



Michigan Metabolomics and Obesity Center SYMPOSIUM

Focus Topic: Pediatric Obesity

Tuesday, October 11, 2016 • 8 a.m. – 4:30 p.m. • Forum Hall, Palmer Commons

7:00 – 8:00 a.m. - Registration/Breakfast

8:00 – 8:15 a.m. - Introduction

Charles F. Burant, MD, PhD

*Director, Michigan Metabolomics & Obesity Center
Professor, Internal Medicine, University of Michigan*

8:15 – 9:15 a.m.

Brian Wansink, PhD

*John Dyson Professor of Marketing, Director
Cornell Food and Brand Lab, Co-Director, Center
for Behavioral Economics in Child Nutrition
Programs, Cornell University*

**Slim by Design: From Mindless Eating to
Mindlessly Eating Better**

9:15 – 10:00 a.m.

Katherine W. Bauer, PhD

*Assistant Professor, Department of Nutritional
Sciences, University of Michigan*

**"It's All Mom's Fault": Examining
Evidence of the Impact of Parenting on
Childhood Obesity**

10:00 – 10:15 a.m. - Break

10:15 – 11:00 a.m.

Darleen Sandoval, PhD

*Assistant Professor, Department of Surgery,
University of Michigan*

**A Novel Role for Pancreatic GLP-1 in
Regulating Glucose Homeostasis**

11:00 a.m. – 12:00 p.m.

Marie-France Hivert, MD, MMSc

*Assistant Professor, Department of Population
Medicine, Harvard University*

**Understanding Pathways of Fetal
Metabolic Programming to Stop the
Transgenerational Risk of Diabetes**

12:00 – 1:30 p.m. - Lunch/Poster Session

1:30 – 2:30 p.m.

Margo Wootan, DSc

*Director, Nutrition Policy, Center for Science in
the Public Interest*

**Child Nutrition Policies: Supporting
Healthy Eating**

2:30 – 3:30 p.m.

David Ludwig, MD, PhD

*Professor of Pediatrics, Professor of Nutrition,
Harvard University*

**Which Comes First: Overeating or
Obesity?**

3:30 – 4:30 p.m. - Discussion

TARGETED AUDIENCE

- Clinicians and allied health professionals with an interest in nutrition, obesity, and metabolism
- Researchers interested in basic and translational research in nutrition, obesity, and metabolic diseases
- Students in health related fields of study

OBJECTIVES

Symposium participants will enhance their performance, understand and be able to implement in their practice:

- The latest basic and clinical supported sciences in treating obesity and metabolic diseases
- The role of metabolomics in the pathophysiology of disease
- Analyze practice experience and improve practice
- Technology in the preclinical assessment of obesity and nutrition-related diseases

CME CREDITS

This activity has been planned and implemented in accordance with the accreditation requirements and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint providership of the University of Michigan Medical School and the School of Public Health. The University of Michigan Medical School is accredited by the ACCME to provide continuing medical education for physicians. The University of Michigan Medical School designates this live activity for a maximum of *6.75 AMA PRA Category 1 Credits™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

**Nutrition Obesity
Research Center**

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For complete information, registration, and abstract submission, please visit:

<http://mmoc.med.umich.edu/EnrichmentSymposium.php> or contact Grace L. Wu at (734) 647-2271 | glwu@umich.edu

ABSTRACT SUBMISSION DEADLINE: OCTOBER 1, 2016

Disclosure of Relevant Financial Relationships with Commercial Companies

The Accreditation Council for Continuing Medical Education (ACCME) requires CME providers to identify and resolve all potential conflicts of interest of planner and presenters prior to a CME activity.

“Relevant financial relationships” are those in which an individual (including spouse/domestic partner) has both:

- (1) a personal financial relationship (any amount) with a commercial interest (any entity producing, marketing, re-selling, or distributing health care goods or services consumed by, or used on, patients) in the past 12 months, whether the relationship has no ended or is currently active.
- (2) control in planning or presenting educational content addressing specific products of the commercial interest (not simply a whole class of products as a group).

The following planners/speakers **have/had NO relevant personal financial relationships**.

Karen E. Peterson, DSc
Brian Wansink, PhD
Katherine W. Bauer, PhD
Marie-France Hivert, MD, MMSc
Margo Wootan, DSc
David Ludwig, MD, PhD

The following planners/speakers **have/had BOTH (1) a personal financial relationship** with a commercial interest and **(2) will control educational content** about the products of the commercial interest.

Charles F. Burant, MD, PhD
Metabolic Solutions Development Company (current consultant)
Zydus-Cadilla Pharmaceuticals (current consultant)
Metabolic Solutions Development Company (current stock shareholder)

Darleen Sandoval, PhD
Novo Nordisk (current grant/research support)
Sanofi (current grant/research support)
Boehringer Ingelheim (previous grant/research support)

Individual designated to resolve conflict of interest: William Herman, MD, MPH

Brian Wansink, PhD

John Dyson Professor of Marketing

Director, Cornell Food and Brand Lab

***Co-Director, Center for Behavioral Economics in Child Nutrition Programs
Cornell University***

Dr. Wansink is the John Dyson Professor of Marketing, the Director of the Cornell Food and Brand Lab and Co-Director of the Center for Behavioral Economics in Child Nutrition Programs and co-founder of the Smarter Lunchrooms Movement at the Dyson School of Applied Economics & Management at Cornell University, Ithaca NY. He earned his Ph.D. in marketing at Stanford (1990) and was marketing professor at the Amos Tuck School at Dartmouth College (1990–1994), the Vrije Universiteit in Amsterdam (1994–1995), and the Wharton School at the University of Pennsylvania (1995–1997) and the Julian Simon Faculty Scholar and Professor of Marketing, Nutritional Sciences and Agricultural and Consumer Economics at the University of Illinois at Urbana–Champaign (1997–2005). He is also the author of over 150 peer-reviewed papers and of the best-selling book *Mindless Eating: Why We Eat More Than We Think* (2006) and the recently released book *Slim by Design: Mindless Eating Solutions for Everyday Life* (Sept. 2014).

Katherine W. Bauer, PhD

Assistant Professor

Department of Nutritional Sciences

University of Michigan

Dr. Bauer is an Assistant Professor in the Department of Nutritional Sciences at the University of Michigan School of Public Health. Her research focuses on understanding the social and behavioral influences on dietary intake, eating behavior, weight control, and obesity among youth. She is particularly interested in how parenting and other aspects of the family environment can support healthful eating and weight among older children and adolescents, as well as the mechanisms by which significant family stressors, such food insecurity, impact children's eating and weight. Dr. Bauer received a Bachelor of Arts degree with High Honors in Psychology from Oberlin College and a Master of Science degree in Health and Social Behavior from the Harvard School of Public Health. She completed her PhD in Epidemiology with a concentration in behavioral epidemiology at the University of Minnesota, where she was also a postdoctoral fellow with the Minnesota Obesity Prevention Training Program. She is a Fellow of The Obesity Society and received the 2016 Future Leaders Award from the International Life Sciences Institute (ILSI) North America. Dr. Bauer received a MMOC Pilot and Feasibility Award in 2016.

Darleen Sandoval, PhD

*Assistant Professor
Department of Surgery
University of Michigan*

Dr. Sandoval is currently an Assistant Professor at University of Michigan. She received her Ph.D. in Exercise Science at Arizona State University and received a Postdoctoral Fellowship at Vanderbilt University in the Division of Endocrinology. Over the last 10y, her research has focused on understanding the role of a peptide called glucagon-like peptide-1 (GLP-1) on glucose homeostasis, how dysregulation of GLP-1 is involved with the onset of type 2 diabetes mellitus, and how increases in GLP-1 seen with bariatric surgery contribute to diabetes resolution. She works within a collaborative team that has produced multiple mouse models targeted to the GLP-1 system including the ability to generate tissue-specific gain- or loss-of-function of the GLP-1 protein and/or its receptor using Cre/loxp technology. Her work has found that CNS GLP-1 receptors are sufficient, but pancreatic GLP-1 receptors are necessary for normal glucose regulation. Her current work finds that intestinal secretion of GLP-1 is dispensable and instead that pancreatic GLP-1 production is necessary for regulation of insulin secretion. Dr. Sandoval is a member of the American Diabetes Association and the Endocrine Society and is a regular member of NIH study section (IPOD).

Marie-France Hivert, MD, MMSc

*Assistant Professor
Department of Population Medicine
Harvard University*

Dr. Hivert is an endocrinologist and clinical investigator, and an Assistant Professor in the Department of Population Medicine at Harvard Medical School/Harvard Pilgrim Health Care Institute. Her main interests are the prevention of obesity and diabetes and understanding the pathophysiology of metabolic disorders related to excess weight, especially regarding the interactions between genetics and lifestyle.

After completing her clinical training in internal medicine and endocrinology at the Université de Sherbrooke, (QC, Canada), she completed a research fellowship at Massachusetts General Hospital on the epidemiology and population genetics of diabetes and pre-diabetes under the mentorship of James Meigs and David Nathan. She received a Masters degree in Medical Sciences (MMSc) from the NIH-supported program 'Scholars for Clinical Sciences' at Harvard Medical School (HMS).

From 2009 to 2013, she was an Assistant Professor in the Department of Medicine at the Université de Sherbrooke, where she established the prospective cohort named Genetics of Glucose regulation in Gestation and Growth (Gen3G), with the aim of understanding determinants of glucose regulation in pregnancy and the consequences on offspring. She moved to Harvard Medical School in 2013, but remains an adjunct member of the faculty at the Université de Sherbrooke, and continues to direct Gen3G. Dr. Hivert and her Gen3G co-investigators reported the role of adipokines in the risk of diabetes in pregnancy, and conducted genomic investigations elucidating the role of maternal hyperglycemia in modulation of offspring leptin.

Margo Wootan, DSc

***Director, Nutrition Policy
Center for Science in the Public Interest***

Dr. Wootan was recently named one of the Most Innovative Women in Food and Drink by Fortune Magazine and recognized by Harvard School of Public Health for her leadership in public policy. She is the director of nutrition policy at the Center for Science in the Public Interest (CSPI), named as the top Ranked Nonprofit for National Childhood Nutrition/Health. Dr. Wootan received her B.S. in nutrition from Cornell University and her doctorate in nutrition from Harvard University's School of Public Health. Wootan has coordinated and led efforts to require calorie labeling at fast-food and other chain restaurants, require trans fat labeling on packaged foods, improve school foods, reduce junk-food marketing aimed at children, and expand nutrition and physical activity programs at CDC. She co-founded and has led both the National Alliance for Nutrition and Activity (NANA) and the Food Marketing Workgroup. Wootan is a powerful voice shaping the national nutrition debate. She is quoted regularly in the nation's major media and appeared in the movies *Super Size Me* and *Fed Up*.

David Ludwig, MD, PhD

***Professor of Pediatrics
Professor of Nutrition
Harvard University***

Dr. Ludwig is a practicing endocrinologist and researcher at Boston Children's Hospital. He holds the rank of Professor of Pediatrics at Harvard Medical School and Professor of Nutrition at Harvard School of Public Health. Dr Ludwig is Founding Director of the Optimal Weight for Life (OWL) program at Children's Hospital, one of the country's oldest and largest multidisciplinary clinics for the care of overweight children. He also directs the New Balance Foundation Obesity Prevention Center. His research focuses on the effects of diet on hormones, metabolism and body weight. In particular, he developed a novel "low glycemic load" diet (i.e., one that decreases the surge in blood sugar after meals) for the treatment of obesity and prevention of type 2 diabetes and heart disease. Dr. Ludwig is Principal Investigator on numerous grants from the National Institutes of Health, has published over 150 scientific articles, and presently serves as Contributing Writer for *JAMA*. He has written 2 books for the public, including the #1 *New York Times* bestseller *Always Hungry? Conquer Cravings, Retrain Your Fat Cells, and Lose Weight Permanently* (Grand Central Publishing, 2016).

Abstracts

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Structural and Functional Characterization of a Human Exonuclease Implicated in Obesity

Obesity affects a significant number of Americans and greatly increases the risk of developing cardiovascular disease, type-II diabetes, fatty liver disease, and certain cancers. Understanding how obesity is regulated opens up new avenues of pharmacological interventions to treat obesity, concurrently reducing the risk of co-morbidities. We have characterized a broadly expressed enzyme with exonuclease activity in *in vitro* assays and a repressive function against reporter RNAs *in vivo*. These observations are consistent with a function in RNA decay. Mice lacking this exonuclease are resistant to diet-induced obesity and other studies have observed a role for this enzyme in regulating intestinal trafficking of dietary fats and promoting adipogenesis. These observations suggest that there may be a novel pathway to regulate lipid metabolism by targeting mRNAs for degradation. Though the broader physiological effects of gene deletion of this exonuclease have been described, discovering its molecular function remains an important goal. We are utilizing structural and functional approaches to determine the first crystal structure of the human homolog of this exonuclease to aid in elucidating its RNA substrate specificity and catalytic mechanism. Additionally, we have developed biochemical and cell-based assays that will be used to study its function and analyze the effects of active site mutants. These studies describe the structure and activity of the human exoribonuclease and provide the foundation for characterizing this enzyme as a potential drug target for obesity and related co-morbidities.

Supportive family relations and subsequent obesity in Black Youth; a 12-year cohort study

Shervin Assari^{1,2}, Cleopatra H. Caldwell^{2,3}, Marc A. Zimmerman^{3,4}

¹ Department of Psychiatry, ²Center for Research on Ethnicity, Culture and Health, School of Public Health, ³Department of Health Behavior and Health Education, School of Public Health, ⁴ Michigan Youth Violence Prevention Center, School of Public Health, University of Michigan,

Background

Most studies that have investigated the link between family relations and risk of obesity among offsprings have mostly used a cross-sectional design, enrolled White samples, focused on childhood obesity, and covered those aspects of family relations that directly influence energy balance and food intake. More longitudinal research is needed on how general aspects of family functioning such as parental support influence risk of obesity in Black youth who live in unsafe urban areas. In the current longitudinal study we tested whether supportive family relations and fear of violence at baseline predict subsequent increase in body mass index (BMI) among Black youth, and if these associations depend on gender.

Methods

Using a risk and resilience model, the current longitudinal study followed 227 young Black youth (109 male and 118 female) for 12 years from year 2000 (mean age 20) to year 2012 (mean age 32). All participants were enrolled from a disadvantaged urban area in the Midwest of the United States. Baseline demographics (age, gender), socio-economics (family structure, and parental employment), psychological symptoms (anxiety and depression), general parental support (maternal support, and paternal support), and fear of violence in the neighborhood were measured. BMI was measured at baseline and at follow up. We used models in the pooled sample as well as specific to genders to test the predictive role of baseline parental support and fear of violence in the neighborhood (year 2000) on change in BMI (from 2000 to 2012).

Results

In the pooled sample of Black youth, high maternal support at baseline was protective against subsequent increase in BMI from 2000 to 2012. The association remained significant net of covariates for female but not male youth. Fear of neighborhood violence at baseline also predicted increase in BMI from 2000 to 2012, an association which remained significant net of covariates for females but not males.

Conclusion

While neighborhood social disorder increases the risk of obesity, parental support is protective against an increase in BMI among African American youth, particularly females. As parental support is a modifiable factor, as evidence-based interventions that enhance parenting exist, family-based obesity prevention programs should be considered for African American youth. Policies and programs should support African American families who live in disadvantaged neighborhoods to remain supportive of each other. Future research should test the efficacy of such programs and policies for reducing obesity among African American families.

Keyword: African Americans, Gender, Obesity, Family Relations, Parental support

TITLE: Differences in healthy weight-control behaviors in overweight or obese adolescents who accurately perceive or misperceive their weight

AUTHORS: Borton, K., Hahn, S. & Sonnevile, K. R.

Weight misperception, or the belief that one is of a normal or healthy body weight despite being overweight or obese, has been established as protective against further weight gain in adolescents, in comparison to those who accurately perceive their elevated weight status. However, less is known about the individual factors that may contribute to this relationship. Using national YRBS data from the CDC, N=4536 adolescents (Male: n=2467, Female: n=2069) enrolled in grades 9-12 who met the criteria for overweight or obesity, were included in this sample. Participants were classified as either accurate perceives of their overweight or obese weight status (n=1368), or misperceivers (n=3103). Significant associations were found between accurate and misperceivers on intentional weight loss, with accurate perceives being more likely to be trying to lose weight ($\chi^2(1)=632.709, p=.000$). Those who misperceived their weight as normal were significantly more likely to eat breakfast every day ($\chi^2(1)=6.008, p=.014$), consume fruit or 100% fruit juice two or more times per day ($\chi^2(1)=8.390, p=.004$), consume vegetables two or more times per day ($\chi^2(1)=10.836, p=.001$), and drink at least one sugar sweetened soda per day ($\chi^2(1)=8.492, p=.004$). Misperceivers were also significantly less likely to spend at least three hours per day playing computer games. No significant difference was found on television viewership ($\chi^2(1)=.043, p=.836$). Identifying both positive and negative health behaviors in both these groups can help researchers determine possible intervention targets that focus on health behaviors for adolescents who are overweight or obese.

Selective deletion of the brain-specific alpha and delta isoforms of the human obesity gene product SH2B1

†Anabel Flores, §Jessica Cote, †Lawrence Argetsinger, †*§ Martin Myers, and †*§ Christin Carter-Su

†Cell and Molecular Biology Training Program, §Neuroscience Graduate Program, †Dept Molecular & Integrative Physiology, and ‡Dept of Internal Medicine, Univ. of Michigan USA

There has been a rise in obesity and its associated co-morbidities (e.g. diabetes, cancer, heart disease) worldwide in the past decades making it critical to gain new insight into regulators of body weight. Mutations in the scaffold protein SH2B1 have been identified in individuals with severe early onset childhood obesity. These individuals also exhibit hyperphagia and insulin resistance. Some of these mutations are found in specific SH2B1 isoforms. The four known isoforms of SH2B1 (α , β , γ and δ) share 631 amino acids and differ only in their C-terminal tails. The unique C-terminal sequences of the 4 isoforms are a consequence of 2 alternative donor sites in exon 8 and exon 9 skipping, which result in different reading frames and different stop codons in exon 10. SH2B1 γ and β isoforms are ubiquitously expressed; interestingly, the α and δ isoforms are expressed primarily in the brain. Previous experiments in our lab have shown that different isoforms of SH2B1 also have different subcellular localization and abilities to enhance neurite outgrowth *in vitro*. To study the role of the brain-specific α and δ isoforms of SH2B1 *in vivo*, our lab used the CRISPR-cas9 system to edit the DNA of mice to prevent the expression of the SH2B1 α and δ isoforms. We designed two potential guide RNAs (C and D) to delete a region in *Sh2b1* required for expression of the α and δ isoforms. The guides were cloned into a px330 vector, which also contained the cas9 nuclease needed to catalyze the site-specific cleavage of double-stranded DNA. We also designed a 4 kb donor template which was mutated to juxtapose exon 9, the sequence in exon 10 that contains the γ and β stop codons but not the subsequent α and δ codons, and the region of exon 10 after the α stop codon. Following homology-directed repair, only the γ and β isoforms of *Sh2b1* should be expressed. The 4kb template was cloned into a Zero blunt TOPO vector. We performed a screen in mouse oocytes to test for the editing efficiency of our constructs. Two screens were done in which either guide C RNA or both guide C and D RNAs plus the donor template plasmid were injected into mouse oocytes, which were grown to the blastocyst stage. To analyze editing of the blastocyst DNA, we amplified the targeted *Sh2b1* region, looked for an appropriately smaller-sized fragment by PCR, and confirmed the presence of the appropriate edit by DNA sequencing. For our screen using a single guide C RNA, 6 out of 11 blastocysts appeared to have the correct edit. For the screen with guides C and D RNAs, 16 out of 20 blastocysts appeared to have the correct edit. To generate mice, each set of constructs was injected into 300 C57BL/6 X SJL F2 mouse oocytes. Genomic DNA was isolated from the tails of the pups. Diagnostic fragments were amplified by PCR and screened for the correct edit. For the mice generated from the single guide C RNA, 3 out of 17 were correctly edited. For the mice generated with guides C and D RNAs, 4 out of 58 were correctly edited. We have confirmed germ-line transmission of the correct edit. We have also confirmed that the mRNA for the γ and β isoforms is transcribed whereas the mRNA for the α and δ isoforms is absent, as expected. We are now metabolically phenotyping the correctly edited mice to determine the contribution of the brain-localized α and δ isoforms of SH2B1 to body weight control, feeding behavior, and energy expenditure.

Health Behaviors Predicts Cardiovascular Risk Profile in Middle-School Children

Rosa S.F. de Visser, MS, Rachel Sylvester, BS, Qingmei Jiang, MS, MA, Eva Kline-Rogers, MS, RN, NP, Jean DuRussel-Weston, RN, MPH, Kim A. Eagle, MD, Elizabeth A. Jackson, MD, MPH

Background: Lifestyle behaviors related to diet, physical activity (PA) and sedentary behavior (SB) are associated with increased cardiovascular (CV) risk in adults; however, less is known about adolescents. We examined PA, SB and diet in association with CV risk factors among middle school students.

Methods: Data from 2,667 students were collected in Project Healthy Schools, an educational health program, from 2004-2015. In addition to self-reported diet, PA and SB, physiological measures included height, weight, and lipids. Students were categorized to the 'Unhealthy Behavior' group (N=855) if 4 or more of the following behaviors were present: <1 day/week vigorous (20 min) PA, <1 day/week moderate (30 min) PA, <1 day/week physical education classes, <1 team sport participation per year, >2 hours/day TV time, >2 hours/day computer time, >2 hours/day video games, <1 serving/day fruit or vegetables, no daily breakfast consumption, >1 serving/day sugary foods and >1 serving/day sugary beverages. The 'Healthier Behavior' group (N=1812) was the reference group and included students with 0 or 1 unhealthy behavior. Outcome measures included overweight and obesity (>85th and 95th percentile respectively), high density lipoprotein (HDL) cholesterol ≤ 40 mg/dl and low density lipoprotein (LDL) cholesterol ≥ 140 mg/dl. Descriptive statistics and risk estimates were performed.

Results: Compared to students in the Healthier Behavior group, those in the Unhealthy Behavior group were positively associated with overweight and obesity (Odds Ratio [OR] 1.41; 95% confidence interval [CI] 1.19-1.67) and negatively associated with increased HDL (OR 0.79; 95%CI 0.6-0.99). A trend towards increased LDL (OR 1.51; 95%CI 0.83-2.77) was also observed.

Conclusions: In PHS, ≥ 4 unhealthy behaviors were detected in 32.1% of the students and were associated with overweight /obesity, decreased HDL-C and a trend towards increased LDL-C. Our findings suggest that unhealthy behaviors can identify middle school students at risk for increased CV risk and may serve as targets for future educational programs.

School Lunch is Associated with Increased Cardiovascular Risk among Middle-School Children

Rosa S.F. de Visser, MS, Rachel Sylvester, BS, Qingmei Jiang, MS, MA, Eva Kline-Rogers, MS, RN, NP, Jean DuRussel-Weston, RN, MPH, Kim A. Eagle, MD, Elizabeth A. Jackson, MD, MPH

Introduction: Millions of children consume school lunches daily. Children from low-income families are eligible for free or reduced-price school meals. While studies show improvement in the nutritional quality of school lunches, the effect of school lunch or lunch brought from home on cardiovascular risk factors among children is unknown.

Hypothesis: We hypothesized that frequently consuming school lunch is associated with increased cardiovascular risk factors when compared with lunch brought from home.

Methods: All 15,742 sixth graders enrolled in Project Healthy Schools, a school-based wellness intervention, were included in this cross-sectional study (2004-2015). We examined 10,169 behavioral surveys and 1,845 physiological screenings. We compared self-reported diet, physical activity (PA), sedentary behaviors and physiologic parameters (height, weight, blood pressure (BP) and heart rate) in 2 groups, children who reported eating school lunch daily and those who eat home-prepared lunch daily. The groups were further stratified by socioeconomic status (SES); low SES (<\$35,000) or high SES (>\$50,000) based on the median household income of the school region. Students in the middle SES range (\$35,000-\$50,000) were excluded from analysis (n=4230).

Results: School lunch students were associated with less healthy behaviors (PA, diet [fruit/vegetable servings, meat and sugary beverage intake], and sedentary activities) and physiologic measures (percent of overweight/obesity, systolic BP and recovery heart rate) compared with students bringing lunch from home in low and high SES groups (Table 1).

Conclusions: In this large cohort of children, we observed frequent school lunch consumption, even after adjustment for SES, was associated with less healthy behaviors and physiologic parameters. Further research is warranted to determine whether healthier school lunches would improve cardiovascular health characteristics and health behaviors in middle-school students.

Table 1. School lunch versus lunch from home in low and high SES groups

Health Behaviours <i>Mean(SD)</i>	Low SES			High SES		
	Home lunch	School lunch	<i>p-value</i>	Home lunch	School lunch	<i>p-value</i>
Vigorous PA (sessions/week)	4.1(4.2)	3.9(2.4)	0.04	4.6(1.9)	4.2(2.2)	<0.001
Team sport participation (teams/last year)	3.0(2.3)	0.9(1.0)	<0.001	1.4(1.1)	1.0(1.9)	<0.001
Fruit/vegetables intake (servings/day)	2.5(1.8)	2.3(1.7)	0.04	3.2(1.7)	2.6(1.0)	<0.001
(Fried) meat intake (servings/day)	1.1(1.2)	1.3(1.2)	<0.001	0.7(0.9)	1.1(1.1)	<0.001
Sugary beverages (servings/day)	0.7(0.9)	0.8(0.9)	<0.001	0.3(0.6)	0.6(0.9)	<0.001
TV and video game time (hours/day)	2.6(1.9)	2.7(1.9)	<0.001	0.3(0.6)	2.2(1.7)	<0.001
Physiologic measures <i>Mean(SD)</i>						
Overweight* and obesity** (%)	8.90%	34.20%	NS	12.90%	15.30%	<0.001
Systolic BP (mmHg)	107.9 (10.3)	108.9 (9.9)	NS	107.2 (10.4)	109.0 (10.7)	0.02
Recovery Heart Rate (beats/min)	110.5 (20.5)	113.6 (20.2)	NS	102.0 (16.3)	104.7 (18.2)	0.04

*Overweight defined as at or beyond the 85th percentile of weight for age and gender; **Obesity defined as at or beyond the 95th percentile of weight for age and gender.

Effect of Saroglitazar on Fatty Acid Metabolism in Zucker Fa/Fa Rats

Saroglitazar is a novel dual PPAR α /g agonist recently approved in India for the treatment of diabetic hypertriglyceridemia. To understand the mechanisms of action of saroglitazar in vivo, we treated Zucker fa/fa (n=10-12 in each group) with vehicle, fenofibrate (F) (150 mg/kg) or saroglitazar (Saro) (0.4 or 4 mg/kg/day) for 14 days. On day 15, rats were gavaged with 5ml/kg of corn oil which contained [U-¹³C]Palmitic Acid (PA) (1 gm/5ml). Plasma was obtained hourly for 8 hours. Adipose and skeletal muscle was collected at 8 hours. Only 4 mg/kg/day Saro increased body weight (p<0.01) and reduced fasting insulin (p<0.01 vs. vehicle) as well as reducing plasma triglyceride (TG) at 0 and 2 hour post corn oil treatment (p<0.01 and p<0.001, respectively). LC-MS and GC-MS were used to assess the incorporation of ¹³C-lipids into plasma and tissue lipids (n=5 for tissue metabolomics studies). The major M+16 isotopomers of the major TG species, TG(52:3) and TG(52:4), rose in the first two h following gavage (likely reflecting chylomicron production) and declined over the next 4 hours with a secondary rise at 6-8 h. In contrast, F-treatment caused a greater increase in M+16 TG species; both low and high dose Saro significantly attenuated the appearance of M+16 TG. In all animals, Major M+16 phosphatidyl choline PC(34:1), carried primarily in HDL and VLDL, rose at similar rates, however the % labeling in the F-treated animals was significantly lower, suggesting a reduction in liver derived lipids by F. Low and high dose Saro significantly increased the accumulation of M+16 palmitate in adipose tissue (by 86% and 247%, respectively, p<0.01). F, low and high dose Saro decreased Gastrocnemius M+16 palmitate labeling, which could be due to induction of lipid oxidation by F and potentially Saro and reduced plasma levels of TG in Saro. In conclusion, Saro significantly reduces fasting and postprandial TG levels through enhanced clearance of TG into adipose tissue and works by a mechanism distinct from that of F, a 'pure' PPAR α activator.

Authors

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Dynamics of the plasma and skeletal muscle metabolome during exercise

Heidi B. IglayReger, Charles R. Evans, Christine A. Parker, Jeffery F. Horowitz and Charles F. Burant

Abstract

Reduced exercise capacity is associated with elevated risk of mortality and adverse health conditions including metabolic syndrome and diabetes. However, the genetic and metabolic determinants of exercise capacity, as well as how these relate to disease risk, remain incompletely understood. One aspect of metabolism which associates closely with exercise capacity is fuel selection. Fatty acids and carbohydrates are both oxidized by skeletal muscle to produce energy to sustain exercise, but individuals with higher exercise capacity oxidize fatty acids to a greater extent than those with lower exercise capacity at the same work intensity. In this study, we used metabolomics in parallel with indirect calorimetry to probe the dynamics of metabolism and fuel selection during exercise in unprecedented detail. An initial group of 50 healthy males, age 18-30 y, were recruited and screened for VO_2 max. Those with the highest (HI) and lowest (LO) VO_2 max (top and bottom 20%) returned to complete an increasing effort cycle ergometry protocol with continuous indirect calorimetry, blood sampling every 3 minutes, and vastus lateralis biopsies before and after exercise. Plasma and muscle samples were analyzed by LC-MS and GC-MS for targeted metabolite quantitation and untargeted metabolite profiling. Plasma free fatty acid levels were lower at baseline and during exercise in HI, whereas respiratory quotient data indicated fatty acid oxidation was greater in this group, suggesting the match between fatty acid supply and mitochondrial fat oxidation capacity is influenced by VO_2 max. Metabolomic profiling of plasma and muscle biopsies revealed distinct dynamics of TCA cycle, amino acid, free fatty acid, acylcarnitine, and other metabolite classes in response to exercise. These findings may contribute to improved understanding of metabolic factors associated with fuel selection and exercise capacity. Future research should more thoroughly investigate the mechanisms underlying these associations.

Metabolomics of mammalian tissues: optimizing mode of anesthesia, tissue collection and extraction strategies

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Rodents are widely used as a model organism to study complex diseases because they share most characteristics with humans, yet they enable genetic and metabolic manipulation that allow disease mechanisms to be probed directly. Rodent tissues are a logical target for study using metabolomics, since alterations in metabolism within specific organs is a key feature of many diseases. However, metabolomics of rodent tissue poses unique challenges pertaining to sample collection and analysis which must be carefully managed to capture an accurate portrait of compounds which may be labile or rapidly turned over in vivo.

Methods: To compare commonly-used methods for tissue collection, C57/BL6 mice were anesthetized (using isoflurane, ketamine or pentobarbital) or euthanized (by cervical dislocation, carbon dioxide, or isoflurane overdose) and skeletal muscle, liver, heart, adipose and serum were collected. Hydrophilic interaction chromatography-time of flight mass spectrometry analysis was used to analyze the polar metabolome of the tissue samples, and data were studied using both targeted and untargeted metabolite profiling.

Results: Different modes of anesthesia induced striking alterations in the metabolome of most mammalian tissues. In skeletal muscle, all modes of euthanasia induced rapid elevation of metabolites associated with glycolysis and glycogenolysis. In liver, accumulation of nucleosides and other nucleotide breakdown products following euthanasia was observed. Based on our findings we recommend that rodent tissue samples for metabolomics be collected under anesthesia as opposed to post-euthanasia when possible. Our experiments demonstrate the importance of proper tissue collection strategies for metabolomics experiments, and highlight the susceptibility of the metabolome to rapid alterations.

A leptin-responsive brainstem circuit that modulates sympathetic responses to noxious stimuli

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Leptin acts via leptin receptor (LepRb)-expressing neurons in the brain to connect physiology and behavior control to the repletion of fat stores. We recently demonstrated that leptin inhibits hypoglycemia-activated parabrachial nucleus (PBN) LepRb neurons; these project to the ventromedial hypothalamus (VMH) and promote sympathetic (SNS) outflow and other counterregulatory (CRR) responses to glucoprivation. Thus, decreased leptin activates this circuit during caloric restriction, enable an appropriate CRR to hypoglycemia in the face of low baseline SNS tone and depleted nutritional reserves. We hypothesized that such a system could also augment the response to other emergencies. Indeed, fasting enhances the hyperglycemic response to noxious stimuli. We found that, like glucoprivation, noxious stimuli activate PBN^{LepRb} neurons; noxious stimuli (but not glucoprivation) also activate adjacent periaqueductal grey (PAG; a site important for processing painful and hypercapnic stimuli) LepRb neurons. These findings suggested (and neuroanatomical tracing studies confirmed) that PAG^{LepRb} neurons lie afferent to the PBN LepRb-VMH pathway. Furthermore, pharmacogenetic activation of PAG^{LepRb} neurons activated PBN neurons and increased locomotor activity, blood glucose, and SNS outflow to the adrenal in a manner similar to the activation of PBN^{LepRb} neurons, as well as enhancing the ventilatory response to hypercapnia. Similarly, ablating LepRb in CCK-expressing PAG and PBN neurons, or in PAG neurons alone, augmented the CRR to noxious stimuli and enhanced the ventilatory response to hypercapnia. Taken together, these data suggest that PAG^{LepRb} neurons stimulate the PBN-VMH circuit and SNS outflow in response to noxious stimuli such as pain and hypercapnia. Decreased leptin action on PAG LepRb neurons during low energy storage enhances the SNS response to such stimuli, ensuring appropriate responses to acute emergencies in the face of depleted nutritional reserves.

Mouse model of the human obesity-associated SH2B1 P322S mutation

Flores, A., Argetsinger, LS., Myers, MG., and Carter-Su, C.

Obesity and associated co-morbidities such as diabetes, cancer and heart disease are on the rise. Insight about molecular mechanisms underlying obesity can be gained by studying human mutations associated with obesity. Twelve mutations have been identified in SH2B1 in individuals with severe childhood obesity. These individuals are hyperphagic and disproportionately insulin resistant. Many exhibit behavioral abnormalities including aggression and learning delay. Experiments using *Sh2b1*-KO mice and cultured neurons suggest SH2B1 may play an essential role in neuronal development and connectivity. I hypothesize that the human mutations in SH2B1 disrupt establishment of neuronal projections involved in regulating body weight. I am studying the P322S mutation in SH2B1 because it has a robust phenotype in *in vitro* assays and it is located in the PH domain of SH2B1, a domain whose function in SH2B1 is unclear. We used CRISPR-Cas9 genome editing to make a mouse model encoding the P322S mutation. We designed two guides to target *Sh2b1* and a 180-nucleotide donor to introduce the P322S mutation. Each guide was cloned into the px330-Cas9 vector and, along with the 180-nucleotide donor, injected into 75 oocytes. Eleven pups were generated. DNA sequencing verified that seven pups contained indels and two carried the P322S mutation. We are metabolically phenotyping the mice and analyzing neurite outgrowth in leptin receptor expressing neurons. Preliminary results show that the *Sh2b1*^{P322S/+} mice have increased body weight compared to their wild-type littermates at 28 weeks of age when fed standard chow (9% fat). In support of the P322S mutation having an impact on the physiology of the mice, pups born to a *Sh2b1*^{P322S/P322S} x *Sh2b1*^{P322S/P322S} breeding pair were small compared to pups born to *Sh2b1*^{P322S/+} x *Sh2b1*^{P322S/+} breeding pairs and half of the litter died within 5 days of age. For *Sh2b1*^{P322S/+} x *Sh2b1*^{P322S/+} breeding pairs, the homozygote pups are similar in size to their littermates but the number of homozygotes born is half of the expected Mendelian ratio. *Sh2b1*(P322S) mice provide a powerful model that should help us identify key functions of SH2B1 that control body weight, neuronal function, and development at the animal, cellular and molecular level.

Adolescent Weight Management Strategies: What Are Teens Doing Today?

Samantha L. Hahn, Katherine W. Bauer, Ph.D.

Nearly half of adolescents report dieting to lose weight. However, we have a very limited understanding of what dieting means to adolescents today and therefore cannot well-distinguish weight control methods that are effective and appropriate from those that are harmful. Further, while social media and other forms of technology are increasingly being used to promote weight control, it is unknown whether and how adolescents are using these information sources. The objective of the current study is therefore to develop a comprehensive taxonomy of adolescent weight control methods, including technology-based approaches, resulting in a psychometrically-sound instrument with which to assess adolescent weight control in clinical and community-based samples. To achieve this objective, we developed a mixed-method, progressive-phase study design that incorporates (1) a comprehensive literature and social media review, (2) in-depth interviews with adolescent-focused health professionals, (3) an online qualitative survey of college freshman, (4) talk-aloud interviews with adolescents, and (5) an online quantitative survey of adolescents. To date, we have completed the literature and social media review and in-depth interviews of health professionals. A review of social media identified novel weight control strategies not previously assessed among adolescents including the use of detox teas, juice cleanses, and waist trainers. Interviews with adolescent health professionals have revealed that teens look to social media frequently for weight loss advice, and that technology-based self-monitoring is becoming increasingly common among adolescents attempting to lose weight. Many clinicians have also indicated that in recent years there has been an increased desire for adolescents to change their body shape and build muscle in addition to losing weight. Additionally, clinicians are confirming that “dieting” means something different to nearly every teen that they work with, and that what teens are doing to “diet” varies by socioeconomic status. For example, those of low socioeconomic status frequently skip meals whereas those of higher socioeconomic status follow specific diet regimens. Completion of this study will provide a novel assessment tool to identify whether weight control behaviors used by contemporary adolescents have positive or negative effects on adolescents’ mental and physical health, including weight.

Metabolomic Responses to Phthalate Treatment in a Placental Cell Model (BeWo): Preliminary Analysis and Future Directions

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The contribution of environmental toxicant exposure to adverse pregnancy outcomes such as preterm birth and preeclampsia is a significant public health concern. Phthalate esters (PEs) are environmental toxicants used as plasticizers in a wide array of consumer products and human exposure is ubiquitous. Although epidemiology studies have found associations between exposure to PEs and preterm birth and preeclampsia, the exact role that PEs play in the etiology of these adverse birth outcomes is unclear. In order to elucidate potential mechanisms of PE toxicity in female reproductive tissues, we utilized a metabolomics based approach to identify responses in a placental cell model (BeWo) after treatment with a PE metabolite commonly found in human blood and urine samples (MEHP). BeWo cells were cultured for 24 hours in the presence of vehicle control (DMSO) or MEHP (90 or 180 μ M). Cell media and cells were then collected and analyzed using untargeted metabolomics analysis via liquid chromatography/mass spectroscopy. Preliminary analysis revealed that metabolites associated with processes such as glutathione metabolism (ascorbic acid), amino acid metabolism (isoleucine, 2-methylmaleic acid, acetoacetic acid) and purine metabolism (2'-deoxyinosine) were significantly decreased by MEHP treatment (ANOVA, $p < 0.05$). These initial findings offer new insight into potential mechanisms of phthalate toxicity in the placenta and provide a promising direction for future research. This research is supported by the National Institute of Environmental Health Sciences of the National Institutes of Health: Superfund Research Program PROTECT Center (P42ES017198) and Environmental Toxicology and Epidemiology Training Grant (T32ES007062).

Elevated glucocorticoids in the presence of obesity leads to worsened metabolic syndrome.

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Background: Elevated glucocorticoids arise from stress, Cushing's syndrome or prescribed medications and are known to cause metabolic syndrome in otherwise healthy individuals. However, effects of chronically elevated glucocorticoids in obese individuals have not been studied. Since obesity and elevated glucocorticoids are so prevalent in the US and have similar associated co-morbidities, it is important to investigate their relationship.

Hypothesis: Chronically elevated glucocorticoids in the presence of obesity lead to worsened insulin resistance and non-alcoholic fatty liver disease.

Methods: Male C57BL/6J mice were fed a high-fat diet for 12 weeks prior to 5 weeks of dexamethasone treatment (a synthetic glucocorticoid), or no treatment. These mice were compared to controls fed a normal chow diet. Body composition, strength and food consumption were assessed weekly. Measures of *in vivo* lipolysis, insulin resistance and NAFLD were performed towards the end of the study or following sacrifice.

Results: Increases in muscle atrophy and insulin resistance were observed in both of the dexamethasone-treated groups; however, these effects were exacerbated in the HFD-fed mice. Decreased fat mass was observed in the HFD-fed, dexamethasone treated mice consistent with elevated lipolysis in that group. When we measured mRNA of lipolytic genes we found up-regulations of *Pnpla2*, which encodes ATGL, the protein responsible for the rate-limiting step in lipolysis, in subcutaneous adipose tissue of both dexamethasone-treated groups, with even further increases seen in the HFD-fed, dexamethasone treated mice.

Conclusion: Chronically elevated glucocorticoids in the presence of obesity lead to worsened metabolic syndrome. This is potentially due to increased ATGL-mediated adipose tissue lipolysis.

Contribution of OEA pathways to the metabolic benefits of vertical sleeve gastrectomy

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The bariatric surgical procedure, vertical sleeve gastrectomy (VSG), causes sustained reductions in body weight and improved lipid profiles in both preclinical and clinical studies. Despite the well-known effectiveness of bariatric surgery, the mechanisms underlying the dramatic impact on lipid homeostasis are not fully understood. The lipid mediator, oleoylethanolamide (OEA) is an anorectic signal physiologically regulated by diet with a profound ability to benefit lipid metabolism. OEA is produced in the small intestine from dietary fats internalized from the lumen by the fatty acid translocase, CD36. Newly formed OEA often targets the intestinal downstream PPAR α pathway. VSG surgery in male rats leads to a regional increase in duodenal but not jejunal OEA levels; however, the functional significance of this is unknown. Therefore, we performed VSG or sham surgery in high fat fed male WT, CD36 KO, and PPAR α KO mice. We predicted that decreased OEA production in the CD36 KO mice or decreased signaling through PPAR α KO mice would attenuate the metabolic benefits of VSG. Unlike in WT mice, VSG did not decrease fat mass in CD36 KO mice, which is likely due to their partial inability to taste or internalize fat. VSG did not alter fasting or postprandial blood glucose levels in CD36 KO mice. PPAR α KO mice have elevated lipid responses after a meal compared to WT mice, and VSG was able to correct this lipid abnormality. However, VSG improved hepatic lipid homeostasis equally in both WT and PPAR α KO mice, likely through pathways decreasing hepatic lipogenesis. Collectively, these data suggest that the involvement of CD36 and PPAR α are not necessary for the metabolic benefits of VSG. Future research will explore the contribution of alternative fat sensing systems after surgical intervention.

The age-dependent effects of preproglucagon on high-fat diet intake

Ki-Suk Kim, Randy J. Seeley, & Darleen A. Sandoval

Objective: Preproglucagon gene (Gcg) encodes various gut peptides, such as glucagon, glucagon-like peptides (GLPs), and oxyntomodulin, which have crucial effects on the food intake and glucose homeostasis. We fed 60% high-fat diet (HFD) to wildtype (WT) and Gcg null mice, which separated into two different groups by their ages, to investigate 1) the role of preproglucagon in HFD intake, and 2) the age-dependent effects of preproglucagon in the HFD intake.

Methods: An older WT mouse group (8 ½ to 9 ½ months old); a younger WT mouse group (5 ½ to 8 months old); an older Gcg null mouse group (8 ½ to 9 months old); and a younger Gcg null mouse group (6 to 7 ½ months), have ingested 60% HFD for 4 weeks. The body weight (BW), and food intake (FI) have measured during the time of HFD intake, and oral glucose tolerance test (OGTT) was performed at the end of the experimental period.

Results: Younger Gcg null mice showed highly increased body weight gain and body weight change during 4 weeks of 60% HFD intake, while older Gcg null mice showed moderate results. Both younger and older WT mice, which correspond the ages to that of Gcg null mice, showed similar body weight changes during period the HFD intake, respectively. The younger Gcg null mice showed increased HFD intake during 4 weeks compare to the younger WT mice. However, the older Gcg null mice showed same HFD intake with the older WT mice. In the OGTT performed after the 4 weeks of HFD intake period, Gcg null mice showed lowered blood glucose respond to the glucose gavage compare to the WT mice. In this result, older Gcg null mice showed marked decreases in the blood glucose response than the younger Gcg null mice.

Conclusion: Absence of preproglucagon increases the HFD intake, and the effect is age dependent.

MMOC Symposium Abstract

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Associations between Longitudinal DNA Methylation Patterns and Obesity Status in the Healthy Families Childhood Obesity Cohort

Previous work has shown that DNA methylation status is a labile epigenetic mark that is altered in response to behavioral and environmental factors. Given that DNA methylation status is established early in development, environment-mediated changes in the developing methylome represent a possible mechanism driving the Developmental Origins of Health and Disease (DOHaD) hypothesis. Recent reports suggest that both cross-sectional DNA methylation and rates of DNA methylation drift with age are associated with obesity status. We tested whether neonatal bloodspot DNA methylation, childhood DNA methylation (12-24, mos. old, 3-6 years of age), and/or adolescent DNA methylation (10-13 years of age) at several candidate gene regions predicted later-life obesity status, as defined by weight-for-length z-score (WLZ) or body mass index z-score (BMIZ). Methylation was quantified at a repetitive element (LINE-1), two imprinted genes (*IGF2*, *H19*), and two non-imprinted genes (*LEP*, *PPARA*) in children from the Healthy Families childhood obesity cohort. Across age, methylation levels in blood significantly decreased ($p < 0.05$) at LINE-1, *PPARA*, *IGF2*, and *H19*, and significantly increased ($p < 0.05$) at *LEP*. However, there was no significant interaction between WFL/BMI z-score and DNA methylation levels across age, suggesting no significant obesity-related epigenetic drift deflection at the investigated genetic loci. For the 3-6 year old age group, neonatal bloodspot *IGF2* methylation demonstrated a moderately significant negative association with later-life obesity likelihood ($\beta = -2.37$, $p = 0.07$); this relationship, though less significant, was consistent when all age groups were combined ($\beta = -0.08$, $p = 0.09$). Unlike the neonatal bloodspots, childhood and adolescent *IGF2* methylation levels were not associated with body weight parameters (BMI z-score) or obesity likelihood. Our results suggest that aging, even early in life, has a significant effect on human candidate gene methylation, and that neonatal bloodspot methylation at the *IGF2* imprinted locus may be a useful biomarker for predicting later-life obesity risk.

Bariatric Surgery: The Key to the Future of Weight Loss

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Over the summer I interned at the University of Michigan Health System; Adult Bariatric Surgery Program. This was a clinical registered dietitian experience that is in coordination with the surgeons and physician assistants who help prepare and select patients to undergo the latest methods in bariatric surgery, which is used to alter the stomach anatomy. This in turn leads to rapid weight loss for 1 year due to calorie restriction, a slow normalizing increase from year 1 to year 3 of only a fraction of weight lost, followed by a sustained weight loss for life. This is due to gut hormone changes involved with defended weight level.

Since my research at the University of Michigan examines bariatric surgery on rats and the corresponding changes in physiology, my goals were to see the human aspect of bariatric surgery while garnering competency in clinical experience. I wanted to learn patient interviewing techniques, apply my nutrition knowledge gained through education and research, discover limitations that exist in the field, and learn how a system of different disciplines (doctors, surgeons, psychiatrists, registered dietitians, and insurance companies) coordinate with each other successfully, creating an environment to improve health safely and effectively.

The role of the bariatric dietitians is to educate patients on the diet needed pre/post-op as well as deducing ways to modify diet. My tasks included conducting diet interviews, teaching a pre-op nutrition education class, writing monthly newsletters, attending support group meetings, and developing a presentation on dumping syndrome. I managed to develop skills in public presentation, patient interaction, and learning to critically think through a problem while asking patients questions. I learned to communicate effectively with all levels of education, show empathy, understand when someone isn't willing to change, and lead my patients to quality health. These learned competencies will allow me to be a more effective physician in the future.

IMPACT OF WEIGHT LOSS AND REGAIN ON OBESE ADIPOSE TISSUE PHYSIOLOGY

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Background: Obesity causes dramatic changes to the adipose tissue immune environment which include the recruitment and activation of M1-like macrophages. The inflammatory polarization of these cells is thought to play a critical role in the severity of insulin resistance associated with obesity. Weight loss can improve insulin resistance, but little is known regarding the effects of weight loss on immune activity in adipose tissue.

Objectives: To evaluate the effects of obesity, weight loss and weight regain on adipose tissue health and physiology.

Design and methods: Diet switch from a 60% high-fat diet (HFD) to a 13% normal-fat diet for 8 weeks was used to induce weight loss in diet-induced obese C57BL/6 mice. Weight regain was achieved by re-challenging formerly obese mice with HFD for 6 weeks. Leukocyte populations were analyzed by flow cytometry and qPCR. Adipose tissue dysfunction and insulin signaling was assessed using qPCR and immunoblots.

Results: Weight loss improved glucose tolerance and whole-body insulin sensitivity but failed to resolve defects in adipocyte health and function. Markers for insulin resistance remained abnormal as did markers for adipose tissue inflammation. Adipose tissue macrophages developed a pro-inflammatory polarization state after diet-induced obesity and their activation persisted despite weight loss. After re-challenge, the adipose tissue of previously obese mice exhibited maintenance of several markers associated with adipose dysfunction and insulin resistance than that of age-matched mice challenged for the same amount of time but without the initial diet-induced obesity and weight loss.

Conclusions: While weight loss can ameliorate obesity-associated adipose tissue defects and inflammation the resolution is incomplete. We propose that the activated inflammatory profile of adipose tissue macrophages, maintained despite weight loss, may be responsible for the significant worsening of adipose tissue physiology following HFD re-challenge. Understanding the effects of obesity that persist following weight loss is clinically relevant as it can provide avenues for the development of therapeutics and lifestyle management strategies to prevent and resolve both childhood and adult obesity.

Perinatal Exposure to Bisphenol A and High Fat Diets Alter Longitudinal Metabolic Health via Differential Gene Expression

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Prenatal exposure to endocrine disrupting chemicals, like bisphenol A (BPA), may increase risk of metabolic disease across the life course via altered regulation of gene expression. Using an isogenic mouse model, we examined the hypothesis that lifelong metabolic effects of perinatal BPA exposure are exacerbated by a Western high fat diet (HFD) and ameliorated by a Mediterranean HFD. Dams (n=98) were randomized to 1 of 6 diets: control (C), Western (W), Mediterranean (M), or each diet with 50ug BPA/kg chow added, CBPA, WBPA, and MBPA, respectively. Metabolic parameters were assessed at postnatal day 10 (PND10, n=118) and 10 mos. (10M, n=133), with extensive metabolic phenotyping at 2, 4, and 8M. Fatty Liver and Insulin Resistance RT² Profiler PCR Arrays were run on hepatic RNA from 10M males and females (n=60). ANOVA with Tukey's post-hoc analysis compared pups' metabolic outcomes between exposure groups; mixed effects models were used to assess repeated measures over time. At PND10, CBPA weighed less than C pups (p=0.001); the impact of BPA was negated by both HFDs. Surprisingly, pups exposed to M had higher weekly weights from 5-10M of age (p < 0.001), higher PND10 serum leptin levels (p = 0.003) and greater hepatic triglycerides (p = 0.002), compared to C. No differences were observed with BPA or W diet. Fasting serum insulin at 8M was greater among M females (p=0.026) and males (p=0.047) than controls.

Hepatic gene expression differences between perinatal exposure groups were more pronounced in 10M female than male offspring and more affected by HFDs than by BPA. In 10M females, W and M diets impacted (p < 0.05) gene expression in the KEGG pathways of fatty acid metabolism, regulation of lipolysis, insulin signaling and resistance and cytokine-cytokine receptor interactions. Interestingly, W females had a greater effect on PPARs, increasing *Pparg* (p = 0.03) while decreasing *Ppargc1a* (p = 0.03) and *Ppara* (p = 0.06) expression, than M females, whose PPAR expression levels did not differ from C. 10M CBPA females had altered expression in similar pathways as HFD, but fewer gene loci were significant. The same patterns of hepatic gene expression were observed in 10M males, but fewer gene loci differences were significant. Thus, perinatal HFD appears to have a greater impact on life course metabolic health than perinatal BPA exposure, and concurrent BPA exposure does not appear to exacerbate effects of either HFD. Sexually dimorphic effects support the need to conduct longitudinal studies in both male and female offspring. Lastly, lipid composition of the HFD matters, resulting in different longitudinal metabolic health outcomes. Shotgun lipidomics on 10M males and females across all six perinatal exposures is currently underway to examine if alterations in fatty acid metabolism and lipolysis gene expression impact circulating lipid levels.

Nothing to Disclose: EHM, CH, DCD

Sources of Research Support: EPA RD83543601 / NIH P01 ES02284401, F31 E025101, T32 HD079342, T32 ES 007062, Michigan Diabetes Research Center (MDRC) Pilot & Feasibility grant.

Gestational Testosterone Excess Selectively Increases Local Estrogenic Action in the Sheep Visceral but not Subcutaneous Adipose Tissue

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Abstract

Female sheep prenatally treated with testosterone (T) develop metabolic complications such as insulin resistance and dyslipidemia during adulthood similar to those seen in women with Polycystic Ovarian Syndrome (PCOS). These changes are associated with decreased adipocyte size, increased plasma triglyceride and accumulation of fat in other tissues. Steroids exert direct effects on adipocytes with both estrogen (E) and T influencing body fat deposition along with adipocyte number, adipogenesis, and/or differentiation. In addition, E also reduces lipogenesis and promotes lipolysis thereby decreasing adipocyte storage capacity. Because gestational T treatment leads to hyperandrogenism and increased circulating levels of E, and adipocytes are sites of steroid action as well as production, there is potential for alterations in local steroid milieu influencing adipocyte differentiation. To test this, the changes in mRNA expression of members of steroidogenic biosynthetic pathway (steroidogenic acute regulatory protein (STAR), P450 side chain cleavage [CYP11], 17 hydroxylase [CYP17], 3 beta hydroxysteroid dehydrogenase [HSD3B], and aromatase [CYP19]) and the receptors for androgens (AR) and E (estrogen receptor 1 [ESR1]) were assessed in the visceral (VAT) and subcutaneous (SAT) adipose tissues from 21 months of age female offspring treated during fetal life with or without T (mothers treated with 100 mg T propionate i.m. twice a week). As gestational T treatment increases maternal T and insulin levels, the relative contribution of these pathways in programming adipocyte-specific steroidogenic defects were assessed in animals generated in parallel from mothers receiving T concurrently with either androgen antagonist, flutamide, or insulin sensitizer, rosiglitazone. In addition, protein expression of CYP19, AR, and ESR1 were also examined. Prenatal T excess increased the mRNA and protein expression of CYP19 and protein levels of ESR1 expression and showed a trend for increased STAR gene expression ($p = 0.056$) in VAT. In contrast, no changes in any of the enzymes or receptors examined were observed in the SAT. Intervention with either flutamide or rosiglitazone did not reverse the prenatal T-induced increase in CYP19 and ESR1 expression. These data provide support for increased aromatization of androgens to estrogens and increased estrogenic actions via ESR1 locally in VAT but not SAT from prenatal T-treated animals. These adipocyte specific alterations seem to be programmed independent of either androgenic or insulin pathways. The prevailing local estrogenic milieu in VAT from prenatal T-treated female sheep could therefore contribute to increased lipolysis promoting elevated plasma lipid concentrations while reducing adipocyte size.

Prenatal but not concurrent cadmium exposure negatively associated with adiposity in adolescents

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NIEHS 1R01ES021446-01, P20 ES018171-01/RD834800, P01 ES02284401/RD 83543601

Introduction:

Cadmium is a highly pervasive toxic metal that remains a large public health concern, especially for children where early life exposure can impact later health outcomes. Many studies have demonstrated the inverse association of early exposure with growth in infancy and childhood, but no study has examined its influence into adolescence. Our study examines whether prenatal and concurrent cadmium exposure is associated with adiposity measures at ages 8-15 years in a well-characterized birth cohort.

Methods:

Our sample included 185 adolescents between 8-15 years of age from birth cohorts in Mexico City. Maternal (third trimester) and adolescent urines were analyzed for cadmium using an Inductively Coupled Plasma Mass Spectrometer. Children provided anthropometry measurements at visit via trained personnel: waist circumference, height and weight, and subscapular, suprailiac, and triceps skinfold thickness. Body mass index (BMI) and z-scores defined as BMI for age and sex were calculated, using the World Health Organization's reference population. Linear regression models were used to determine the association of prenatal and concurrent urinary cadmium levels with anthropometric measurements, adjusting for cohort, SES, child age and sex, maternal BMI and smoking history.

Results:

Among 86 males and 99 females, mean age was 10 years. Pregnant women and children had median urinary cadmium concentrations of 0.19 $\mu\text{g/L}$ (IQR 0.12-0.27 $\mu\text{g/L}$) and 0.14 $\mu\text{g/L}$ (IQR 0.11-0.18 $\mu\text{g/L}$), respectively. Adjusted linear regression models showed inverse relationships between prenatal cadmium exposure and child adiposity measures: an IQR increase in prenatal cadmium resulted in decreases in BMI z-scores (-26.89%, $p=0.03$), waist circumference (-3.03%, $p=0.03$), and subscapular (-11.18%, $p=0.03$), suprailiac (-10.74%, $p=0.05$), and triceps (-8.10%, $p=0.02$) skinfold thickness. When stratified by sex, these relationships remained significant in females but not males. No significant relationships were discovered between concurrent urinary cadmium exposure and adiposity measures in children.

Conclusions:

Prenatal but not concurrent cadmium exposure was negatively associated with adiposity measures in adolescents. These results emphasize the influence of *in utero* exposures on child growth that persists into adolescence.

Michigan Metabolomics and Obesity Center 2016 Annual Symposium

Abstract submission

Title: Dietary reversal improves peripheral neuropathy in a murine model of type 2 diabetes

Authors: Phillippe D. O'Brien, PhD, Lucy M. Hinder, PhD, John M. Hayes, BS, Carey Backus, BA, Liz S. Bruno, BS and Eva L. Feldman MD, PhD.

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Background: Peripheral neuropathy (PN) is a common complication of type 2 diabetes (T2D) for which no effective treatment currently exists. Recently clinical studies have demonstrated that lifestyle intervention consisting of dietary modification and exercise can improve innervation of intraepidermal nerve fibers in patients with T2D. To explore the relative contribution of diet in improving nerve function, *in vivo* studies are needed so that the physiological mechanisms underlying improved nerve function can be elucidated.

Objective: To determine whether dietary reversal (DR) can improve nerve function in high fat diet fed mice injected with low-dose streptozotocin (STZ), a novel model of T2D.

Methods: Male C57BL6/J mice were fed either a standard diet (10% kcal fat; **CTRL**) or a high fat diet (60% kcal fat; **HFD**). At 12wk a subset of HFD mice were administered with STZ (1x75mg/kg, 1x50mg/kg; **HFD-STZ**). At 16wk subsets of HF and HF-STZ mice were placed on a 10% kcal fat diet for 8wk (**HFD-DR** and **HFD-STZ-DR**, respectively) until study conclusion at 24wk when terminal neuropathy phenotyping was performed on all groups.

Results: By study conclusion HFD mice exhibit signs of impaired glucose tolerance and robust PN while HFD-DR mice displayed an improved metabolic profile which corresponded with improved peripheral nerve function. As a consequence of STZ administration, HFD-STZ mice develop an initial increase in hyperglycemia compared to HFD mice however PN presentation was that of a similar degree to that seen in HFD mice. Similar to HFD-DR mice, after 8wk of DR HFD-STZ-DR mice had significantly improved metabolic profile and PN was corrected.

Conclusion: DR of HF-STZ mice can improve the metabolic profile and restore peripheral nerve function supporting the idea that dietary intervention is a feasible strategy in improving peripheral nerve health both in patients with T2D.

Michigan Metabolomics and Obesity Center 2016 Annual Symposium

Abstract submission

Title: High fat diet-fed female C57BL6/J mice develop early peripheral neuropathy in the absence of systemic insulin resistance

Authors: Phillippe D. O'Brien, PhD, John M. Hayes, BS, Carey Backus, BA, Liz S. Bruno, BS and Eva L. Feldman MD, PhD.

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Background: Peripheral neuropathy (PN) is a common complication in patients with impaired glucose tolerance (IGT) and type 2 diabetes (T2D). Though the development of IGT and T2D does not discriminate between gender affecting both males and females alike, females are less susceptible to developing metabolic impairments, a feature that is attributed to the protective effects of estrogen. It is currently not known whether females also display protection in the development of PN.

Objective: To characterize and compare the development and progression of PN in male and female mice placed on a HFD. As HFD-fed female mice are resistant to HFD-induced metabolic changes we hypothesized that these mice would also exhibit resistance to developing PN when compared to their male counterparts.

Methods: Male and female C57BL6/J mice were fed either a standard diet (10% kcal fat; **CTRL**) or a high fat diet (60% kcal fat; **HFD**) from 5wk. At 16wk, 24wk and 36wk, neuropathy phenotyping was performed on all groups complemented with longitudinal metabolic assessment including insulin tolerance testing (ITT). Neuropathy phenotyping consisted of hindpaw latency to heat stimulus, motor and sensory nerve conduction velocity (NCV), and terminal intraepidermal nerve fiber (IENF) counts.

Results: Assessment of insulin resistance through ITT demonstrated that female HFD mice exhibited relatively normal insulin responsiveness early during the disease course while male HFD mice exhibited insulin resistance. Despite this, at 16wk female HFD mice displayed a similar pattern of PN to that of their male counterparts with similar fold-changes in hindpaw latency, sensory and motor NCV.

Conclusion: Despite exhibiting resistance to HFD-induced metabolic changes female HFD mice display a robust peripheral neuropathy comparable to HFD-male mice. These data suggest that systemic insulin resistance does not contribute to PN. Current studies are investigating insulin signaling in the peripheral nerve of HFD-fed female mice.

DEVELOPMENTAL PROGRAMMING: INFLAMMATION IN VISCERAL ADIPOSE TISSUE OF PRENATAL TESTOSTERONE-TREATED FEMALE SHEEP INDICATES PREDISPOSITION TOWARDS IMPAIRED INSULIN SENSITIVITY LATER IN LIFE

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Background: Prenatal exposure to excess testosterone (T) disrupts programming of the metabolic system in the female sheep. These include insulin resistance (IR) with tissue-specific changes in insulin sensitivity with muscle and liver but not adipose tissue being IR at 21mo. of age. Insulin signaling disruption in IR may occur as a result of proinflammatory cytokine secretion and oxidative stress. **Objectives:** We hypothesize that the insulin sensitive status of metabolic tissues may be associated with their state of inflammatory and oxidative stress in prenatal T-treated female sheep. **Method:** To test this premise and determine if such perturbations are programmed by androgen or insulin, control, prenatal T- (from days 30-90 of gestation), prenatal T plus androgen receptor antagonist, flutamide-, and prenatal T plus insulin sensitizer, Rosiglitazone-treated female sheep were studied at 24mo. of age. Expression levels of inflammatory cytokines and antioxidant enzymes in metabolic tissues were analyzed by real time RT-PCR. **Results:** Increases in proinflammatory cytokines and antioxidant enzymes were not evident in muscle [except superoxide dismutase 1 (SOD1)], liver and subcutaneous adipose tissues. Paradoxically, increased expression of proinflammatory cytokines such as interleukin (IL) 1 beta, IL6 and chemokine ligand 2 was observed in visceral adipose tissues (VAT). Expression of antioxidant enzymes, such as glutathione reductase, SOD1 and 2 were also increased in VAT of prenatal T-treated sheep and this increase was not prevented by co-treatment with flutamide or Rosiglitazone. **Conclusions:** These data suggest that (1) IR state of muscle and liver is not modulated by changes in proinflammatory cytokines, (2) prenatal T- increases expression of proinflammatory cytokines supportive of an inflammatory state in the VAT, (3) the concomitant increase in antioxidant enzymes in VAT is suggestive of a compensatory response to negate underlying inflammation-induced oxidative stress thereby accounting for insulin sensitive state in VAT, (4) disruptions in proinflammatory cytokine and antioxidant enzyme expression in the VAT appear to be independent of modulation via the androgen and insulin pathways, and (5) Increased proinflammatory cytokine expression, which promotes infiltration of immune cells, might potentially tip the balance subsequently and predispose VAT for development of impaired insulin sensitivity later in life. Support: NIH P01 HD44232

Influence of Parental Education on Nutritional and Lifestyle Choices of Project Healthy Schools Students

Author Information: Ryan Rogers, Qingmei Jiang, Nathaniel Costin, Rosa de Visser, Rachel Sylvester, Jean DuRussel-Weston, Eva Kline-Rogers, Kim A. Eagle, and Elizabeth A. Jackson

Background: Prior research has shown that dietary beliefs and activity levels of parents significantly mediate dietary and physical behaviors of their children. However, there is limited research regarding the influence of parental education on children's health. This study analyzes the differences in dietary and lifestyle behaviors of 6th grade students with more educated parents compared to those with less educated parents.

Methods: Data were taken from 6048 students in 41 schools involved in Project Healthy Schools (PHS), a middle school-based intervention program in Michigan. Students were divided into two groups based on their parents' education levels, as selected in a health behavior survey given to the students. Students were included in the "More Educated" group if they had at least one parent with a college or Master's/Professional degree. Students with neither parent having completed a college degree were classified as the "Less Educated" group. Students' baseline survey responses to nutritional and lifestyle questions were compared using a Wilcoxon rank-sum test.

Results: Students with more educated parents reported eating more fruits and vegetables per day, exercising more frequently, and spending less time in front of a screen. The more educated group were more likely to eat breakfast (0.70 vs. 0.53 on a 0-3 scale $p<.0001$), less likely to eat school lunch (1.11 vs 1.39 on a 0-2 scale; $p<.0001$), and less likely to have a TV in their bedroom (0.53 vs. 0.70 on a 0-1 scale; $p<.0001$).

Conclusions: It was observed that students with more educated parents made healthier dietary choices, exercised more often, spent less time in front of screens, and participated more often in other healthy lifestyle choices. These observations may be partially attributed to higher income levels in more educated parents, which may increase students' access to healthier resources; however, this study certainly highlights the importance of parental influence on a child's health. Future efforts will encourage more parental involvement in Project Healthy Schools.

Table 1. Student Health Behaviors by Parental Education Level

	Less Educated (n=1280)	More Educated (n=2831)	p-value
Dietary Habits (Servings/Day)			
Vegetables	1.1668	1.4004	<.0001
Fruit	1.2492	1.4841	<.0001
Reg. Soda	0.7685	0.5645	<.0001
Diet Soda	0.1904	0.1577	0.0777
Chocolate Desserts	0.5533	0.4527	0.0043
Physical Activity (Sessions/Week)			
Vig. 20 min Exercise	3.9676	4.4556	<.0001
Mod. 30 min Exercise	2.8166	3.0316	0.0124
Weight Training	2.5810	2.9260	<.0001
Screen Time (Hours/Day)			
TV/Movie	2.3500	2.0328	<.0001
Computer Time	1.4680	1.3017	0.1954
Video Game	1.5233	1.2964	0.0006
Mobile Device	2.0837	1.7898	.0001

Title: Toddler Overweight Prevention: Developing a Model by Socioeconomic Gradients

Background. Childhood obesity is a major health issue associated with increased risk of long-term health consequences; early prevention is crucial for public health. Little is known about variations in predictors of overweight across gradients of social class. This study addresses this gap using the ecological framework of childhood obesity to assess how characteristics vary across socioeconomic gradients.

Methods. Child, parent, and community characteristics of toddlerhood were examined within SES quintiles in a national sample. Data were collected from 9,850 families via in-home and self-administered assessments. The sample was stratified by SES quintile (1-low, medium-low, medium, medium-high, 5-high). Taylor series linearization was applied to logistic regression models within quintiles using weight status as the dependent variable (overweight/normal). Parameter estimates were compared across quintiles to assess differences in predictors of overweight.

Results. Children were 2.03 years-old, on average. African Americans and Hispanics comprised the majority in SES 1 and SES 2; Caucasians were the majority in quintiles 3-5. Higher rates of overweight were observed in SES 1 (29.2%), and reduced as SES increased (16.9% - SES 5).

Regression results showed child characteristics of race/ethnicity were significant in SES 1 and 4. Compared to Caucasians, African Americans had decreased odds of overweight (55%) in SES 1, and Hispanics and those of an “other” race had decreased odds of overweight in SES 4 (47% and 60%, respectively). Better motor development was associated with increased odds of overweight in SES 1 (71%) and SES 2 (43%); better mental development was associated with decreased odds of overweight in SES 2 (22%). Both SES 1 (32%) and 5 (36%) showed decreased odds of overweight for females, compared to males.

Parameter estimates for parent characteristics also varied across quintiles. In SES 1, being unmarried predicted increased odds of overweight. Normal maternal BMI was associated with decreased odds in both SES 1 and 2, although odds were much higher in SES 2 (51%), compared to SES 1 (29%). Lack of employment was associated with increased odds of overweight in SES 2, whereas parental feeding practices were significant across 3 quintiles. In SES 2 (55%), SES 4 (169%), and SES 5 (77%), introduction of solid foods before 4 months was associated with increased odds of overweight. Being put to bed with no bottle was associated with decreased odds of overweight in SES 2. More children in the home was associated with increased odds of overweight in SES 1, and having more adults in the household was associated with increased odds of overweight in SES 5. At the community level, in SES 1, not having WIC/SNAP predicted increased odds of overweight (81%), and higher community engagement reduced odds of overweight (17%) in SES 2.

Conclusions. Using an ecological and social gradient of health framework, this study indicates there are distinct predictors of overweight within each SES quintile, which yields important implications for public policy, intervention developers, and researchers. Existing policies and practices should be reformed to address the specific social determinants of health for families at different levels of SES.

The Kielin/Chordin-like Protein KCP can Attenuate High Fat Diet

Induced Obesity and Metabolic Syndrome in Mice

Obesity and its associated complications, such as insulin resistance and non-alcoholic fatty liver disease, are reaching epidemic proportions. In mice, the TGF- β superfamily is implicated in the regulation of white and brown adipose tissues differentiation. The KCP protein is a secreted regulator of the TGF- β superfamily pathways that can inhibit both TGF- β and Activin signals while enhancing the Bone Morphogenetic protein (BMP) signaling. However, the effects of KCP on metabolism and obesity have not been studied in animal models. Thus, we examined the effects of KCP loss or gain of function in mice that were maintained on either a regular or a high fat diet. Loss of KCP sensitized mice to obesity and associated complications such as hepatic steatosis and glucose intolerance. In contrast, transgenic mice that expressed KCP in the kidney, liver and brown adipose tissues were resistant to developing high fat diet induced obesity and had significantly reduced white adipose tissue. KCP over-expression was able to shift the pattern of Smad signaling in vivo, to increase the levels of P-Smad1 and decrease P-Smad3, resulting in resistance to high fat diet induced hepatic steatosis and glucose intolerance. The data demonstrate that shifting the TGF- β superfamily signaling with a secreted inhibitor or enhancer can alter the profile of adipose tissue to reduce obesity and can inhibit the initiation and progression of hepatic steatosis to significantly reduce the effects of high fat diet induced metabolic disease.

LC-Data Independent Acquisition (DIA- SWATH™) HR-MS/MS Increases Untargeted Metabolomics Coverage and Identification Confidence

LC-high resolution mass spectrometry (HRMS) has become a major technology platform in metabolites profiling. LC-HRMS is utilized to generate accurate masses to determine molecular formulae of metabolites along with their retention times (RTs) and integrated areas to determine relative amounts and initial annotations of metabolites after searching public or in house databases. Further work is needed to confirm the founded metabolites by tandem MS/MS and standard comparisons. Data dependent acquisition (DDA) and DIA are two MS/MS methods to generate larger numbers of MS/MS spectra for metabolites identifications. New SWATH™ (Sequential Window acquisition of All Theoretical fragment ion spectra) is more effective form of DIA that allows untargeted qualitative and quantitative metabolites analysis of complex samples like human plasma. LC- SWATH™ MS/MS technology allows collecting high resolution quantifiable MS/MS data for all detectable metabolites with in a complex sample, especially low level metabolites with higher coverage of predicated or un-predicated metabolites. High throughput untargeted metabolite ID workflows have become a reality with LC-SWATH™ MS/MS for data acquisition, data deconvolution and processing with Sciex MetabolitePilot and other software tools. Commercial available MS/MS metabolite databases such as Metlin and NIST14 small molecules database nist_msms and nist_msms2 are available for LC-SWATH™MS/MS applications. In addition, other public databases such as LipidBlast, MassBanks, etc provide additional MS/MS information for metabolomics identification. LC-SWATH™ MS/MS in combination with the MS/MS databases and software tools give the most comprehensive coverage and confident identification for untargeted metabolomics analysis with HRMS, isotope pattern, RT, HRMSMS all taken into account in one analysis. In this is work, results from a red cross plasma and the Children's Health Exposure Analysis Resource (CHEAR) plasma samples will be demonstrated.

ChREBP mediates metabolic adaptation to fructose intake and prevents fructose-rich diet-induced hepatotoxicity

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Epidemiologic and animal studies implicate overconsumption of fructose in the development of non-alcoholic fatty liver disease, but the molecular mechanism remains largely unknown. We present evidence supporting the essential function of the lipogenic transcription factor ChREBP in adaptation to fructose and protection against fructose-induced hepatotoxicity. High-fructose diet (HFrD) activated hepatic lipogenesis via a ChREBP-dependent manner in wildtype mice, while inducing steatohepatitis in *Chrebp-KO* mice. In *Chrebp-KO* mouse livers, HFrD reduced levels of molecular chaperones and activated the CHOP-dependent unfolded protein response (UPR), whereas administration of chemical chaperone or *Chop* shRNA reversed liver injury. Gene expression profiling revealed elevated expression of cholesterol biosynthesis genes in *Chrebp-KO* livers after HFrD. Furthermore, inhibition of cholesterol biosynthesis by atorvastatin reduced hepatic CHOP expression and rescued liver injury in HFrD-fed *Chrebp-KO* mice. Therefore, our findings demonstrate a pivotal role of ChREBP in hepatoprotection against HFrD by preventing overactivation of cholesterol biosynthesis and CHOP-mediated pro-apoptotic UPR.

A Novel Method Enabling the Use of Small Muscle Samples to Identify Insulin Effects on Akt2 and AS160 Subcellular Localization

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Akt protein kinases play overlapping and distinct roles in many physiological processes. Akt1 and Akt2 are highly expressed in skeletal muscle, and Akt2 is more important for insulin-stimulated glucose transport. In adipocytes Akt-isoform diversity in insulin-mediated subcellular localization is crucial for isoform-specific function, but little is known about Akt isoform-specific localization in muscle tissue. Subcellular localization by fractionation is challenging, in part because existing methods typically require grams of tissue. Therefore, the purpose of this study was to develop a method to evaluate subcellular protein localization requiring only 50 mg muscle and assess insulin effects on Akt localization. Rat soleus strips were isolated and incubated with or without insulin. Muscle lysates subsequently underwent ultra-speed centrifugation. Membrane (Na⁺, K⁺ ATPase and insulin receptor) and cytosolic (LDH) fraction markers were used to validate the purity of fractions. With insulin-stimulation, Akt2, but not Akt1, had significantly ($P < 0.05$) increased abundance in the membrane, but not cytosol. Similar Akt isoform specificity has been previously demonstrated for insulin-stimulated adipocytes, but this is a novel result for muscle tissue. In addition, Akt substrate of 160 kDa (AS160; also called TBC1D4), an Akt2 substrate and key regulator of insulin-regulated glucose transport, was significantly ($P < 0.05$) increased in membrane, but not cytosol, with insulin-stimulation. In conclusion, we developed a novel method for assessing subcellular localization of insulin signaling proteins in small muscle samples. This method will enable experiments aimed at advancing understanding of the roles of Akt localization for altered insulin sensitivity with physiological conditions, e.g., obesity or exercise.

Differentiating Adipocytes in Collagen Hydrogels

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It has long been recognized that body fat distribution and regional adiposity play a major role in the control of metabolic homeostasis. However, the ability to study and compare the cell autonomous regulation and response of adipocytes from different fat depots has been hampered by the difficulty of inducing preadipocytes isolated from the visceral depot to differentiate into mature adipocytes in culture. Here, we present an easily created 3-dimensional (3D) culture system that can be used to differentiate preadipocytes from the visceral depot as robustly as those from the subcutaneous depot. The cells differentiated in these 3D collagen gels are mature adipocytes that retain depot-specific characteristics, as determined by imaging, gene expression, and functional assays. This 3D culture system therefore allows for study of the development and function of adipocytes from both depots in vitro and may ultimately lead to a greater understanding of site-specific functional differences of adipose tissues to metabolic dysregulation.