in growth and WAT formation, lower blood glucose levels and decreased expression of both white and brown adipocyte markers but induced expression of UCP-1 in both WAT and BAT. In contrast, SRAKO males have normal WAT formation with increased expression of p160 coactivators (SRC-2 and SRC-3), and changes of expression of obesity-related inflammation genes. SRAKO males also have increased BAT activity with elevated expression of a subset of brown adipocyte markers including PGC-1α, UCP-1, PRDM16 and Dio2, and with moderate insulin resistance. Therefore, SRA/SRAP emerge as novel regulators of adipocyte development and potentially of energy balance.

Abstract #15

DEVELOPMENT OF CARBON 13 METABOLIC FLUX ANALYSIS METHODS FOR USE WITH SKELETAL MUSCLE: APPLICATION TO RATS BRED FOR HIGH OR LOW INTRINSIC OXIDATIVE CAPACITY

Charles R. Evans,[1,2] Katherine A. Overmyer,[2] Jie Cao,[1] Nathan R. Qi,[1,2] Steven L. Britton,[3] and Charles F. Burant[1,2]

[1]*Michigan Metabolomics and Obesity Center, University of Michigan, Ann Arbor, MI 48109*
[2]*Division of MEND, Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109*
[3]*Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI 48109*

In humans, oxidative capacity is a well-established measure of metabolic health. Low oxidative capacity is prevalent in people with the metabolic syndrome, obesity and type 2 diabetes. To help understand the causes of the metabolic syndrome, we have investigated a line of low capacity runner (LCR) and high capacity runner (HCR) rats developed by a program of artificial selection for exercise endurance. Detailed phenotyping studies performed in our lab suggest that fatty acid oxidation and the balance between anaplerotic and cataplerotic flux of the TCA cycle differ between skeletal muscle from the LCR and HCR animals. We hypothesize that these alterations are related to the connection between low oxidative capacity and the metabolic syndrome. To this end, we are developing a method to directly measure metabolic flux in skeletal muscle via use of stable isotope tracers. The method, termed carbon 13 metabolic flux analysis (^{13}C-MFA), uses liquid chromatography – mass spectrometry (LC-MS) to measure incorporation of the stable isotope into a wide variety of metabolic intermediates including those of glycolysis, the TCA cycle, and lipid synthesis. We describe the use of ^{13}C-MFA for in vitro experiments in isolated skeletal muscle, as well as in vivo experiments in which stable isotope tracers are used to investigate metabolic flux in a living animal. Results reveal the utility of ^{13}C-MFA flux analysis for study of metabolite turnover and give preliminary insights into alterations in metabolism associated with changes in oxidative capacity.

Abstract #16

AGING IS ASSOCIATED WITH AN INCREASE IN T CELLS AND INFLAMMATORY MACROPHAGES IN VISCERAL ADIPOSE TISSUE

Carey N. Lumeng, Jianhua Liu, Lynn Geletka, Colin Delaney, Jennifer DelProposto, Anjali Desai, Kelsie Oatmen, Gabriel Martinez-Santibanez, Annabelle Julius, Sanjay Garg, and Raymond L. Yung. University of Michigan, Ann Arbor MI.

Background: Age-related adiposity has been linked to chronic inflammatory diseases in late-life. To date, the studies on adipose tissue leukocytes and aging have not taken into account the heterogeneity of adipose tissue macrophages (ATMs), nor have they examined how age impacts other leukocytes such as T cell in fat. **Objective:** To examine leukocyte composition of visceral fat in young and old mice. **Design/Methods:** Visceral fat pads from neonatal (3d), Young (6mo) and Old (22mo) C57Bl/6 mice were assessed for macrophage and T cell composition using flow cytometry and immunofluorescence microscopy. Adipocyte size was measured using ImageJ. **Results:** Neonatal mice demonstrate asynchronous adipose tissue development and have large numbers of ATMs present in developing fat. Old mice had increased adiposity and larger adipocytes than young. With age there were significant qualitative changes in ATMs that generate a decrease in resident Type 2 (M2) ATMs. The profile of ATMs in old fat shifts towards a pro-inflammatory environment with increased numbers of CD11c+ (Type 1) and CD206-CD11c- (double negative) ATMs. The mechanism of this aging-induced shift in the phenotypic profile of ATMs was found to be related to a decrease in PPARγ expression in ATMs and alterations in chemokine/chemokine receptor expression profiles. Furthermore, we have revealed a profound and unexpected expansion of adipose tissue T (ATT) cells in visceral fat with aging that includes a significant induction of regulatory T cells (Tregs) in fat. **Conclusions:** Our findings demonstrate that macrophages are a central component of adipose tissue development. We also have found a unique inflammatory cell signature that is induced in the physiologic context of aging adipose tissue that differs from those induced in setting of diet-induced obesity.

Abstract #17

NEUROPEPTIDE Y PRODUCTION BY MACROPHAGES AND ITS INFLUENCES ON ACTIVATION STATE

Kanakadurga Singer, David L Morris, Tianyi, Wang, Carey N. Lumeng

Background: Neuropeptide Y (NPY) contributes to the link between obesity and stress via its peripheral effects on adipose tissue function, but the mechanism for this is unclear.
Objective: In this study, we examined the hypothesis that NPY influences metabolism by modifying macrophage activation state.
Methods: M1 (LPS) and M2 (IL-4) activation was assessed in bone marrow derived macrophages (BMMP) and dendritic cells (BMDC) by RT-PCR and ELISA obtained from C57Bl/6 mice fed a normal diet (ND) or high fat diet (HFD). Immunofluorescence of adipose tissue explants from ND and HFD mice was performed. Adipose tissue was separated into stromal-vascular fraction (SVF) and adipocytes after collagenase digestion.
Results: Immunofluorescence demonstrated a network of nerves associated with blood vessels, adipocytes, and crown-like structures in fat. Npy and NPY receptor gene expression was higher in the SVF compared to adipocyte fractions and was increased in the SVF from visceral fat compared to inguinal fat in obese mice. The NPY cleaving enzyme DPP4 was expressed on macrophages. In vitro, addition of NPY to BMMP or BMDC had minimal effects on M1 or M2 activation profiles. However, NPY receptor inhibition had effects on macrophage activation. Y5R antagonists increased Tnfa expression in BMMP, while Y2R antagonists increased Il6 expression in BMDC. NPY mRNA and protein expression was detected in BMMP and BMDC. BMDCs from HFD animals had overall increased M1 genes (Tnfa, iNos) expression compared to ND animals.
Conclusions: These results suggest that NPY has anti-inflammatory effects on macrophages via the Y2/Y5 receptors and that macrophages are a regulated source of NPY production. In addition, bone marrow derived cells from ND and HFD animals are primed to respond differently to polarization stimuli.

Abstract #18

HIGH CONCENTRATIONS OF A PHYSIOLOGIC FATTY ACID MIXTURE DO NOT IMPAIR INSULIN SIGNALING IN C2C12 MYOTUBES

Allison C. Everett, Sean A. Newsom, Alexander Hinko, and Jeffrey F. Horowitz
Substrate Metabolism Laboratory, School of Kinesiology, University of Michigan, Ann Arbor, MI

Excessive fatty acid availability has been found to suppress insulin action in skeletal muscle, in part through the accumulation of lipids known to impair insulin signaling. The primary aim of this study was to determine the effects of graded doses of a physiologic mix of fatty acids (i.e., resembling the plasma fatty acid profile of a healthy human) on lipid accumulation and factors that regulate insulin action in skeletal muscle cells. C2C12 myotubes were incubated in graded doses (0, 0.1, 0.2, 0.4, 0.8, 1.2, 1.6, and 2.0mM) of a physiologic fatty acid mixture (25% palmitic, 15% stearic, 5% palmitoleic, 30% oleic, 25% linoleic) for 12h, followed by a 15min incubation with or without insulin (100nM). Muscle cell triacylglycerol (TAG) and diacylglycerol (DAG) were isolated using standard chromatography techniques and lipid concentrations were determined via gas chromatography/mass spectrometry. Phosphorylation of key insulin signaling proteins (Akt, AS160, GSK) were quantified using standard immunoblotting procedures. Not surprisingly, we found that muscle cell TAG and DAG concentration increased markedly with increasing fatty acid concentrations. Despite the large increase in lipid accumulation, insulin-stimulated phosphorylation of key insulin signaling proteins was not impaired, even after incubation in the highest fatty acid dose (0 vs. 2mM; $pAkt^{Thr308}$/Akt: 3.8±1.1 vs. 6.0±0.6 arbitrary units (AU); $pAS160^{Thr642}$/AS160: 2.0±0.2 vs. 3.6±0.9 AU; pGSK-$3\beta^{Ser21/9}$/GSK-3β: 3.3±0.6 vs. 4.5±0.7 AU). In conclusion, exposing muscle cells to a high concentration of fatty acid resembling the lipid profile of a healthy human markedly increased the accumulation of lipid, but did not impair muscle cell insulin signaling.

Abstract #19

REGULATOR OF SEX LIMITATION (*RSL*), A KRÜPPEL-ASSOCIATED BOX ZINC FINGER REPRESSOR, LINKS SEX DIFFERENCES IN GENE EXPRESSION TO METABOLISM

Christopher J. Krebs, Ania Owczarczyk, Diane M. Robins

Krüppel-associated box zinc finger proteins (KRAB-zfps) are the largest class of transcription factors encoded in mammalian genomes, yet very few have been assigned biological roles. These repressors silence gene expression via interaction with KRAB-associated protein 1 (KAP1), a corepressor that recruits a complex of chromatin modifying enzymes to genomic targets. We identified the first function for a KRAB-zfp by cloning Regulator of sex-limitation (Rsl) 1 and Rsl2 from a mutant mouse altered in sexually dimorphic liver gene expression. Further analysis revealed that 8% of the mouse liver transcriptome is Rsl-responsive, with many genes residing in pathways of steroid and lipid metabolism.

Although mice mutant in both Rsl1 and Rsl2 (rsl) appear normal, reproduction and intermediary metabolism differ from wild type (wt). Female rsl mice enter puberty early and are more attractive to males; both traits are due to combined effects on hormonal and pheromonal cues. rsl mice are lean and respond differentially to fasting, with altered expression of key enzymes in glucose and lipid metabolism. rsl females, but not males, gain more weight on a high fat diet. Liver-specific cDNA transgenes showed that Rsl2 restored puberty onset to wt whereas both Rsl1 and Rsl2 attenuated weight gain on high fat. While these effects are liver-intrinsic, overall physiology is impacted by Rsl actions in extrahepatic sites.

To determine the molecular basis of KRAB-zfp action, we are exploring how Rsl repression interacts with hormonal induction via STAT5b on the enhancer of the mouse sex-limited protein (Slp) gene, the hallmark of Rsl action. Chromatin immunoprecipitation (ChIP) experiments indicate that Rsl binds concurrent with STAT5b activation of Slp but KAP1 and STAT5b bind reciprocally dependent on growth hormone pulses. Thus presence of KAP1 in the complex appears to determine repression vs. activation. Prior to STAT5b activation at puberty, Rsl directs gene silencing by targeting methylation to a site 2 kb downstream in the Slp promoter. This detailed view of Rsl action on Slp expression forms a basis for identifying global genomic targets and actions by ChIP-Seq.

In sum, KRAB-zfp molecular and biological actions can be causally linked via the unique Rsl mouse model. This will allow us to determine how these important but poorly understood mammalian transcription factors modulate a broad array of genes that affect complex traits, such as reproduction, and differential susceptibility to common diseases, such as diabetes.

Abstract #20

DIFFERENTIAL ROLES OF HYPERGLYCEMIA AND HYPOINSULINEMIA IN DIABETES INDUCED RETINAL CELL DEATH: EVIDENCE FOR RETINAL INSULIN RESISTANCE

Patrice E. Fort, Mandy K. Losiewicz, Chad EN Reiter, Ravi SJ Singh, Makoto Nakamura, Steven F. Abcouwer S, Alistair J. Barber A, Thomas W Gardner

Purpose: Diabetes pathology derives from the combination of hyperglycemia and hypoinsulinemia or insulin resistance leading to diabetic complications including diabetic neuropathy, nephropathy and retinopathy. Diabetic retinopathy is characterized by numerous retinal defects affecting the vasculature and the neuro-retina, but the relative contributions of the loss of retinal insulin signaling and hyperglycemia have never been directly compared. In this study we tested the hypothesis that increased retinal insulin signaling and glycemic normalization would exert differential effects on retinal cell survival and retinal physiology during diabetes.
Methods & Results: We found that both subconjunctival insulin administration and systemic glycemic reduction using the sodium-glucose linked transporter inhibitor phloridzin affected the regulation of retinal cell survival in diabetic rats. Both treatments partially restored the retinal insulin signaling without increasing plasma insulin levels. Retinal transcriptomic and histological analysis also clearly demonstrated that local administration of insulin and systemic glycemia normalization use different pathways to counteract the effects of diabetes on the retina. While local insulin primarily affected inflammation-associated pathways, systemic glycemic control affected pathways involved in the regulation of cell signaling and metabolism.
Conclusion: These results suggest that hyperglycemia induces resistance to growth factor action in the retina and clearly demonstrate that both restoration of glycemic control and retinal insulin signaling can act through different pathways to both normalize diabetes-induced retinal abnormality and prevent vision loss.

Abstract #21

TARGETED LC/MS BASED METABOLOMIC ANALYSIS REVEALS ALTERED METABOLIC SUBSTRATE UTILIZATION IN RENAL CORTEX IN DIABETIC NEPHROPATHY

Carolyn Buller, Anuradha Vivekanandan-Giri, Jaeman Byun, Hongyu Zhang, Jharna Saha, Frank C. Brosius III, Subramaniam Pennathur.
Department of Internal Medicine, Division of Nephrology
University of Michigan, Ann Arbor, 48109.

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease in the United States. While it is well-recognized that the diabetic state leads to altered carbohydrate, amino acid and fatty acid metabolism, the utilization of these substrates *in vivo* in diabetic renal tissue has not been systematically studied. In this study, we utilized a pathophysiologically relevant animal model which exhibits characteristic pathologic features of DN. We developed a comprehensive targeted metabolomic platform for quantitative analysis of glycolytic, tricarboxylic acid (TCA) and fatty acid oxidation intermediates by electrospray ionization tandem mass spectrometry (LC/ESI/MS/MS) in the multiple reaction monitoring (MRM) mode following a single extraction. We determined metabolite levels in control and diabetic renal cortex and mitochondria isolated from renal cortex in a type 2 diabetic (C57BLKS db/db) model. There was a marked (* $p< 0.05$) elevation of all glycolytic and TCA intermediates measured in renal cortex compared to control littermates. Interestingly, lactate levels were significantly elevated consistent with increased aerobic glycolysis. In contrast, no differences were found in fatty acid metabolites (acyl CoAs and acyl carnitines), suggesting that fatty acid oxidation is relatively unchanged in diabetic kidneys. Importantly, mitochondria isolated from the kidney cortex exhibited similar changes in metabolite profile raising the possibility that mitochondrial metabolic enzyme or regulatory network alterations may affect substrate utilization in DN. In summary, we have developed a sensitive, high-throughput method of analyzing substrate metabolism. Increases in renal metabolites may serve as potential biomarkers of DN.

Abstract #22

INSULIN ACTION IN KISSPEPTIN1 NEURONS IS IMPORTANT FOR THE REGULATION OF PUBERTY AND REPRODUCTION

Xiaoliang Qiu, Abigail Dowling, Joseph Marino, Laura Nedorezov, Latrice Faulkner, Jennifer Hill

The neuropeptide kisspeptin, coded by gene Kiss1 is necessary for reproduction, fertility, and puberty. Humans and mice with loss-of-function mutation of Kiss1 are infertile due to hypogonadotropic hypogonadism. Insulin receptors and insulin signaling proteins are widely distributed throughout the hypothalamus which is enriched of Kiss1 neurons, and plays a pivotal role in regulation of energy balance, metabolism, and reproduction. In order to study whether insulin-sensing plays an important role in Kiss1 neuron, we generate Kiss1 neuron specific insulin receptor knockout mice (Kiss1-Cre, IR $^{flox/flox}$ mice). Here, we show that Kiss1-Cre, IR $^{flox/flox}$ female mice have delayed vaginal opening and first estrus cycle, and male mice also have late sexual maturation. However, the reproduction in the knockout mice is normal. Furthermore, these mice have no discernable abnormity in body weight, food intake, and glucose metabolism. We conclude that insulin signaling in Kiss1 neuron may serve as a metabolic sensing signal in the puberty initiation, but not reproduction, food intake, body weight and metabolism.

Abstract #23

NOVEL MECHANISMS OF PYRUVATE DEHYDROGENASE REGULATION BY MITOCHONDRIAL SIRTUINS

Daniel X. Tishkoff and David B. Lombard

Department of Pathology and Institute of Gerontology, University of Michigan, Ann Arbor, MI USA 48109

Mitochondria are cytoplasmic organelles that perform numerous functions crucial to cellular and organismal homeostasis, notably generation of most cellular ATP and supply of metabolic intermediates for macromolecular synthesis. With age, acquired defects in mitochondrial number and function contribute to a broad spectrum of pathologies: sarcopenia, insulin resistance and type 2 diabetes (T2D), cardiac dysfunction, and neurodegeneration. Despite the central importance of mitochondria to health, mechanisms regulating mitochondrial energy production and metabolite flux remain incompletely understood.

Pyruvate Dehydrogenase Complex (PDC) is a mitochondrial holoenzyme that catalyzes oxidization of pyruvate to acetyl-CoA, thus linking glycolysis to the Krebs cycle. PDC consists of E1α/E1β heterotetramers surrounding a core of E2, E3, and E3bp subunits (Patel and Korotchkina, 2006). PDC activity is tightly regulated. E1α phosphorylation by pyruvate dehydrogenase kinases (PDKs) represses PDC activity, and represents a key known mechanism of PDC regulation. Conversely, pyruvate dehydrogenase phosphatases (PDPs) dephosphorylate E1α and activate PDC. Roughly 50% of overall daily caloric intake passes through PDC (Patel and Korotchkina, 2006). PDC produces acetyl-CoA for lipid synthesis under conditions of caloric excess. Regulation of PDC activity is a key mechanism regulating glucose oxidation in mammals *in vivo* (Wieland et al., 1972). A large body of evidence links hypofunctional PDC to metabolic syndrome, T2D, cardiac ischemia, and cancer (Roche and Hiromasa, 2007).

The sirtuins are a family of NAD^+-dependent lysine deacetylases that promote longevity in yeast and regulate core processes in mammals (Lombard et al., 2011). Three mammalian sirtuins – SIRT3, SIRT4, and SIRT5 –localize to mitochondria (Lombard et al, 2007). We have identified a novel means of PDC regulation: specifically, SIRT4 and SIRT5 play key roles in modulating PDC activity *in vivo*. This suggests that pharmacologic targeting of these sirtuins may represent a useful strategy to increase glucose metabolism in T2D and other conditions where impaired PDC function is implicated.

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Abstract #24

IN UTERO BISPHENOL A EXPOSURE: EFFECTS ON METABOLIC HOMEOSTASIS THROUGHOUT THE LIFE-COURSE

Olivia S. Anderson[1], Brisa N. Sanchez[2], Karen E. Peterson[1], Zhenzhen Zhang[2], and Dana C. Dolinoy[1]

[1]Department of Environmental Health Sciences, University of Michigan, Ann Arbor, Michigan, USA
[2]Department of Biostatistics, University of Michigan, Ann Arbor, Michigan, USA

Submitted by:
Olivia S. Anderson, M.P.H., R.D.
Doctoral Candidate
University of Michigan School of Public Health
Department of Environmental Health Science
oliviasa@umich.edu

Animal data indicate that the risk of developing adult-onset diseases such as obesity and metabolic syndrome may be influenced by persistent adaptations to prenatal exposure of altered environmental conditions. One such exposure of interest is bisphenol A (BPA), a chemical used in the production of polycarbonate plastic and epoxy resins. *In utero* exposure to BPA has been linked to disturbances in metabolic homeostasis in animal models, including increased adipogenesis and glucose intolerance across different life-stages. Despite growing evidence, controversy still exists about BPA's ability to act as an obesogen, a chemical foreign to the body that has the ability to interrupt energy balance. Utilizing non-agouti isogenic *a/a* offspring from heterozygotic matings of *a/a* dams with A^{vy}/a sires, we examined the effects of *in utero* BPA exposure on metabolic homeostasis throughout the life-course. Only non-agouti *a/a* offspring were evaluated because BPA-induced physiological changes in A^{vy}/a offspring would be difficult to interpret due to a unique mechanism of obesity associated with the insertion of an epigenetically controlled retrotransposon in the agouti promoter. Following *in utero* exposure to either 50 ng/kg (n=20), 50 ug/kg (n=21), or 50 mg/kg (n=18) BPA through maternal diet, offspring energy expenditure, activity and body composition was measured through sophisticated animal phenotyping at three time-points (3, 6, and 9 months of age). Statistical analysis for offspring energy expenditure, activity and body composition was conducted using a linear mixed regression model for repeated measures adjusting for sex, and additional models were run with sex-exposure interactions. Results indicate that offspring exposed to 50 mg/kg BPA had higher oxygen consumption at 3 months among males and females, and at 9 months among males (p=0.002, and 0.04, respectively), and offspring exposed to 50 ug/kg BPA at 9 months among males (p=0.0005) compared to controls (n=19). Ambulatory activity was increased in ug exposed offspring at 3 and 9 months among females (p=0.0006, and 0.024, respectively), and in mg exposed offspring at 9 months among females (p=0.0021) compared to controls. Vertical activity was increased in mg exposed offspring at 3 months among both female and males, and at 6 and 9 months among females (p=0.0006, 0.02, and 0.02, respectively) and in ug exposed offspring at 9 months among females (p=0.002). Although not reaching significance, fat mass in ug exposed offspring is decreased compared to controls at 3 months among females and at 6 months among males (p=0.07, and 0.07, respectively). While recent literature connects early BPA exposure to obesity and obesity-related outcomes, our life-course analysis indicates BPA is associated with lean and hyperactive phenotypes, making it necessary to further evaluate BPA as a potential obesogen.

Abstract #25

INTRACELLULAR MECHANISMS INVOLVED IN REPRODUCTIVE DYSFUNCTION CAUSED BY DELETION OF INSULIN AND LEPTIN SIGNALING IN POMC NEURONS

Latrice D. Faulkner[1] and Jennifer W. Hill[1,2]
[1]Center for Diabetes and Endocrine Research, Department of Physiology and Pharmacology, College of Medicine, The University of Toledo, Toledo, OH 43614; [2]Department of Obstetrics and Gynecology, College of Medicine, The University of Toledo, Toledo, OH 43614.

During times of metabolic stress, animals must divert energy away from processes such as reproduction and channel energy towards survival. The hypothalamus contains reproductive circuits that have the ability to respond to metabolic cues allowing energy status to modify. The neurons that mediate this regulatory communication are not well understood. Hypothalamic pro-opiomelanocortin (POMC) neurons are critical regulators of energy balance and glucose homeostasis that also communicate with neurons controlling reproductive function [7-10]. Insulin and leptin are two vital messengers that relay information regarding energy stores to the CNS and both act in the brain to affect fertility[1]. Given the redundancy in the signaling pathways engaged by leptin and insulin[13-17], we hypothesized that previous studies deleting either one of the receptors may have underestimated the significance of insulin and leptin action on POMC neurons.

To this end, we characterized male mice that lack both insulin and leptin receptors only in POMC neurons. We crossed mice lacking leptin receptors in POMC neurons (Pomc-Cre, $Lepr^{flox/flox}$ mice)[11] with mice carrying a loxP-modified insulin allele ($IR^{flox/flox}$)[12] to create Pomc-Cre, $Lepr^{flox/flox}$ $IR^{flox/flox}$ mice. These mice were compared to littermate controls carrying the floxed alleles but without altered gene expression ($Lepr^{flox/flox,}$ $IR^{flox/flox}$ mice). Utilizing this model, we have shown that male mice on a mixed background lacking both insulin and leptin receptors in POMC neurons ($Lepr^{flox/flox,}$ $IR^{flox/flox}$ mice) exhibit increased body weights, excess hepatic glucose production, and systemic insulin resistance. Remarkably, $Lepr^{flox/flox,}$ $IR^{flox/flox}$ males also show reduced fertility associated with elevated testosterone levels, testis weights, and LH levels. Our results suggest that the absence of leptin and insulin signaling in POMC neurons increases basal LH release and disrupts reproductive function via reduction in the inhibitory opioid tone on GnRH neurons. Thus, perception of signals of energy status by POMC neurons is not only required for normal body weight and glucose homeostasis, but also permits normal fertility. Additional studies are required to determine whether this action is mediated via direct or indirect neuronal connections and the intracellular mechanisms involved.

Abstract #26

Lipolysis regulates adipose tissue T cells trafficking and inflammation
Kae Won Cho and Carey Lumeng; University of Michigan, Ann Arbor, MI

Background: Adipose tissue inflammation plays an important role in the development of metabolic diseases such as type 2 diabetes and cardiovascular diseases. Adipose tissue T cells (ATTs) and adipose tissue macrophages (ATMs) are the main source of inflammation in obese visceral fat. An integrated understanding of how ATMs and ATTs interact with each other in physiologic and pathologic settings remains unclear. **Objectives:** Since lipolysis can trigger ATM activation, we hypothesized that CD4+ ATT cells might also respond to lipolytic signals and collaborate with ATMs to promote adipose tissue inflammation. **Design/method:** Intravital microscopy techniques were developted to visualize the T cell migration in fat. Lean or obese mice were adoptively transferred with CFSE labeled T cells and then, injected with saline or β3-adrenergic receptor agonist (CL 316243). 16 h post-injection, the distribution of CD4+ T cells in adipose tissue was determined by confocal microscopy. ATT cell movement was assessed in fat explants using two-photon intravital microscopy (2P-IVM). Qualitative changes of ATMs were determined in adipose tissue explants treated with vehicle or β-adrenergic receptor agonist using immunofluorescent staining. **Results:** In visceral adipose tissue, CD4+ and CD8+ T cells localized to fat associated lymphoic clusters (FALCs) that harbor large numbers of ATMs. In saline treated mice, CD4+ ATT cells rapidly migrated towards the center of the FALC at rates of 3~10 μm/min. In contrast, with CL treatment, CD4+ T cells were nearly absent from the FALCs demonstrated slower motility (1~2 μm/min). Analysis of adipose tissue regions devoid of FALCs showed significant numbers of labeled CD4+ T cells only with CL treatment. In adipose tissue explants, adrenergic receptor agonist markedly induced the CD11c expressing cells around adipocytes in structures similar to crown-like structures. **Conclusions:** Lipolytic signals lead to the rapid translocation CD4+ ATT cells from FALCs to be in proximity to adipocytes. Dynamic regulation of ATT cell movement may be a core physiologic response to lipolysis and may link inflammation and metabolism.

Abstract #27

THE GROWTH HORMONE-RESPONSIVE TRANSCRIPTIONAL REPRESSOR BCL6 REGULATES GENES ASSOCIATED WITH LIPID METABOLISM AND ADIPOGENESIS.

Christopher R LaPensee[1], Siming Li[2], Jiandie D Lin[2], Alexander L Dent[3], Jessica Schwartz[1] Dept of Molecular and Integrative Physiology[1], Dept of Cell and Developmental Biology, and Life Sciences Insititute[2] Univ Michigan; Dept of Microbiology and Immunology[3], Indiana Univ School of Medicine.

Growth Hormone (GH) regulates normal growth and metabolism. We recently reported that the transcriptional repressor B-cell Lymphoma 6 (Bcl6) is present in adipocytes and regulated by GH, suggesting a novel mechanism of transcriptional regulation by GH. Bcl6 KO mice were found to exhibit a striking reduction in adipose tissue mass compared to wild-type (WT) mice, suggesting a link between Bcl6 and fat mass. We investigated whether alterations in lipid metabolism and/or adipogenesis contribute to this relationship. Lower hepatic triglycerides in male Bcl6 KO mice suggested that Bcl6 might contribute to the regulation of lipid metabolism. Since Bcl6 inhibits and GH increases expression of Suppressor of Cytokine Signaling (Socs2), we examined other GH-induced genes associated with lipid metabolism which we found contained predicted Bcl6 binding sites in their promoters. Among these, expression of fatty acid/Δ^5 desaturase (Fads1) and acyl CoA synthetase 5 (Acsl5), as well as Socs2, was elevated in liver of Bcl6-KO mice, suggesting similar patterns of regulation of some genes in lipid metabolic pathways mediated by Bcl6. During adipogenesis, we found that Bcl6 mRNA expression increased in 3T3-F442A adipocytes. In addition, expression of mRNA for adipogenic transcription factors C/ebpα, Ppparγ, and the adipocyte-specific marker aP2, were much lower than WT in the limited amount of adipose tissue obtained from Bcl6 KO mice. The anti-adipogenic genes Pref-1 and Gata3 were higher in Bcl6 KO adipose tissue, also consistent with a role of Bcl6 in adipogenesis and with reduced adipose tissue in Bcl6 deficiency. Together, these studies indicate that Bcl6 contributes to regulation of genes associated with lipid metabolism and adipogenesis, and suggest that this transcriptional repressor may play a role in GH-regulated lipid metabolism.

Abstract #28

EFFECTS OF GENETIC BACKGROUND ON INSULIN RESISTANCE AND FERTILITY IN A POLYCYSTIV OVARIAN SYNDROME MOUSE MODEL

Authors: A.R. Dowling [1], L.B. Nedorezov[1], J.S. Marino[1], XiaoLiang Qiu[1], and J.W. Hill[1,2].

[1]Center for Diabetes and Endocrine Research, Department of Physiology and Pharmacology, College of Medicine, University of Toledo, Toledo, Ohio 43614; [2]Department of Obstetrics and Gynecology, College of Medicine, The University of Toledo, Toledo, OH 43614.

Abstract:

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder in reproductive age women, and is characterized by hyperandrogenemia, insulin resistance, and reproductive abnormalities. Which of these factors triggers the pathogenesis of PCOS remains unclear. A mouse model of PCOS has been developed using chronic administration of dehydroepiandrosterone (DHEA) in the BALB/cByJ strain. We hypothesized that the PCOS phenotype would be more pronounced on the diabetes-prone C57/Bl6 background. C57Bl6 and BALB/cByJ female mice at 25 days of age were injected daily with DHEA (6mg/kg body weight) or vehicle for 20 consecutive days. In order to determine which strain more closely mimics PCOS, body weight, body composition, food intake, fasting insulin, glucose tolerance, serum estradiol, progesterone and leutinizing hormone were all compared to the control groups of each strain. Hyperandrogenemia induced glucose intolerance in the C57Bl6 but not the BALB/cByJ strain. Both strains exhibited accelerated puberty and caused acyclicity. Analysis of gene and protein expression was then performed for genes known to be linked to PCOS and/or that differ between the strains, including HSD17B6, POMC, ACVR2A, SGTA, Cyp17, Cyp11A, StAR, Bcl2, C4b, Gpd1, LHreceptor, Ncam1, Mbp, and Adh1. Among the differences seen, the expression of Fem1B, a gene associated with PCOS in humans, was upregulated in whole ovary of the C57BL6 strain, but not the BALB/c strain. This gene plays a role both in sex determination and glucose tolerance in mice, and thus provides a potential pathway for the induction of insulin resistance in PCOS. Nevertheless, the similarity of the reproductive phenotype in these two strains suggests that susceptibility to glucose dyregulation per se is not a causal factor in the development of PCOS in this model.

Abstract #29

THE ROLE OF MACROPHAGES IN ADIPOSE TISSUE REMODELING

Gabriel Martinez-Santibañez, Jennifer B. DelProposto, Lynn Geletka, and Carey N. Lumeng

Cellular and Molecular Biology Graduate Program, Department of Molecular and Integrative Physiology, Department of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, MI

Background: Adipose tissue fibrosis correlates with inflammation and obesity, and may promote the development of diabetes and metabolic syndrome. The extracellular matrix in adipose tissue is a critical regulator of adipocyte function and metabolism, since it has been shown that the loss of ECM remodeling flexibility promotes metabolic disease in mice. The cellular and molecular mediators of adipose tissue ECM in lean and obese states are unclear. In many tissues, macrophages contribute to ECM remodeling by the secretion of collagenases and protease inhibitors, and there is an extensive network of adipose tissue macrophages in fat associated with fibrotic regions.

Hypothesis: We hypothesize that ATMs contribute to the remodeling of the extracellular matrix during physiological and pathological expansion of adipose tissue.

Methods: C57/BL6 mice were fed a high fat diet (60%) for 8 or 16 weeks. In a second study, mice were fed high fat diet for 16 weeks before weight loss was induced by feeding a normal diet (4.5%) for 2 weeks. In both studies, visceral (epididymal), mesenteric, and subcutaneous (inguinal) fat depots were collected for gene expression analysis. Elastin in fat samples was stained with Verhoeff's elastic stain.

Results: We observed altered expression of many genes encoding Matrix Metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) during with diet-induced obesity. Specifically MMP-12, 13, 14, and TIMP-1 were significantly increased in visceral fat depots while MMP-2, 9, and 7 were decreased. Fat depot specific effects were observed with the most prominent expression differences seen in visceral fat. Since MMP-12 (macrophage metalloelastase) was >100 fold increased in the stromal-vascular fraction of fat with obesity, we examined the distribution of elastin fibers in visceral fat. With weight gain, we saw a significant increase in elastin deposition, while, after weight loss, there was a decrease in elastin staining.

Conclusions: The expression of MMPs and TIMPs is dynamically regulated during the expansion of various fat depots with diet-induced obesity that correlates with changes in ECM composition. Future studies will examine how distinct ATM sub-populations in fat couple the adipose tissue inflammation to ECM remodeling.

Abstract #30

AE Rothberg, LN McEwen, WH Herman. University of Michigan

The Impact of Weight Loss on Health Utility Scores: Implications for the Design of Cost-Utility Analyses of Medical Weight Loss Programs.

In cost-utility analysis, preferences for health states, termed health utilities, are combined with measures of duration to calculate quality-adjusted life-years (QALYs). Utility theory provides a well-established approach for the measurement of health preferences, and indirect approaches, such as multi-attribute utility models, are considered to be especially appropriate for cost-utility analyses because they reflect the values of the general public. A disadvantage of such models is that individuals with a disease or condition may underestimate the impact of the condition on the domains of health-related quality-of-life (HRQL) assessed such as mobility, usual activities, pain, and anxiety or depression. Failure to accurately assess the impact of a condition on HRQL may underestimate the value of an intervention to prevent or treat the condition. We hypothesized that obese individuals might underestimate the impact of obesity on HRQL and conducted a study to assess the relationship between body mass index (BMI) and HRQL measured both before and after substantial weight loss with the EQ-5D, a widely used multi-attribute utility model, and the visual analog scale.

We studies 49 obese subjects (age 49 $\pm$ 7 years, mean $\pm$ SD) with baseline BMI 39.5 $\pm$ 4.8 kg/m^2 (range 32.3-57.3) and follow-up BMI 32.4 $\pm$ 4.3 kg/m^2 (range 25.8-45.7) after a medical weight

loss intervention. Fifty-three percent were women and 86% white. The Table shows the relationship between HRQL and BMI as assessed by the VAS before and after weight loss.

	Body Mass Index (kg/m^2)				
	30	**35**	**40**	**45**	**50**
Before weight loss	66	64	62	60	58
After weight loss	80	75	70	65	60

After weight loss, HRQL was substantially higher for any given BMI, with the greatest difference observed at the lowest BMI levels.

Failure to account for the improvement in HRQL after weight loss will underestimate the benefit of weight loss programs in cost-utility analyses. To provide a more accurate picture of the benefits of weight loss interventions, cost-utility analyses should use utility scores measured both before and after weight loss.

Character count: 2,222 (Permitted 2,300)

Abstract #31

Sleep, physical activity and cardiometabolic risk in obese adolescents: Sex matters

Heidi B. IglayReger[1], Mark D. Peterson[1], Bethany J. Sallinen[2], Erik C. Heinlein[1], Christine A. Robert[1], Fauziya Hassan[3, 4], Susan J. Woolford[5], Paul M. Gordon[1]

[1]The Laboratory for Physical Activity and Exercise Intervention Research, Department of Physical Medicine and Rehabilitation; [2]Division of Child Behavioral Health, Department of Pediatrics; [3]Division of Pediatric Pulmonology; [4]Michael S. Aldrich Sleep Disorders Center, [5]Child Health Evaluation and Research Unit, Department of Pediatrics; at the University of Michigan, Ann Arbor, Michigan

Background: Though roughly 30% of obese adolescents are diagnosed with metabolic syndrome, there is a paucity of objective data regarding physical activity (PA) and sleep and their influence on cardiometabolic risk. We sought to determine sex-specific associations between sleep, PA and cardiometabolic risk in obese adolescents.

Methods: Metabolic syndrome characteristics (BMI, mean arterial pressure, HOMA-IR, HDL-C and triglycerides) were measured in obese adolescents (n=244; 69% female; mean age 14 ± 2 years) and standardized residuals (z-scores) were summed to create a continuous cardiometabolic risk score (cMetS), while controlling for age. Sleep and PA were objectively measured in free-living conditions for 7 days using SenseWear monitors. Participants who wore monitors for ≥ 22 hours on two consecutive days were included in analyses (n=62; 66% female). ANOVA and multiple regression were used to assess baseline characteristics as well as the association of race, PA and sleep with cMetS.

Results: cMetS did not differ by sex (n=62, $p>0.05$). Within females, sleep variables (total sleep, number and length of session) were unrelated to cMetS; however, PA emerged as a predictor of risk, even after controlling for the number ($\beta= -0.44$; $p<0.01$) and length ($\beta= -0.45$; $p<0.01$) of weekend sleep sessions (combined model $\beta= -0.45$; $p<0.05$). Conversely, for males the number ($\beta=0.63$; $p<0.01$) and length ($\beta= -0.73$; $p<0.01$) of weekend sleep sessions predicted cMetS, though PA did not. Neither weekday sleep nor total sleep predicted cMetS.

Conclusions: Sleep and PA affect cardiometabolic risk differently in obese adolescent males and females. Further research is needed to clarify sex-specific factors that influence cardiometabolic risk.

Abstract #32

Training decreased resistance exercise-induced macrophage recruitment and increased macrophage M2 polarization in skeletal muscle

Dongmei Liu[1], Maureen Sartor[1], Heidi IglayReger[1], Emidio Pistilli[2], Laurie Gutmann[3], Gustavo Nader[4], Eric Hoffman[5], and Paul Gordon, FACSM[1].

[1]University of Michigan, Ann Arbor, Michigan; [2]University of Pennsylvania, Philadelphia, PA; [3]West Virginia University, Morgantown, WV; [4]Karolinski Institute, Stockholm, Sweden; [5]Children's National Medical Center, Washington, DC

Macrophages play a crucial role in skeletal muscle repair and regeneration. Different types of macrophages have been implicated in the healing process with pro-inflammatory M1 involved in phagocytosis of tissue debris and anti-inflammatory M2 associated with myofiber growth. It is unknown if different macrophages are involved in resistance exercise (RE) training-induced muscle growth. **PURPOSE:** To examine the gene expression associated with macrophage recruitment and M1/M2 polarization in trained and untrained skeletal muscle following RE in humans. **METHODS:** Fourteen young men (n=7) and women (n=7) who underwent a 12-week progressive unilateral arm RE training (FAMuSS study) participated in the study. Six days after the last FAMuSS exercise session, subjects performed an acute bout of either unilateral (4 women and 3 men) or bilateral (3 women and 4 men) RE. Muscle biopsies were taken 4 hr post-exercise from both arms in all subjects. Expression profiling was performed using the Affymetrix Human Genome U133 Plus 2 chip. Absolute expression values were calculated using MAS.5 and normalized by RMA. Differential gene expression between trained exercised (**TE**) and untrained non-exercised (**UTNE**) or trained exercised and untrained exercised muscle (**UTE**) was analyzed using an intensity-based Bayesian moderated (paired) t-statistic. **RESULTS:** In **TE** vs. **UTNE** muscle monocyte chemotaxis regulators increased (*CCL2*, fold=↑2.46, p<0.0001; *CX3CL1*, fold=↑1.34, p=0.0004; *VEGFA*, fold=↑1.60, p<0.0001; and *PLAU*, fold=↑1.31, p=0.01); M1 macropahge marker *CD16b* decreased (fold=↓2.97, p=0.002) and M2 marker *CD206* increased (trend only) in females (fold=↑1.27, p=0.05). A separate analysis indicated an increase in *CD206* in males at a later time point (fold=↑1.53, p=0.001). In **TE** vs. **UTE** muscle, monocyte chemotaxis regulators were reduced (*CCL2*, fold=↓3.26, p=0.0001; *PLAU*, fold=↓1.79, p=0.0005), and M1 marker *CD16b* was lower (fold=↓2.14, p=0.03), and M2 markers were higher (*CD163*, fold=↑1.58, p<0.0001; *CD206*, fold=↑1.67, p=0.0003). **CONCLUSION:** Macrophage infiltration and polarization appear to be involved in the muscle response to acute RE. Moreover, training has the potential to minimize the monocyte recruitment and augment the M2 polarization of existing macrophages.

Abstract #33

Homeostasis model assessment of insulin resistance (HOMA-IR) is inversely associated with the adaptive strength response to resistance exercise in adults.

Peterson MD, Gordish-Dressman H, Hubal MJ, Pistilli E, Angelopoulos TJ, Clarkson PM, Moyna NM, Pescatello LS, Seip RL, Visich PS, Zoeller RF, Thompson PD, Devaney JM, Hoffman EP, and Gordon PM.

Laboratory for Physical Activity and Exercise Intervention Research (PAEIR): University of Michigan, Medicine

Background: Emerging data have revealed a negative association between insulin resistance (IR) and muscle function, however there is a lack of research to examine the independent influence of IR on adaptation to resistance exercise (RE).

Objective: The purpose of this investigation was to examine the contribution of homeostasis model assessment of insulin resistance (HOMA-IR) on adaptive response to resistance exercise.

Design: Analyses included 697 adults (281 males, 416 females; age = 23.7 ± 5.6 yrs). HOMA-IR, subcutaneous adipose tissue (SAT) and muscle mass (MRI-derived SAT and biceps muscle volume), and dynamic biceps strength (1 repetition maximum [1RM]) were analyzed at baseline and following 12-weeks of unilateral RE.

Results: Adaptation to RE revealed a significant negative association between HOMA-IR and changes for strength capacity (β = -0.05; p = 0.02), controlling for sex, age, body mass index, and SAT, as well as baseline muscle characteristics (i.e. muscle mass and strength) as covariates. Changes in muscle size after RE were not significantly associated with HOMA-IR.

	Model: Predictor(s)	β	*t*	*p*	F	Adjusted R^2	VIF
Post-Intervention Muscular Strength (1RM)							
Males	Baseline Strength (1RM)	0.68	12.70	< 0.01	58.30	0.65	1.5
	Baseline Muscle Volume	0.19	2.70	< 0.01			2.5
	Baseline Adiposity	-0.16	-1.90	0.06			3.9
	Age	-0.09	-1.90	0.06			1.0
	Baseline BMI	0.16	1.60	0.11			5.0
	HOMA-IR	-0.11	-2.17	0.03			1.4
Females	Baseline Strength (1RM)	0.59	12.55	< 0.01	37.20	0.39	1.3
	Baseline Muscle Volume	-0.04	-0.61	0.54			1.8
	Baseline Adiposity	0.01	0.11	0.91			4.1
	Age	-0.18	-4.06	< 0.01			1.1
	Baseline BMI	0.12	1.30	0.19			4.5
	HOMA-IR	-0.11	-2.51	0.01			1.2

Conclusions: Among non-diabetic adults, localized adiposity was an independent negative predictor of baseline strength. Moreover, despite significant increases in muscle mass and strength after 12-weeks of resistance exercise among all participants, HOMA-IR was negatively associated with strength adaptive-responses among both males and females.

Abstract #34

A Continuous Metabolic Syndrome Score to Identify Predictors among Obese Adolescents: The Relative Contribution of Physical Activity and Cardiorespiratory Fitness.

Peterson MD, IglayReger H, Woolford S, Robert C, Muth T, and Gordon PM.

Background: At present there are no standard criteria to define or characterize metabolic syndrome (MetS) among pediatric populations. As a result there is wide variability in the purported prevalence within this population, which creates a unique challenge for modeling and identifying the association between potential risk factors and MetS outcomes. The use of continuous metabolic risk scores (cMetS) have been proposed as an alternative to binary classification of MetS, and allows for a more sensitive, powerful statistical means to assess predictors of metabolic health/risk.

Purpose: The purpose of this study was to identify the independent associations between physical activity (PA) and cardiorespiratory fitness (CRF) on cMetS, among a large group (n = 256; age = 14.13 ± 1.7 years) of obese adolescents (body mass index [BMI] ≥95th percentile for age and sex).

Methods: A cMetS was derived by standardizing individual metabolic syndrome variables (i.e. BMI, mean arterial pressure [MAP], homeostasis model assessment of insulin resistance [HOMA-IR], HDL-C and triglyceride levels) by regressing them onto age, sex, and race. Standardized residuals (i.e. z-scores) for individual variables were summed to create a cMetS. HOMA-IR was chosen instead of glucose because most children have normal fasting glucose, and is related to IR. Each factor was weighted equally and a higher cMetS was indicative of diminished metabolic health.

Results: A listwise sample of n = 60 males and females had complete data for regression analyses. Univariate regression demonstrated that both PA (β = -0.25, p = 0.01) and CRF (β = -0.25, p = 0.02) were independently associated with cMetS. However, after entering both into multiple regression, only CRF remained a significant predictor (β = -0.36, $p < 0.01$), controlling for PA.

Conclusions: These findings indicate not only the usefulness of cMetS for characterizing metabolic health in an obese adolescent sample, but also the relative importance of PA and CRF on cardiometabolic health risk. Efforts should thus be made to encourage both PA and exercise to improve cardiorespiratory fitness among obese adolescents.