UAB Diabetes Research Day 2014

May 23, 2014 – Doubletree Hotel, Heritage Hall I

SCHEDULE

8:00 AM – 9:00 AM	**Registration and Breakfast**
9:00 AM – 10:45 AM	**Session I** (Co-chairs: Tim Garvey & Adam Wende)
9:00 AM	**Welcome** Anath Shalev, MD Professor of Medicine, Director, UAB Comprehensive Diabetes Center
9:05 AM – 9:45 AM	**"Translational Bioenergetics in Diabetes: From the Beta Cell to Chronic Kidney Disease"** **Victor Darley-Usmar, PhD** Professor of Pathology, Director, Center for Free Radical Biology, Vice-Chair of Research Department of Pathology, UAB
9:45 AM – 10:00 AM	"Food-Intake at Night on Workdays is Associated with Cardiometabolic Syndrome Risk Factors in Night-Shift Nurses" Hylton E. Molzof, Department of Psychiatry, UAB (Gamble Lab)
10:00 AM – 10:15 AM	"Adipose and Metabolic Dysfunction in a Western Diet-Induced Model of NAFLD" Yuwen Luo, Boshell Metabolic Diseases and Diabetes Program, Auburn University (Greene Lab)
10:15 AM – 10:30 AM	"Sad Fat: The Relationship between Depression, Visceral Fat, and Markers of Inflammation before and after Weight Loss" Holly E.S. Resuehr, Department of Nutrition Sciences, UAB (Gower Lab)
10:30 AM – 10:45 AM	"Tribble 3 Contributes to Foam Cell Formation and Programs Macrophages towards Lipid Accumulation over Inflammation" Dennis Steverson Jr., Department of Nutrition Sciences, UAB (Garvey Lab)

10:45 AM – 11:00 AM	**Coffee Break**
11:00 AM – 12:20 PM	**Session II** (Chair: Stu Frank)
11:00 AM – 12:00 PM	**Keynote Address** **"Pathways to Diabetes Revealed through Mouse Genetics"** **Alan Attie, PhD** Professor of Biochemistry, University of Wisconsin-Madison
12:00 PM – 12:15 PM	"Fibroblast Growth Factor 21 Mediates the Beneficial Effects of Exercise on Diet-Induced Glucose Intolerance" Kirk Habegger, Assistant Professor of Medicine, UAB
12:15 PM – 12:30 PM	**Announcement of the Winner of the 2014 Eliezer S. Award for Best Diabetes Research Abstract**
12:30 PM – 2:30 PM	**Lunch**
1:00 PM – 2:30 PM	**Poster Session**

ORAL PRESENTATION ABSTRACTS

Fibroblast Growth Factor 21 Mediates The Beneficial Effects Of Exercise On Diet-Induced Glucose Intolerance

[1]Christine Loyd, [1]Jack Magrisso, [1]Michael Haas, [1]Sowmya Balusu, [2]Nobuyuki Itoh, [1]Darleen Sandoval, [1]Silvana Obici, [1]Diego Perez-Tilve, & [3]Kirk M Habegger

[1]Metabolic Disease Institute, Div. of Endocrinology, Diabetes and Metabolism, Dept. of Medicine, University of Cincinnati, Cincinnati OH, USA
[2]Department of Genetic Biochemistry, Kyoto University Graduate School of Pharmaceutical Sciences
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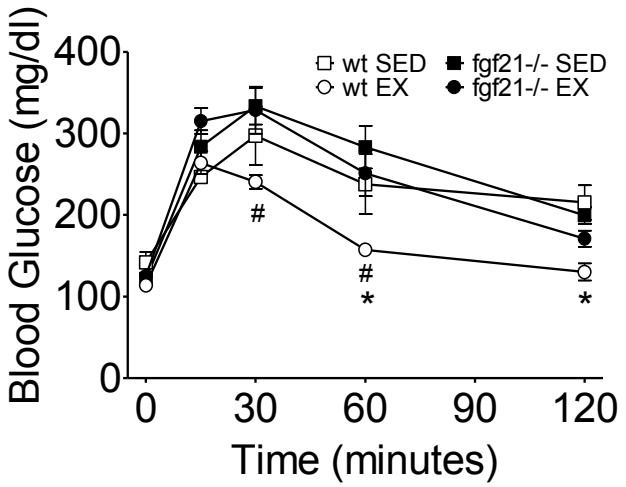
Exercise is a well-defined preventative and therapeutic intervention against metabolic syndrome. Yet, the molecular pathways underlying the beneficial effects of exercise are unclear. Recent evidence indicates that glucagon signaling in the liver plays an important role in the metabolic benefits associated with exercise. We have previously shown that fibroblast growth factor 21 (FGF21) mediates some of the beneficial metabolic effects of glucagon. Thus, we aimed to test the role of FGF21 in mediating benefits of chronic exercise on glucose and lipid metabolism. Wildtype (*wt*) and FGF21-deficient (*fgf21*$^{-/-}$) mice were fed high-fat diet (HFD) and allowed access to inoperable (sedentary) or operable (exercise) running wheels for 12 weeks. Exercise was effective in suppressing body weight and fat mass gain, and improving plasma lipidemia in *wt* and *fgf21*$^{-/-}$ mice. Exercise likewise improved glucose tolerance in *wt* mice; however this effect was lost in *fgf21*$^{-/-}$ mice. We identified that *fgf21*$^{-/-}$ mice exhibited reduced adaptations to chronic exercise in skeletal muscle. Exercise stimulated a notable increase in AMPK activation in skeletal muscle of *wt* mice, but strikingly, not in that of *fgf21*$^{-/-}$ mice. These results clearly show that systemic FGF21 activity is required for exercise benefits on glucose tolerance by stimulating AMPK activation in skeletal muscle.

This abstract is also presented as poster number 15.



Adipose and metabolic dysfunction in a western diet-induced model of NAFLD

Yuwen Luo[1], Jian Zhang[1], Robert L. Judd[1], Emily C. Graff[1], and Michael W. Greene[1]

[1]Boshell Metabolic Diseases and Diabetes Program, Auburn University, Auburn, AL 36849, USA, [2]Bassett Research Institute, Cooperstown, NY

Nonalcoholic steatohepatitis (NASH), a more severe form of nonalcoholic fatty liver disease (NAFLD) that is associated with obesity, insulin resistance, and diabetes, is characterized by steatosis, inflammation, oxidative stress, apoptosis, and fibrosis. It has been postulated that excessive sugary drink consumption may contribute to the development and severity of NAFLD. However, its role in adipose tissue expansion and dysfunction is not known. To better understand the role of consumption of liquid sugar in high fat diet-induced NAFLD progression, we investigated metabolic and histologic parameters and gene expression from hepatic and adipose tissues in mice fed a low or high fat western diet (HFWD) without or with liquid sugar [fructose and sucrose (F/S)] at two time points (2 and 12 week). HFWD+F/S impaired the storage capacity of epididymal white adipose tissue (eWAT) and exacerbated HFWD-induced glucose intolerance and insulin resistance. Consistent with these results, HWFD+F/S fed mice developed more profound eWAT inflammation characterized by macrophage infiltration, a dramatic increase in crown-like structures, and upregulated proinflammatory gene expression. Hepatic triglyceride, plasma alanine aminotransferase, and normalized liver weight were significantly increased only in HFWD+F/S fed mice. Hepatic oxidative stress, assessed by superoxide dismutase activity, 4-hydroxynonenal, NADPH oxidase activity, and gene expression was highest in HFWD+F/S fed mice. HFWD+F/S also resulted in increased hepatic fibrosis and elevated collagen I, collagen III and TGFβ gene expression. Our results highlight the effect of sugary water consumption in adipose tissue dysfunction and NAFLD progression.

This abstract is also presented as poster number 42.

Food-Intake at Night on Workdays is Associated with Cardiometabolic Syndrome Risk Factors in Night-Shift Nurses

Molzof, H. E., Johnson, R.L., & K. L. Gamble

Nurses who work night-shift are at a higher risk of developing a range of metabolic disorders, including cardiometabolic syndrome (CMS), in which patients exhibit multiple risk factors for heart disease, stroke, and diabetes. For example, night-shift nurses exhibit a significantly greater cumulative incidence rate of CMS compared to day-shift nurses—9.0% vs. 1.8% over a four-year period (Pietroiusti et al., 2010). Here, we tested the hypothesis that altered food-intake rhythms in night shift workers are associated with CMS risk factors. Female nurses (N = 17; 8 day-shift and 9 night-shift; matched for age and BMI) reported 24-h recall of daily food intake over a 10-day assessment period. The time, quantity, and nutrient content of meals were analyzed using Nutrition Data System for Research (NDSR) software. On a middle day off from work, blood samples were collected after an overnight fast in order to determine several metabolic parameters including glucose, triglycerides, and cholesterol in addition to systolic and diastolic blood pressure. For both day- and night-shift nurses, we compared food intake during the day or night on free-days versus work-days in terms of total grams, proteins, carbohydrates, fats, and sugars. For night shift nurses only, total food intake (g) at night (between 9pm and 9am) on work-days was significantly associated with increased fasting triglyceride levels, fasting glucose, total cholesterol, LDL cholesterol, and diastolic blood pressure. This result was not accounted for by further analysis of specific macronutrients. On days off, nocturnal intake of fat (only) was significantly associated with these same CMS risk factors in night shift nurses. Moreover, these results were specific to night shift nurses since linear regression analysis of the same variables was not significant for nightly food intake in day-shift nurses. In summary, these findings suggest nocturnal food intake on work nights or nocturnal fat intake on days off are associated with increased cardiometabolic risk factors in night-shift nurses. Future classification of food-intake strategies could be beneficial for identifying nurses most at risk of developing CMS.

This abstract is also presented as poster number 41.

Sad fat: The relationship between depression, visceral fat, and markers of inflammation before and after weight loss

Holly E. S. Resuehr[1], Amy Goss[1], Paula Chandler-Laney[1], Gordon Fisher[2], Gary Hunter[2], Richard Shelton[3], Barbara Gower[1]

[1]Department of Nutrition Sciences, [2]Department of Human Studies, [3]Department of Psychiatry, University of Alabama at Birmingham

Objective: Depression has been linked to excess adiposity, intra-abdominal adipose tissue (IAAT), and inflammation. However, the causative nature of these relationships remains unclear. This study was designed to test the hypothesis that improvement in depressive symptoms with weight loss is due to a reduction in markers of inflammation (MOI), which occurs secondary to reduction in IAAT. The specific associations tested were: 1) whether a decrease in depressive symptoms with weight loss is associated with decreases in both visceral fat and MOI; 2) whether a reduction in IAAT is associated with reduction in MOI; and 3) whether improvement in depressive symptoms with weight loss is associated with reduction in MOI independent of changes in IAAT.
Method: This was a longitudinal study designed to look at healthy, overweight (BMI 27-30 kg/m^2), premenopausal women before and after weight loss through diet or diet plus exercise interventions. Depressive symptoms were evaluated using the Beck Depression Inventory-II (BDI). Body composition was measured with DXA, and IAAT with CT scanning.
Results: Total body adiposity, MOI, and BDI scores were all significantly reduced after weight loss. Change in BDI score was independently associated with change in IAAT after adjustment for change in total fat mass (Std β=-0.22, $p<0.05$). Change in BDI score was also independently associated with change in TNF-α after adjusting for the change in total fat and change in IAAT (Std β=-0.22, $p<0.05$).
Conclusions: The improvement (decrease) in depressive symptoms with weight loss was significantly and independently correlated with reduction in both IAAT and TNF-α. Thus, loss of body fat may relieve depressive symptoms through multiple mechanisms.

Tribble 3 Contributes to Foam Cell Formation and Programs Macrophages towards Lipid Accumulation over Inflammation

Dennis Steverson Jr., Ling Tian, Yuchang Fu, W. Timothy Garvey

Insulin resistance is central in the pathophysiology of cardiometabolic disease; however, common mechanisms that explain the parallel development of both type 2 diabetes and atherosclerosis have not been elucidated. We have previously shown that tribbles homolog 3 (TRB3) can exert a chronic pathophysiological role in promoting insulin resistance in muscle and fat cells and also has an acute physiological role to alternatively regulate glucose uptake in fat and muscle during short-term fasting and nutrient excess. Since TRB3 is expressed in human atherosclerotic plaques, we explored its role in foam cell formation to assess its potential contribution to atherogenesis. We have employed human THP-1 monocytes which transition to lipid-laden macrophage foam cells when exposed to oxidized LDL (oxLDL). We first observed that TRB3 was upregulated by 2 fold increase ($p<.01$) within 24 hours of treatment with oxLDL. To determine whether TRB3 actively participated in foam cell formation, we overexpressed TRB3 in THP-1 monocytes and found that this led to a 1.2-fold increase in cholesterol accumulation after 48 hour ($p<.01$) compared with control cells. This was due to increased expression of genes promoting lipid accumulation such as Adipocyte Lipid Binding Protein (ALBP). At the same time, TRB3 overexpression suppressed the inflammatory posture of macrophages as evidenced by reduced expression of Tumor Necrosis Factor alpha (TNF-a) and Monocyte Chemoattractant Protein-1 (MCP-1) (both $p<.01$). In conclusion: 1) TRB3 is upregulated in macrophage foam cells and promotes lipid accumulation associated with increased expression of ALBP and suppression of ABCA-1. 2) TRB3 also suppresses cytokine expression and therefore orients the macrophage to assume a more primary role for lipid accumulation while maintaining a secondary role as an inflammatory immune cell.

This abstract is also presented as poster number 43.

POSTER ABSTRACTS

Poster Number 1

A Novel Approach to Measuring Heat Exposure, Thermal Preference and Time Spent Outdoors

Molly C. Bernhard[1], Shia T. Kent[2], Leslie A. McClure[3], Mary B. Evans[4], Julia M. Gohlke[1]

[1]Environmental Health Sciences, University of Alabama at Birmingham (UAB), [2]Epidemiology, UAB, [3]Biostatistics, UAB, [4]Center for the Study of Community Health, UAB

A better understanding of temperatures an individual experiences and its association with body composition may lead to greater understanding of barriers to outdoor physical activity. Diabetics have higher rates of heat illnesses and deaths during heat waves than the general public. Current methods for estimating heat exposure and time spent outdoors such as weather station data, activity logs, or GIS instruments may lack accuracy, are expensive or raise privacy concerns. We set out to determine whether a small, inexpensive temperature/sunlight monitor attached to the shoe could be used to estimate heat exposure, thermal preference, and time spent outdoors simultaneously. Community partners in rural and urban areas recruited participants (N=80) to wear monitors for one week. Height, weight and body composition measurements were taken and participants recorded their activities and locations (inside or outside) on an hourly basis. In response to an exit survey, most participants (86%) found wearing the monitor on their shoe was very comfortable and reported the monitor was not hard to remember to wear (81%) and 68% reported becoming more aware of the time they spend indoors and outdoors upon receiving a graph of the monitor's output at the turn-in session. When data from the individual monitors were compared to data from the closest weather station, weather stations overestimated average heat exposure. In contrast, daily maximum temperatures from a nearby weather station underestimated maximum temperatures experienced by urban participants. In conclusion, we have demonstrated an improved, inexpensive method for heat exposure and time spent outdoor estimation.

Poster Number 2

Inhibition of the CA^{2+}- Independent Phospholipase $A_2\beta$ ($iPLA_2\beta$) by a Novel Fluoroketone-Based Compound Reduces Downstream Products of $iPLA_2\beta$ and Reduced Incidence of Type 1 Diabetes

Robert N. Bone[1,2], Ying Gai[2,3], Victoria Magrioti[4], Hubert M. Tse[2,5], George Kokotos[4], Sasanka Ramanadham[2,3]

Departments of [1]Pathology, [3]Cell, Developmental, & Integrative Biology, [5]Microbiology, and the [2]Comprehensive Diabetes Center, University of Alabama at Birmingham, Birmingham, AL, USA and [4]Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Athens, Greece.

Type 1 Diabetes (T1D) is an autoimmune disease that leads to ablation of insulin-producing pancreatic islet β-cells, but the mechanism(s) involved are not fully understood. Our findings that group VIA Ca^{2+}-independent phospholipase A_2 beta ($iPLA_2\beta$) derived signals contributed to β-cell apoptosis & that $iPLA_2\beta$ expression is increased in islets of diabetes-prone non-obese diabetic (NOD) mice prompted us to assess whether $iPLA_2\beta$ activation contributes to the development of diabetes in this model of autoimmune T1D. FKGK18, a recently available fluoroketone-based reversible inhibitor of $iPLA_2\beta$ was administered (20 mg/kg, i.p.) tri-weekly to female NOD mice. By 25 weeks of age, 80% of vehicle-treated mice developed while 10 of 11 FKGK18 treated mice remained diabetes free at 25 weeks. The reduced incidence of diabetes was associated with higher circulating insulin levels, improved glucose tolerance, and preservation of β-cell mass. Islet infiltration was dramatically reduced with treatment, reflected by reduced CD4+ T-cells and B-cells in the islets of FKGK18-treated mice. The reversible nature of FKGK18 inhibition prompted us to measure PGE2 metabolites (PGEM) in urine to assess the *in vivo* inhibitory ability of the drug. We find that the PGEM were decreased ~25%, suggesting decreased generation of PGE2 due to inhibition of arachidonic acid hydrolysis in the presence of FKGK18. We further find that generation of cytokines by inflammatory cells is significantly reduced by FKGK18, suggesting a direct effect of $iPLA_2\beta$-derived lipid signals on immune cell function. Taken together, our findings suggest that generation of $iPLA_2\beta$-derived lipid signals play critical roles in the pathogenesis of autoimmune-mediated type 1 diabetes.
Acknowledgements: NIH R01-DK69455, The Iacocca Foundation, & the UAB Comprehensive Diabetes Center.

Poster Number 3

Telemetric Continuous Blood Glucose Monitoring of Postprandial Responses in Rats

Rachel A. Brewer[1], Samir Rana[2], Inga Kadish[3], Thomas van Groen[3], Nathan D. Miyasaki[1], Daniel L. Smith Jr.[1,4,5,6]

[1]Department of Nutrition Sciences; [2]Psychiatry – Behavioral Neurobiology; [3]Cell, Developmental and Integrative Biology; [4]Nutrition Obesity Research Center; [5]Diabetes Research Center; [6]Comprehensive Center for Healthy Aging; University of Alabama at Birmingham

Introduction: Accurate blood glucose (BG) monitoring is important for a variety of disease models. Current methods for assessing BG levels in rodents often involve invasive procedures with repeated handling which may cause stress and inadvertently elevate BG.
Aim: To evaluate telemetric continuous glucose monitors (CGM) for measuring postprandial blood glucose levels.
Methods: Telemetric CGM (DSI, HD-XG) were surgically implanted into the lower descending aorta of 10-week-old, male Sprague-Dawley rats (n=8). Circulating BG levels were sampled every 10 seconds (for up to 3 months). Calibrations were performed weekly with a handheld BG monitor (Nova, StatStrip Xpress). Animals were fed a chow diet with alternating 2-week exposures to acarbose (0.1% w/w in diet), an α-glucosidase/α-amylase inhibitor. Postprandial blood glucose (PPG) was determined weekly for 60 minutes following 12-hour (light phase) fasts. Comparisons were made within individual animals.
Results: The slope of the rate of initial increase in BG and PPG area under the curve (AUC) were underestimated with the handheld monitor (vs. CGM). CGM revealed a series of PPG peaks-troughs, including a rapid rise immediately upon return of food, which were not captured with the handheld monitor. The first PPG peak was <15 minutes after meal initiation, with the first trough occurring <30 minutes. Consistent with previous studies, acarbose treatment blunted these PPG responses (AUC and slope).
Conclusions: CGM by implantable telemetry is an effective method of evaluating BG and reveals more nuanced patterns in postprandial variability than handheld monitoring alone, while reducing stress associated with handling needed for traditional glucose measures.

Funding: The Ellison Medical Foundation (Smith, New Scholar in Aging Award), Core Support - P30 NS47466

Poster Number 4

How We Talk About People with Conditions: Trends Over Time Using Google Ngrams

Andrew W Brown, Ph.D.

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People first language (PFL) is the practice of referring to a person separately from a health- or physical-condition he or she may have. Some people advocate for using PFL out of respect for individuals, and the journal *Diabetes* codified in their guide to authors that “The term *diabetic* should not be used as a noun.” However, PFL can make for awkward prose, and clinically relevant terms can be stigmatizing. The purpose of this study was to investigate trends in the usage of different descriptors of four conditions over time in the Google Books Ngram Viewer: asthma, autism, diabetes, and obesity. The five categories of descriptors investigated are: condition-defining (“the diabetics”), condition-adjective (“the diabetic patients”), PFL-adjective (“the woman who is diabetic”), PFL-neutral (“the child who has diabetes”), and PFL-negative (“the man who suffers from diabetes”). From 1800 to 2008 (the latest year available), the proportion of literature referencing these four conditions increased by degrees of magnitude. The use of PFL-neutral language describing people with asthma, autism, and diabetes increased over time, particularly since 1980, but not for obesity. People with obesity are consistently referred to with PFL-adjective and condition-adjective language. Condition-defining language was used to describe people with diabetes more than other conditions, with a notable decrease in condition-defining language since 1980. In conclusion, language describing individuals with conditions has varied markedly across time and across conditions, though the influence of this change in language usage on patients with and opinions of these conditions remains uncertain.

Poster Number 5

Superoxide Deficiency Hinders Anti-Viral Responses of Non-Obese Diabetic Macrophages during Diabetogenic Coxsackie B4 Virus Infection

Ashley R. Burg, Lindsey E. Padgett and Hubert M. Tse, PhD

Department of Microbiology, Comprehensive Diabetes Center, University of Alabama at Birmingham, Birmingham, AL

Type 1 diabetes (T1D) is defined by the autoimmune destruction of pancreatic beta-cells. While the events that initiate the autoimmune attack remain elusive, studies have suggested that pancreatic viral infections may trigger this destructive process. The innate immune anti-viral response can create an inflamed environment, producing reactive oxygen species (ROS) and pro-inflammatory cytokines. We recently demonstrated a critical role for NADPH oxidase (NOX)-derived ROS production in T1D pathogenesis, as superoxide-deficient Non-Obese Diabetic (NOD.*Ncf1*^{m1J}) mice are highly T1D-resistant. Interestingly, bone marrow-derived macrophages from these mice have reduced viral RNA sensing capacity against the viral dsRNA-mimic, poly(I:C). Therefore, we hypothesize that the absence of NOX-derived ROS will reduce the diabetogenicity of viral infections by dampening innate immune anti-viral responses that contribute to T1D and pancreatic beta-cell destruction. We performed both *in vivo* and *in vitro* viral infection studies with Coxsackie B4 virus (CB4), a hypothesized viral trigger of T1D in both humans and rodents. Following a 6-hour infection with CB4 *in vitro*, NOD.*Ncf1*^{m1J} macrophages displayed significant 1.4- and 2-fold decreases in protein expression of the viral RNA sensors, TLR3 and RIG-I, respectively, compared to NOD macrophages. By 24 hours this resulted in a 2-fold decrease (p<0.0005) in TNF-alpha production, and a dramatic 11-fold decrease (p<0.0001) in IFN-beta release by NOD.*Ncf1*^{m1J} macrophages. This defective response by NOD.*Ncf1*^{m1J} macrophages was recapitulated *in vivo*, as CB4-infected NOD.*Ncf1*^{m1J} mice had a 2.5-fold decrease in the percentages of both TNF-alpha- and IL-1beta-producing macrophages infiltrating the pancreas. These results suggest that depletion of ROS may curtail the viral-induced initiating events in T1D. Future studies will further define this role of ROS in potentiating the innate immune response to diabetogenic viral infections that trigger T1D onset.

Poster Number 6

The Burden of Diabetes in Rural Alabama: A Patient Perspective

Caresse Campbell, MPH, Maria Pisu, PhD, Monika Safford, MD, Jewell Halaynch , MD, Robert Weech-Maldonado, PhD, Meredith Kilgore, PhD

Background: Patients bear considerable economic costs (i.e., not reimbursed by third party payers) for the management of diabetes. This economic burden is rarely documented and poorly understood. Rural communities in the South are disproportionately affected by diabetes due to high rates of poverty, low educational attainment, and limited access to insurance and specialty medical care. The purpose of this study was to (1) describe the patient economic burden of a rural population in Alabama and (2) determine if disease self-management (DSM) is associated with lower economic burden in this population.
Methods: We conducted secondary data analysis of data collected at baseline from 424 adults participating in the Encourage trial. Patient economic burden included three domains: (1) direct costs (medical and non-medical out-of-pocket costs, and time for doctor visits and DSM), (2) indirect costs (productivity losses) and (3) intangible costs (quality of life, QOL, measured by EQ-5D). We analyzed secondary data collected at baseline from participants of Encourage, a trial that tested the effectiveness of a peer support intervention to improve DSM. DSM was measured by a score based on 5 variables: adherence to medication, exercise, diet, self-monitored blood glucose (SMBG) and self-examination of feet. The association between patient economic burden and DSM was tested using a generalized linear model (GLM), adjusting for demographic factors, insurance, insulin use, depressive symptoms, and health status.
Results: The sample was predominantly female (76%) and African-American (87%); 45% had annual household incomes below $20,000. Total monthly costs were $745 (SD=$707; median $623). Monthly mean direct medical out-of-pocket costs were $121 (SD = $139; median = $76). Mean direct non-medical out-of-pocket costs were $31 per month; the most frequent expenses were for transportation (96%), and exercise (22%). Time costs were calculated at $16.21 per hour. Mean monthly time for travel, waiting to receive care and DSM, was 29 hours; mean monthly time cost was $467 (SD= $356; median = $394). Approximately 30% reported indirect costs with a monthly mean cost of $454 (SD= $935; median= $194). The mean QOL score was 0.76 (SD=0.20): almost 20% reported a score of 1 (perfect health).
Approximately 35% reported sub-optimal DSM, 40% intermediate DSM and 25% ideal DSM. Mean total cost for sub-optimal DSM patients was $579 (SD=$423); for intermediate DSM patients was $842 (SD=$702); and for ideal DSM patients was $821 (SD=$942). Ideal DSM patients spent significantly more on direct medical cost (p=0.001) direct non-medical cost (p=0.008), time costs (p=<0.0001) and total cost (p=<0.0001) compared to their counterparts. QOL score were not significantly different across DSM groups.
Conclusions: Diabetic patients incurred substantial economic burden with out-of-pocket costs that averaged $1,824 annually. This is a considerable financial burden for this rural low a population. Ideal self-management did not alleviate but was rather associated with higher costs in the short run. Policies are needed to counter the cost consequences associated with improved self-management. Future studies should investigate whether cost reductions are realized in the longer term such as lower diabetes related complications.

Poster Number 7

Late-night Carbohydrate Intake is Associated with Poorer Glucose Tolerance Among Obese, but not Normal Weight, African American Women in Late Pregnancy

Paula C. Chandler-Laney [a], Camille R. Schneider [a], Barbara A. Gower [a], Joseph R. Biggio [b]

Departments of Nutrition Sciences[a] and Medicine[b], University of Alabama at Birmingham

Objective: Obesity and late-night food consumption are each associated with impaired glucose tolerance. Late-night carbohydrate consumption may be particularly detrimental to obese (Ob) pregnant women because they have lower insulin sensitivity than do women of normal weight (NW). The purpose of this study was to test the hypothesis that night-time carbohydrate consumption would be associated with poorer glucose tolerance in late pregnancy and that this association would be exacerbated among obese women.
Research Design and Methods: Forty non-diabetic African American women were recruited and stratified by weight status (NW: <25kg/m^2; Ob: ≥30kg/m^2) in early pregnancy. Food diaries were used to assess free-living dietary intake in the 3rd trimester and serum glucose area under the curve was assessed following a 75g oral glucose test (OGTT).
Results: Women in the Ob group reported greater average 24-hour energy intake (3055 vs 2415 kcals, P<0.05). Late-night caloric intake was very prevalent with only one woman reporting no caloric consumption between 8pm and 6am on either two nights. For women in the Ob group only, results of a multiple linear regression analyses showed that night-time carbohydrate consumption was positively associated with post-OGTT glucose, and inversely associated with the ability of pancreatic β-cells to secrete sufficient insulin to compensate for reduced insulin sensitivity (P<0.05). Daytime carbohydrate intake was not associated with post-OGTT glucose or insulin action for either group.
Conclusions: If replicated, these results suggest that late-night carbohydrate intake may be a potential target for intervention to improve metabolic health of obese women in late pregnancy.

Poster Number 8

Thioredoxin-Interacting Protein Self-Induction: Role of ChREBP and AMP-Kinase

Junqin Chen, Gu Jing, Guanlan Xu and Anath Shalev

Comprehensive Diabetes Center and Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Alabama at Birmingham, Birmingham, AL 35294

Thioredoxin-interacting protein (TXNIP) has emerged as a regulator of key cellular processes involved in diabetes development and plays a critical role in pancreatic beta cell biology and glucose toxicity. We have previously found that high glucose increases TXNIP expression and elevated TXNIP induces beta- cell apoptosis; whereas TXNIP-deficiency protects against beta-cell apoptosis, and prevents type 1 and type 2 diabetes. We further reported that glucose-induced TXNIP expression is mediated by the carbohydrate response element-binding protein (ChREBP). Surprisingly, using our unique rat INS-1 beta cell line overexpressing human TXNIP as well as primary mouse islets and quantitative real-time RT-PCR, luciferase reporter studies and chromatin immunoprecipitation, we have now discovered that TXNIP stimulates its own expression by increasing ChREBP binding to the carbohydrate response element in its promoter. We further found that TXNIP reduced phosphorylation and increased nuclear translocation of ChREBP, key processes conferring ChREBP-mediated transcriptional activation of target genes. In contrast, AMP-activated protein kinase (AMPK) has been reported to promote phosphorylation and inhibition of ChREBP, suggesting that AMPK inhibition might play a role in the observed TXNIP effects on ChREBP. Indeed, we now found that AICAR (5-Aminoimidazole-4-carboxamide 1-β-D-ribofuranoside), an AMPK activator, blunted the TXNIP-conferred self-induction. Moreover, TXNIP overexpression in INS-1 beta cells decreased AMPK phosphorylation/activation, suggesting that TXNIP-induced TXNIP expression was mediated by inhibition of AMPK and the resulting decrease in ChREBP phosphorylation, increase in nuclear ChREBP translocation and enhanced TXNIP transactivation. Taken together, we have discovered a novel pathway that sheds new light onto the vicious cycle of beta cell glucose toxicity, increased TXNIP, and diabetes progression.

Poster Number 9

Antimicrobial peptide and mucin expression differ between the diabetic NOD mouse and non-diabetic NOR mouse

Joseph G Daft and Robin G Lorenz M.D, PhD

UAB Department of Pathology, UAB Comprehensive Diabetes Center

Type 1 Diabetes (T1D) is defined as the selective immune destruction of insulin producing beta cells within the islet. Alterations in the intestinal microbiota, increased intestinal permeability, and an aberrant immune system are thought to play key roles in the development of T1D. Recent studies have shown that mice that develop T1D have a different microbiota compared to mice that do not develop T1D. However why mice that are on the same diet in the same facilities have different microbiota is unknown. We hypothesize that altered antimicrobial peptide (AMP) and mucin production early in life determines ones microbiota, protecting some, but not others from T1D.
Non-obese diabetic (NOD) female mice were compared to age and sex matched Non-obese diabetic resistant (NOR) mice. AMP and mucin gene expression was measured in the ileum and colon of the two strains and protein expression was examined by immunohistochemistry. At 2 weeks of age there is a significant increase in the AMP, defcr-4, in the ileum of NOD mice compared to NOR mice. In addition a decrease in the mucins, Muc 1, 2, and 3 was measured in the colons of NOD mice compared to NOR mice. This correlates with alterations that we have measured in Bacteroidetes and Firmicutes between the two strains of mice. We postulate that these differences in AMP and mucin expression between the two strains leads to alterations in microbiota, which leads to differences in disease outcome.

Poster Number 10

Runx2 Inhibits Insulin Signaling Pathway and Adipocyte Differentiation

Min Ding, Farah Y. Ghori-Javed, Mitra Adhami, Haiyan Chen and Amjad Javed

Department of Oral and Maxillofacial Surgery, School of Dentistry, University of Alabama, Birmingham

Introduction: Alterations in insulin signaling pathways are associated with enhanced fat synthesis and a concomitant decrease in bone formation. Fat producing adipocytes and bone forming osteoblasts are derived from a common mesenchymal progenitor. Runx2 is essential for commitment and differentiation of mesenchymal cells (MC) to mineralizing cell types, but its roles in regulation of adipogenesis and insulin signaling is unknown.
Objective: To investigate the role of Runx2 in regulation of adipocyte differentiation and insulin signaling pathway.
Methods: Biochemical, cellular and molecular approaches were used for this study.
Results: The Runx2-null MC exhibited a preferential commitment to the adipocyte lineage instead of osteoblast. Robust activation of both early and terminal marker genes and adipocyte formation were observed upon stimulation with low dosage of PPARγ agonist rosiglitazone. Surprisingly, insulin strongly inhibited adipocyte differentiation in Runx2-null MC, which is in sharp contrast to the well-established requirement of insulin for the 3T3L1 adipocyte differentiation. This paradoxical effect of insulin in the Runx2-null MC was associated with a selective utilization of exon 11 splice variant of the insulin receptor, and impaired internalization of the activated insulin receptor. Western blotting data shows that Akt and ERK pathways were also differentially engaged in Runx2-null versus 3T3-L1 adipocytes. Reconstitution of Runx2-null MC by Runx2 inhibited rosiglitazone-stimulated adipocyte differentiation, including both the cell number and size of the lipid droplets. Interestingly, Runx2 reconstitution also completely inhibited expression of both Akt and ERK expression. Thus Runx2 may inhibit adipocyte differentiation via negative regulation of the Akt and ERK pathway.
Conclusions: Runx2 inhibits adipocyte differentiation by regulating the insulin and ERK/Akt signaling pathways.

Adipocyte morphology of subcutaneous and visceral fat depots is associated with type 2 diabetes mellitus in humans

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Aims/hypothesis: Regional deposition of adipose tissue, adipocyte cell size and cell size distribution may contribute to increased risk for insulin resistance. The aim of this study was to compare adipocyte cell size and size distribution from multiple fat depots and to determine the association with type 2 diabetes mellitus, anthropomorphic data, and subjects' metabolic profile.
Methods: Clinical data and adipose tissue from subcutaneous fat, omentum, and mesentery were collected from thirty subjects with morbid obesity. Adipocytes were isolated by collagenase digestion and sized by microscopic measurement of cell diameter.
Results: Overall, adipocytes from subcutaneous fat were larger than those from omentum or mesentery. For the subcutaneous and omental fat depots, there was a significant increase in % small cells (14.9% vs 31.4%, p=0.006 and 14.0% vs 30.5%, p=0.015, respectively) and corresponding decrease in % large cells for nondiabetic vs diabetic patients. There was a similar trend for mesentery but it did not reach statistical significance (p=0.090). For omentum and mesentery, there was also a significant decrease in the diameter of the small cells. Fasting glucose was positively correlated with fraction of small cells in omentum and mesentery, and HbA_{1C} was positively correlated with fraction of small cells in the omental fat depot. There was no correlation between large cell diameter with clinical parameters in any of the fat depots.
Conclusions/interpretation: These results indicate size distribution of adipocytes, specifically an increase in the fraction of small cells, is associated with the presence of type 2 diabetes mellitus.

Poster Number 12

miR-200b Increases Beta Cell Apoptosis and is Induced by TXNIP

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Beta cell death is a prominent feature of both type 1 and type 2 diabetes and we have found that Thioredoxin Interacting Protein (TXNIP), plays a critical role in this process. Recently, we also discovered that TXNIP regulates beta cell microRNAs (miRs) and a miR microarray revealed an increase in members of the miR-200 family.
In the present study, we demonstrate that TXNIP positively regulates beta cell levels of the miR-200 family members, miR-200a, miR-200b, miR-200c, miR-141, and miR-429. These results were found in INS-1 beta cells constitutively overexpressing TXNIP and in primary mouse islets, and show that TXNIP deficient HcB-19 mice have decreased levels of miR-200 family members as measured by qPCR. In contrast, islets of obese diabetic B6 ob/ob mice, which have increased TXNIP levels, also exhibit an up-regulation in their expression of miR-200 family members.
Furthermore, we determined that overexpression of miRNA-200 family members, and in particular miR-200b, caused apoptosis in INS-1 beta cells as assessed by Bax/Bcl2 mRNA ratio, cleaved caspase-3 protein levels, and terminal deoxynucleotidyl transferase dUTP nick end labeling. We also investigated which downstream targets of miR-200 may cause an increase in beta cell death. We found that one potential target, Zinc finger E-box binding homeobox 1 (Zeb1) mRNA was decreased in response to overexpression of members of the miR-200 family in INS-1 beta cells. Similar results were also found at the protein level, as measured by Western blot. We also discovered that knocking down Zeb1 using small interfering RNA induced apoptosis as assessed by an increase in cleaved caspase-3 demonstrating that Zeb1 down-regulation can mimic the effects of miR-200 on beta cell apoptosis.
Taken together, the results of this study indicate that TXNIP causes an increase in the beta cell expression of miR-200 family members, that these miRs increase beta cell apoptosis, and that this may be mediated through the targeting of Zeb1.

Poster Number 13

Race-Sex Disparities in Statin Use Among US Adults with Diabetes: the REGARDS Study

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Background: The Look Ahead trial recently showed that lifestyle intervention did not lower the risk of coronary heart disease (CHD) events in diabetes, emphasizing the importance of risk factor control. This is particularly important for blacks, who have higher risks of CHD mortality than whites. We examined race-sex differences in the statin use in a large national biracial cohort with diabetes.
Methods: REGARDS recruited 30,239 black and white participants ≥ 45 years of age from the 48 continental US from 2003-2007. This analysis included participants with physician/nurse diagnosed diabetes or treatment with diabetes medication or insulin. We described the prevalence of statin use and calculated prevalence ratios and 95% confidence intervals comparing white men (WM) with other race-sex groups, adjusted for factors that influence health services use (sociodemographics, access to care, severity of diabetes, functional status, obesity, depressive symptoms, and elevated high density lipoprotein cholesterol).
Results: The race-sex distribution of the 6,497 participants with diabetes is shown in the Table. Compared to WM (56%) all other race-sex groups were less treated (WW 47%, BM 44%, BW 45%) Results were similar after adjustment, but showed that healthcare utilization factors account for much of the difference in statin use between black men and white men, but the lower statin use among women compared to white men is not explained (Table).
Conclusion: These disparities in statin use among people with diabetes warrant further investigation, particularly among blacks, who are especially burdened by diabetes.

Table. Prevalence ratios (95% CI) for statin use among participants with diabetes in REGARDS, n = 6,497.

	Race-Sex group			
	White Men (n = 1,611)	**White Women (n = 1,220)**	**Black Men (n = 1,410)**	**Black Women (n = 2,256)**
Model 1 - Pre-disposing factors†				
Adjusted‡	1 (ref)	0.85 (0.79, 0.92)	0.79 (0.73, 0.85)	0.81 (0.76, 0.86)
Model 2 - Pre-disposing + Enabling factors‡				
Adjusted	1 (ref)	0.86 (0.80, 0.92)	0.81 (0.75, 0.87)	0.83 (0.77, 0.89)
Model 3 Pre-disposing + Enabling + Need factors††				
Adjusted	1 (ref)	0.87 (0.82, 0.92)	0.94 (0.88, 1.00)	0.88 (0.83, 0.93)

† Model 1 adjusts for pre-disposing factors (race-sex group, age, income, education, REGARDS region).
‡ Model 2 adjusts for pre-disposing factors and enabling factors (health insurance, poverty, and rural residence).
†† Model 3 adjusts for pre-disposing, enabling, and need factors (smoking, obesity, depression, elevated HDL-C, systolic BP, awareness of hyperlipidemia, Physical Component Score, history of CHD, and diabetes severity).

Poster Number 14

Cardiometabolically Healthy Obese and Incident Diabetes, CHD, and Mortality

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Obesity is a chronic disease that affects 35% of US adults. There has been an escalating debate as to whether a proportion of obese individuals are free of cardiometabolic disease, termed the 'metabolically healthy obese' (HO). Here, we assessed prevalence of HO in US adults, and its association with incident diabetes, CHD, stroke, and mortality using data from 3 large cohorts, CARDIA, ARIC, and NHANES. First, subjects who were obese at baseline remained obese; in CARDIA young adults (mean 25y) only 1.5% became lean at year 20, and in older adults in ARIC (mean 54 y) only 0.4% became lean after 10 years. Secondly, the percentage of HO adults was substantial; in NHANES the % HO remained fairly constant averaging 16% from 1988-2012. Thirdly, risk factors were more predictive of outcomes than obesity. During 18.7 years follow-up in ARIC, compared with HO, the multivariable adjusted hazard ratio in UHO was increased for diabetes (5.6, 95% confidence interval, CI 4.1-7.5), CHD (6.6, CI 3.4-12.8), stroke (5.2, CI 2.1-12.8), and mortality (2.6, CI 1.9-3.6). Among healthy subjects, HRs were similar among HO, overweight, and lean for CHD, stroke, and mortality, but were decreased for diabetes in lean (0.5) compared with HO. Among unhealthy, HRs for diabetes, CHD, stroke, and mortality were comparable among UHO, overweight, and lean. **In conclusion**, (i) obese people rarely become lean; (ii) HO prevalence has been stable in the US at ~16%; (iii) Metabolic syndrome risk factors confer much higher risk of diabetes and CVD than obesity.

Grant support: VA Merit Review, UAB DRC (P30-DK079626), NIH DK-038765 and DK-083562

Poster Number 15

Fibroblast Growth Factor 21 Mediates The Beneficial Effects Of Exercise On Diet-Induced Glucose Intolerance

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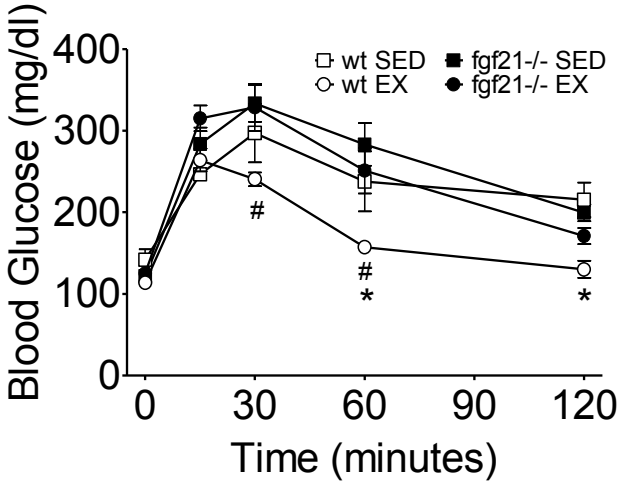
Exercise is a well-defined preventative and therapeutic intervention against metabolic syndrome. Yet, the molecular pathways underlying the beneficial effects of exercise are unclear. Recent evidence indicates that glucagon signaling in the liver plays an important role in the metabolic benefits associated with exercise. We have previously shown that fibroblast growth factor 21 (FGF21) mediates some of the beneficial metabolic effects of glucagon. Thus, we aimed to test the role of FGF21 in mediating benefits of chronic exercise on glucose and lipid metabolism. Wildtype (*wt*) and FGF21-deficient (*fgf21*$^{-/-}$) mice were fed high-fat diet (HFD) and allowed access to inoperable (sedentary) or operable (exercise) running wheels for 12 weeks. Exercise was effective in suppressing body weight and fat mass gain, and improving plasma lipidemia in *wt* and *fgf21*$^{-/-}$ mice. Exercise likewise improved glucose tolerance in *wt* mice; however this effect was lost in *fgf21*$^{-/-}$ mice. We identified that *fgf21*$^{-/-}$ mice exhibited reduced adaptations to chronic exercise in skeletal muscle. Exercise stimulated a notable increase in AMPK activation in skeletal muscle of *wt* mice, but strikingly, not in that of *fgf21*$^{-/-}$ mice. These results clearly show that systemic FGF21 activity is required for exercise benefits on glucose tolerance by stimulating AMPK activation in skeletal muscle. (This was also a talk in Session II.)



Poster Number 16

Acute Glucagon-Receptor Agonism Enhances Rodent Insulin Action

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Glucagon is the key counter-regulatory hormone opposing insulin action. The best-known actions of glucagon are stimulation of hepatic glycogenolysis and gluconeogenesis, while simultaneously inhibiting glycogen synthesis. In marked contrast to these classic effects, we recently found that daily treatment of db/db mice with a potent, long acting, and selective glucagon-receptor (GcgR) agonist decreased body weight, and enhanced the rate of glucose disappearance (k_g) during an insulin tolerance test. These novel and non-canonical effects were evaluated following acute, and therefore bodyweight independent, GcgR agonism. Mice receiving IUB288 displayed the predicted immediate increase in blood glucose. However, this initial hyperglycemia was cleared within 60 minutes and blood glucose returned to basal levels. Treated animals had significantly improved glucose tolerance in response to a subsequent IP glucose challenge. To assess the role of insulin action on the enhanced glucose tolerance after GcgR agonism, we treated mice with varying doses of the compound followed 60 min later by an intra-peritoneal insulin injection. GcgR agonism improved insulin sensitivity, with a significant increase in k_g in both lean and diet-induced obese mice and rats. Administration of a fixed dose of GcgR agonist (10 nmol/kg) enhanced glucose disappearance and skeletal muscle glucose uptake following doses of insulin that did not cause a reduction of blood glucose in untreated animals. Circulating c-peptide was un-changed 60 min after GcgR agonism, suggesting that glucagon-stimulated insulin secretion was not responsible for the enhanced rate of glucose disappearance and overall insulin action. Taken together, these data reveal for the first time that GcgR agonism stimulates enhanced glucose tolerance that is mediated in part by enhanced insulin action. This heretofore-unappreciated aspect of glucagon biology has implications for the use of GcgR agonism in therapeutic strategies for diabetes.

Poster Number 17

O-GlcNAcylation of Runx2 is Essential for Vascular Calcification

Jack Heath, Xia Mao, Lu Huang, Yong Sun, Liang Deng, Yabing Chen

Osteogenic differentiation of vascular smooth muscle cells (VSMC) contribute predominantly to vascular calcification, a characteristic of advanced atherosclerosis, chronic kidney disease, and diabetes. Using Runx2 shRNA and SMC-specific Runx2 deletion mice, we have demonstrated an essential role of Runx2 in regulating vascular calcification in vitro and in vivo. The present studies aim to pinpoint the Runx2 functional domain that is critical for its osteogenic function in VSMC and to elucidate the underlying molecular mechanisms.

A series of Runx2 deletion mutants were generated and stably transfected into VSMC. Full-length Runx2 protein and Runx2 deletion mutants containing amino acids 1-495 or 1-432, but not Runx2 1-391, induced VMC calcification, indicating that the Runx2 osteogenic functional domain is located between amino acids 391-432. Site-directed mutagenesis analysis identified that mutations at the amino acids 412, 413, 425 and 426-428 markedly reduced Runx2 transactivity and inhibited Runx2-induced VSMC calcification. Mutations at amino acids 412, 413 and 425 were found to inhibit Runx2 post-translational modification by O-GlcNAcylation. Immunoprecipitation revealed that Runx2 O-GlcNAcylation at 412, at 413, and proximal to 425 was critical for its binding to Smad 1/5/8 and Smad 4, but not Smad 2/3. Using lentivirus-mediated shRNA, we determined that knockdown of Smad1/5/8, but not Smad 2/3, inhibited Runx2-induced VSMC calcification, suggesting that BMP but not TGF-beta signaling is critical for the osteogenic function of Runx2 in VSMC. Taken together, these data demonstrate that Runx2 amino acids 412, 413, 425 and 426-428 are key for its osteogenic function in VSMC, and Runx2 O-GlcNAcylation at 412, 413 and 425 is essential for its interaction with Smad proteins to determine Runx2 transactivity and VSMC calcification.

We have identified a novel Runx2 post-translational modification by O-GlcNAcylation at key residues that are essential for its osteogenic function. Our studies provide molecular insights into the function of Runx2 in regulating VSMC calcification, which may shed light on novel targets that are amenable to drug discovery for vascular calcification.

Poster Number 18

IL-1beta Regulates Beta-cell TXNIP Expression

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In type 1 diabetes the autoimmune reaction and the release of proinflammatory cytokines (interleukin (IL)-1beta, tumor necrosis factors (TNF) alpha, and interferon (IFN) gamma) leads to beta-cell destruction and apoptosis. Recently, we identified thioredoxin-interacting protein (TXNIP) as an important regulator of beta-cell apoptosis and found that its deletion prevented type 1 and type 2 diabetes. TXNIP is highly induced by glucose via the carbohydrate response element binding protein (ChREBP), but the effects of cytokines on TXNIP expression have remained largely unknown. We therefore cultured INS-1 beta-cells with a cocktail of IL-1beta (1 ng/ml), TNFalpha (5 ng/ml) and INFgamma (5 ng/ml) under low (5 mM) and high (25 mM) glucose conditions and measured TXNIP by quantitative real-time RT-PCR. Cytokine cocktail treatment increased TXNIP expression at 5 mM, but not at 25 mM glucose. Interestingly, we found that this discrepancy was due to IL-1beta, which up-regulated TXNIP mRNA at 5 mM glucose, but down-regulated TXNIP at 25 mM glucose. To investigate the molecular mechanism involved in this surprising IL-1beta effect at high glucose, we performed luciferase assays using INS-1 beta-cells transfected with a TXNIP promoter-driven reporter plasmid containing different truncations and mutations. Consistent with the observed decrease in TXNIP mRNA, a 6.7-fold reduction in TXNIP promoter activity was observed with IL-1beta treatment at 25 mM glucose. Mutation of the E-box ChREBP binding site in the TXNIP promoter blunted this effect, indicating that IL-1beta may inhibit TXNIP expression through ChREBP. Indeed, IL-1beta treatment resulted in a decrease in ChREBP nuclear localization and ChREBP binding to the TXNIP promoter at 25 mM glucose as assessed by nuclear fractionation and ChREBP chromatin immunoprecipitation (ChIP). Taken together, these results reveal for the first time that cytokines have differential effects on pro-apoptotic beta-cell TXNIP expression and that the effects are dependent on the concomitant glucose concentration.

Ldb1-Mediated Transcriptional Complexes During β-cell Development and Function

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Transcription factors are critical for regulating genes involved in pancreatic endocrine cell fate determination and mature beta-cell function. Islet biologists are developing strategies to produce transplantable beta-cells *de novo* that improve diabetes outcomes. Success will require a clear understanding of endogenous gene regulatory mechanisms that drive functional beta-cell development. This includes continued studies of islet-enriched transcription factors plus increased appreciation for interacting coregulators. We have shown that the LIM-homeodomain Islet-1 (Isl1) transcription factor is required for late islet endocrine cell development and survival, which largely requires interaction with the LIM domain binding protein 1 coregulator, or Ldb1 [1-3]. In other tissues, Ldb1 and Isl1 form complexes with additional factors to impact target gene transcription, yet very few binding partners have been described in beta-cells. To address this, I utilized a crosslinked-immunoprecipitation/mass spectrometry approach, termed ReCLIP [4], to enrich and identify proteins directly interacting with Ldb1 and Isl1. Mass spectrometry datasets revealed numerous interacting candidates that may participate in beta-cell Ldb1::Isl1 complexes, including a class of Single-Stranded DNA Binding Proteins (e.g. SSBP2-4). SSBPs are coregulators that potentiate Ldb1::LIM factor target gene *trans*-activation and complex stability in other tissues [5, 6]. However, nothing is known of SSBP expression or activity in pancreatic islets. Western blotting and immunofluorescence analyses demonstrated that at least SSBP3 appeared to be enriched in beta-cell lines, developing endocrine cells and in mouse islets. Future studies will enlist beta-cell lines and pancreas tissue to test the significance of SSBP3 (and other) coregulators to the regulation of Ldb1 or Ldb1::Isl1 target genes, including *MafA*, *Glut2*, *Glp1r* and *Insulin*. I will also examine SSBP occupation of target promoters by ChIP, perform reporter gene experiments testing SSBP activity, plus analyze SSBP impact on Ldb1::LIM stability in beta cells. This project will further elucidate the components and activity of Ldb1::Isl1 complexes, which are required for beta-cell development, maturation and function.

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Poster Number 20

The Development of a Bio-Inspired Hybrid Nanosack for Enhancing the Efficacy of Pancreatic Islet Transplantation in the Omentum

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The omentum is an attractive transplantation site in pancreatic islet transplantation (PIT) as it allows a large implantation volume, ease of surgical manipulation, and some immune privilege. However, revascularization is one of major challenges at the omentum site due to its low vascularity. Fibroblast growth factor-2 (FGF-2) has shown to be a good mitogen for endothelial cells and stimulate angiogenesis. So, the controlled release of FGF-2 in the omentum could enhance islet revascularization. In this study, to enhance the efficacy of pancreatic islet transplantation in the omentum, a hybrid nanosack was developed with a combination of a self-assembled peptide amphiphile (PA) nanomatrix gel capable of encapsulating islets with a nurturing microenvironment and a poly (ε-caprolactone) electrospun (ePCL) nanofiber sheet with porous crater-like structures for blood vessel infiltration and a mechanically stable for surgical manipulation. Crater-like structures were fabricated by gas foaming/salt leaching technique, which allowed vein endothelial cell penetration throughout the ePCL nanofiber sheet. The release profile of FGF-2 from the hybrid nanosack was assessed by ELISA assay, and showed a multi-stage FGF-2 release kinetics; an initial burst release followed by a sustained release. To evaluate whether the hybrid nanosack could enhance angiogenesis in the omentum, the hybrid nanosack was implanted within the rat omentum. After two weeks, an angiogenesis was observed surrounding the hybrid nanosack in the omentum, analyzed by micro-CT. These results demonstrate that the hybrid nanosack enhances vascularization in the omentum. Therefore, this hybrid nanosack will provide great potential to improve islet engraftment at the omentum.

Poster Number 21

TXNIP Induces IAPP Expression via miRNA-124a and FOXA2

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Islet amyloid polypeptide (IAPP), also known as amylin, is a regulatory peptide that is co-expressed and co-secreted with insulin by beta-cells. It tends to aggregate into insoluble amyloid fibrils and smaller non-fibrillar oligomers that are toxic and strongly associated with the progressive loss of pancreatic beta-cell mass in type 2 diabetes. This makes IAPP a potential target and critical factor in the development of type 2 diabetes. However, while glucose and PDX1 are known to induce IAPP transcription, the regulation of IAPP expression is still not fully understood. Interestingly, in our human islet microarray analysis, we not only found a marked induction of IAPP, but also a dramatic increase in thioredoxin-interacting protein (TXNIP) expression in response to glucose. We then went on to show that TXNIP also plays a major role in beta-cell apoptosis and beta-cell loss of diabetes and most recently discovered that TXNIP inhibits insulin transcription and regulates beta-cell function through miR-204. This raised the question whether TXNIP might also be involved in the upregulation of IAPP. In fact, using INS-1 cells, primary mouse and human islets, and beta-cell-specific *Txnip* knockout mice, we found that TXNIP induces beta-cell IAPP expression both *in vitro* and *in vivo*. Our studies further revealed that TXNIP regulates IAPP expression by inducing the expression of a specific transcription factor, forkhead boxA2 (FoxA2) and promoting FoxA2 enrichment at the *IAPP* promoter as assessed by real-time RT-PCR, Western blotting and chromatin immunoprecipitation. Moreover, we also determined the mechanism by which TXNIP induces FoxA2 expression and found that a microRNA, miR-124a, is downregulated by TXNIP and directly targets *FoxA2*. Thus, our findings have identified a novel gene regulatory system implicating TXNIP, miR-124a and FoxA2 in the control of IAPP expression and thereby shed new light onto this important pathway.

Poster Number 22

Toll-like Receptor 4-Induced Endoplasmic Reticulum Stress Contributes to Endothelial Dysfunction

Hyun-Ju Jang[1] and Jeong-a Kim[1, 2, 3]

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Impairment of vasodilator action of insulin is associated with endothelial dysfunction and insulin resistance. Our previous reports demonstrate that saturated fatty acids promote pro-inflammatory responses that contribute to endothelial dysfunction and insulin resistance. Endoplasmic reticulum (ER) stress is implicated as one of the mechanisms for pathophysiology of various cardiometabolic syndromes. Because toll-like receptor 4 (TLR4) plays an important role in pro-inflammatory response and ER stress, we examined whether TLR4-induced ER stress is involved in saturated fatty acid (SFA)-induced endothelial dysfunction. Treatment with SFA (palmitate) stimulated pro-inflammatory responses and ER stress, which was suppressed by knock-down of TLR4 in primary human aortic endothelial cells (HAEC). Then, we examined the role of TLR4 in vasodilatory responses in intact vessels isolated from wild type (WT, C57BL/6) and TLR4-KO mice after feeding high fat (HFD) or normal chow diet (NCD) for 12 weeks. Arterioles isolated from HFD WT mice showed the impairment of vasodilation in response to insulin compared to the arterioles isolated from NCD WT type mice. Interestingly, the arterioles isolated from HFD TLR4-KO mice did not show the impairment of insulin-stimulated vasodilation. There were no differences in acetylcholine (Ach)-, or sodium nitroprusside (SNP)-stimulated vasodilation between groups. Next, we examined whether ER stress is involved in SFA-induced impairment of vasodilator actions of insulin. Infusion of palmitate showed the impairment of vasodilatory response to insulin, which was ameliorated by co-infusion with tauroursodeoxycholic acid (TUDCA), an ER stress suppressor. The results suggest that TLR4-induced ER stress may contribute to the SFA-mediated endothelial dysfunction.

Poster Number 23

Cellular Bioenergetics and Bioenergetic Health Index as Potential Biomarkers of Chronic Kidney Dysfunction

Tanecia Mitchell[1], Leslie Jackson[2], Philip Kramer[1], Balu K. Chacko[1], Michelle Johnson[1], Saranya Ravi[1], Dana Rizk[2] and Victor Darley-Usmar[1]

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Diabetes is a systemic disease associated with severe metabolic and bioenergetic dysfunction in a broad range of tissues and can cause chronic kidney disease (CKD), where renal function declines over time. Patients with CKD have an increased risk of developing infections, cardiovascular diseases, and end stage renal disease. The objective of this study was to evaluate mitochondrial function in circulating monocytes from CKD patients and to investigate whether these cells could be predictors of bioenergetic health and/or CKD progression. To test this, monocytes were isolated from peripheral blood from twenty-six healthy volunteers and sixteen individuals diagnosed with Type 2 Diabetes and CKD with an estimated glomerular filtration rate of 30-45 ml/min/1.73 m^2. The cellular bioenergetic patterns, oxidative burst, and bioenergetic health index of monocytes were determined using the Seahorse XF96 Extracellular Flux Analyzer. Monocytes from CKD patients had decreased maximal mitochondrial respiration and reserve capacity compared to healthy volunteers. In addition, the monocytic oxidative burst and bioenergetic health index were significantly lower in CKD patients. These findings suggest that the bioenergetic health of monocytes in CKD patients are compromised compared to healthy volunteers and could be a potential biomarker to monitor CKD progression or the bioenergetic health of an individual. Understanding these mechanisms could be useful in CKD patient management and help delineate potential therapeutics to mitigate these affects. (T32 HL007457, DK076169)

Poster Number 24

Mitochondrial Fission may be a Crucial Factor in Diabetes-Cancer Association

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The association between type 2 diabetes (T2D) and cancer has emerged from epidemiological studies but the causal link is not clear. Two mutually exclusive possibilities have been proposed: a) hyperglycemic situation in diabetes provides proliferative advantage to the cancer cells which prefer glucose as carbon source; b) the systemic hyperinsulinemia induced by the insulin resistant diabetic state boosts insulin dependent cell proliferation in tumors. Insulin dependent glucose metabolism can be altered by mitochondrial structural changes while hyperglycemia can in turn directly affect mitochondrial structure-function. The steady state mitochondrial structure is dictated by the balance of the mitochondrial fission and fusion events regulated by specific proteins. Using a mosaic strategy in *Drosophila* we have previously shown that the loss of the mitochondrial fission protein DRP1 causes aberrant cell proliferation in an epithelial cell layer. Now we have demonstrated that a) DRP1 loss elevates pAKT levels, indicative of elevated insulin signaling, in the aberrantly proliferating cells; b) high sugar diet induced insulin resistance and hyperinsulinemia attenuates the aberrant cell proliferation in the DRP1 null non-stem clones but fails to do so in the DRP1 null stem cell clones. Our results indicate that the effect of insulin resistance and hyperinsulinemia on any oncogenic mutation may depend whether the mutation initially arises in stem or in non-stem population of a differentiated tissue. This conception has profound implications in understanding the association of T2D and cancer. We are currently pursuing the mechanism behind the effect of DRP1 loss in stem and non-stem populations.

Poster Number 25

Modulation of Autoimmune Diabetes By Innate-Like B Cell Derived Antibodies Against N-Acetyl Glucosamine

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The immunodominant epitope of Group A Streptococcus (GAS) is the cell wall associated N-acetyl-Glucosamine (GlcNAc) of Group A Carbohydrate (GAC). Neonatal GAS exposure has been demonstrated to protect in multiple murine models of Type 1 Diabetes (T1D), and human cases of T1D have been negatively correlated with cases of Scarlet fever in several studies. Through serum-transfer experiments from GAS-immunized to naïve NOD mice, we have found that T1D protection elicited by GAS exposure is conferred by the antibody response to GAC. Anti-GAC antibodies recognize common GlcNAc components of mammalian glycan chains and are consequently highly reactive with epitopes from pancreatic islets of several species, including human. Epitopes recognized by GAC-specific IgM include a subset of, likely immature, insulin-containing granules; this post-translational modification (PTM) is therefore associated with granules containing multiple T1D-related auto antigens. We have found that anti-GAC antibodies are highly reactive with apoptosis-associated beta cell neoepitopes in mice, and with immature human pancreatic glycans, through open source data available to us through the Consortium for Functional Glycomics. Through in vitro and in vivo studies, we have discovered that GlcNAc-modified autoantigens generated during beta cell apoptosis drive lectin-dependent complement pathway initiation, and we find that GlcNAc-specific antibodies modulate activation of the complement pathway on dying beta cells. Opsonization of apoptotic-beta cell neoeptitopes reduces the capacity of dendritic cells to activate diabetogenic T cells with beta cell derived antigen, and protects from T1D development. In our preliminary studies with T1D patients, we have observed that T1D patients possess significantly lower levels of GAS- but not GlcNAc-reactive IgM antibodies, suggesting the involvement of particular natural anti-GlcNAc antibody-producing clonotypes in T1D prevention. Subsets of anti-GlcNAc antibodies, may serve as biomarkers for identifying at risk-patients whose repertoire may lack these idiotype positive antibodies. Furthermore, boosting this idiotype may serve as a therapy to protect at risk patients from developing T1D.

Poster Number 26

Low HDL Cholesterol may Lead to Disruption of Bone Remodeling in Obese Early Pubertal Girls

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Background: Cardiovascular risk factors and compromised bone phenotype are apparent in obese children. Identification of cardiovascular-bone linking factors is warranted. The objective is to determine the association between circulating factors of lipid metabolism and bone turnover markers, as well as qualitative and quantitative bone measures in obese early-pubertal females.
Methods: 43 peri-pubertal overweight/obese girls aged 7-11 years who underwent DXA to measure fat mass and BMC, as well as pQCT to measure qualitative bone parameters were included. Lipids (TC, HDL, LDL, HDL), bone turnover markers (CTX, P1NP), and inflammatory cytokines (TNFα, IL-6) assessed from venipuncture.
Results: TC, LDL, and HDL were negatively associated with total and trabecular area at 4% radial length ($p \leq 0.05$). HDL was positively associated with P1NP ($p<0.01$), total and trabecular area at 66% radial length, yet negatively associated with total and trabecular density ($p \leq 0.05$). Stratification by HDL level revealed significant differences in total and trabecular area at 66% radial length between normal- and low-HDL groups. TNFα and IL-6 contributed to the observed differences ($p \leq 0.05$).
Conclusion: The findings herein indicate an influential effect of the lipid and inflammatory profile on bone formation and expansion in overweight/obese girls, which may account for increased risk of fracture in this population.

Poster Number 27

Obesity Induced Insulin Resistance Contributes to Increased Tumor Growth in a Mouse Model of Human Colon Cancer

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Epidemiological evidence suggests that obesity is linked with colon cancer incidence. Here, we assess the effects of obesity on tumor xenograft growth and examine potential mechanisms driving obesity-linked tumor growth in a novel mouse model of human cancer.

Homozygous Rag1^{tm1Mom} (Rag1) mice were used and fed either a low fat western diet (LFWD) or a high fat western diet (HFWD). In one group, mice were assigned to either diet for 4 weeks, human colon cancer xenografts implanted subcutaneously, harvested after 28 days, and metabolic changes associated with insulin resistance determined. In a second group, mice were placed on either diet for 8 weeks; human colon cancer xenografts were then implanted orthotopically and harvested after 21 days.

Rag1 mice fed a HFWD weighed more, had increased intra-abdominal fat with progressive impairment of both glucose and insulin tolerance. In the subcutaneous model, tumor growth rate and final tumor weight from HFWD fed mice were both significantly greater than in the LFWD mice. Tumor-bearing mice fed a HFWD displayed hyperinsulinemia and increased free fatty acid (FFA) levels, with a significant positive correlation between tumor weight and insulin levels and a positive correlation between insulin to FFA level and tumor weight while a negative correlation between insulin sensitivity and tumor weight was observed. In the orthotopic model, final tumour weights were significantly greater in the mice rendered obese by a HFWD.

These results suggest that diet-induced obesity-linked insulin resistance stimulates human colon tumor growth in a novel obesity model of human colon cancer.

NADPH Oxidase-Deficient Macrophages Display a Dampened M1 Macrophage Phenotype in Type 1 Diabetes

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Macrophages are indispensible in the pathogenesis of Type 1 diabetes (T1D), an autoimmune disease characterized by the destruction of insulin-secreting pancreatic beta-cells. As one of the first islet-infiltrating cells, macrophages present antigen to diabetogenic T cells and generate reactive oxygen species (ROS) and pro-inflammatory cytokines to directly lyse pancreatic beta-cells. In contrast to the pathogenicity of M1 macrophages, alternatively activated M2 macrophages, which generate immunosuppressive cytokines, protect Non-Obese Diabetic (NOD) mice against spontaneous diabetes and represent a promising cellular therapy in T1D; however, the environmental cues that govern macrophage polarization remain largely unknown. Our laboratory previously demonstrated the importance of NADPH oxidase (NOX)-derived ROS in T1D pathogenesis, as NOD mice deficient in NOX-derived superoxide (*Ncf1*^{m1J}) were protected against spontaneous T1D due to blunted pro-inflammatory cytokine and Type I interferon synthesis with Toll-like receptor (TLR) agonist stimulation. Thus, we hypothesized that ROS was essential for M1 macrophage differentiation; therefore, ROS ablation would induce a dampened M1 and/or elevated M2 phenotype. Analysis of sera from 16-week-old female NOD.*Ncf1*^{m1J} mice revealed a 15-fold reduction in CCL5, a marker of pro-inflammatory M1 macrophages (p<0.001), concomitant with 3-fold enhancement in CCL17, an M2 marker, compared to sera from age- and sex-matched NOD mice (p=0.0036). The islet-resident macrophage profile of 16-week female NOD.*Ncf1*^{m1J} mice indicated a significant (p<0.01) dampening in M1 macrophage transcripts, such as *Cxcl10* (2-fold), *Ccl5* (4-fold), *Tnfa* (8-fold), and *Nos2* (8-fold) concomitant with a significant (p<0.01) elevation in M2 macrophage transcripts, including *Ccl17* (3-fold), *Retnla* (5-fold), *Arg1* (1.5-fold), and *Cd206* (2.5-fold). To further define the role of NOX-derived superoxide on macrophage differentiation, NOD.*Rag.Ncf1*^{m1J} mice were transferred with diabetogenic BDC-2.5 CD4 T cells, which destroy beta-cells by recruiting pro-inflammatory M1 macrophages into the pancreas of recipient mice. A 2-fold dampening in pro-inflammatory M1 macrophages at 5 days, concomitant with a 3-fold elevation in anti-inflammatory M2 macrophages was observed in BDC-2.5-transferred NOD.*Rag.Ncf1*^{m1J} mice compared to NOD.*Rag* mice. Interestingly, treatment of NOD.*Rag* and NOD.*Rag.Ncf1*^{m1J} BDC-2.5-transferred recipients with a potent catalytic antioxidant induced a 3- and 2-fold elevation in M2 macrophages within NOD.*Rag* and NOD.Rag.*Ncf1*^{m1J} pancreata, respectively. These results provide evidence that NOX-derived superoxide is essential for a pro-inflammatory M1 macrophage differentiation; thus, targeting macrophage redox status may represent a promising therapy in halting T1D.

Poster Number 29

Temporal Trends and the Analysis of Community-Based Trials

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Context: There is a growing interest in real-world trials of community-based interventions. However, the lack of control inherent in such settings may create issues relating to temporal trends that are not commonly discussed in reporting the results of such studies.
Methods: The ENCOURAGE trial assessed whether education and volunteer community-based peer coaching was more effective at improving diabetes outcomes than education alone. The trial was set in Alabama's 'Black Belt', a predominantly rural and low-income region without efficient centralized health-care delivery. Study participants were randomized at the community level to minimize contamination and because the communities were scattered across a considerable geographic area, recruitment, baseline and follow-up data collection took place one community at a time, with baseline and followup data collection planned to be over approximately one year.
Results: Multiple significant time trends emerged during analysis: 1) secular trends over time; 2) seasonal variation in some biometrics such as HbA1c and BMI; 3) actual time between baseline and follow-up, which varied between 11 and 40 months. The latter is particularly important for the intervention group because any intervention effect is expected to begin, reach a maximum and then decrease. When analyses incorporated adjustment for these 3 temporal characteristics, we observed an effect in the intervention group that was both significant ($p<0.05$) and non-linearly associated with the time between baseline and follow-up; the trial would be interpreted as negative without these adjustments.
Conclusions: In real-world trials where recruitment and follow-up schedules may not occur as designed, complex temporal issues may greatly influence the analysis. Ongoing and future trials can minimize this potential temporal confounding if recruitment and follow-up times are monitored and balanced between study arms.

Poster Number 30

Evaluating Glucose Metabolism in C57BL6/J and C3H/HeN Mice: Implications for Interpretation of Glucose and Insulin Tolerance Data

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According to the scientific literature C57BL6/J mice are susceptible to high fat diet (HFD) induced insulin resistance while C3H mice remain relatively insulin sensitive. This study aimed to confirm this phenotypic difference between the strains by evaluating basal levels of glucose and insulin and conducting measurements of systemic glucose and insulin sensitivity before and after 12 weeks of chow or HFD feeding. Results show that after 12 weeks of HFD, C57 mice have impaired glucose tolerance and insulin sensitivity as reported previously in the literature. However, C3H mice also appeared to have impaired insulin sensitivity, being non-responsive to the insulin administration, despite remaining glucose tolerant. Consequently we determined serum glucose and insulin levels in both C57 and C3H mice and found the latter to have significantly higher levels of insulin and lower levels of glucose compared to the former. Hence, we conclude that the methods for determining relative glucose and insulin tolerance between strains may need to be revisited because basal differences appear to exist between these two strains under both chow fed and HFD fed conditions. For example, despite having different blood glucose and serum insulin levels under both chow and HFD conditions, typically the same dose of glucose (1- 3g/kg) and insulin (1U/kg) are used in both strains for assessing glucose and insulin tolerance respectively. We suggest that comparison studies should evaluate basal glucose and insulin levels and titrate glucose and insulin dosages for the glucose and insulin tolerance tests to give a more accurate comparison of the relative insulin sensitivity of different strains.

Poster Number 31

Effect of Resistant Starch on Postprandial Polymorphonuclear Cell Response and Viability to in vitro Oxidative Stress

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Background: Consumption of a high fat/high carbohydrate meal results in an exaggerated postprandial immune and oxidative stress response. This may potentially increase the vulnerability of cells to stressors such as oxidants and other foreign pathogens. Resistant starch (RS), a non-viscous fermentable fiber, has beneficial effects on insulin sensitivity, endothelial function, and colon health. This study investigated the effects of RS on the acute immune response and lymphocyte cell viability to an *in vitro* oxidative stress response following consumption of a high fat/high carbohydrate meal.
Methods: In a randomized crossover design, participants were given 30g/d RS, 15 g/d RS, or a placebo for four consecutive weeks separated by a 4 week washout. Following each dietary treatment, participants underwent a meal challenge test [42% fat (33% saturated fat), 17% protein, 41% CHO]. Blood samples were drawn under the fasted, and 1-hr and 4-hr postprandial conditions. The acute postprandial polymorphonuclear cell (PMN) responses and lymphocyte cell viability to *in vitro* oxidative stress were assessed during each phase.
Results: There was a time effect for cell viability such that under the high RS condition, there was a significant decrease in lymphocytes (p=.008), and a significant increase in neutrophil concentration (p=.001) at 1 hr following the meal; all values recovered to baseline 4 hours postprandial. With the placebo, lymphocytes remained decreased and neutrophils remained increased 4 hours postprandial.
Conclusion: There was and exacerbated and prolonged lymphocyte and neutrophil response following the high fat/high carbohydrate meal that is thought to represent immune suppression. Under the high dose RS condition, the lymphocyte and neutrophil counts returned to baseline 4 hours postprandial suggesting that was protected from the high dose RS.

Poster Number 32

Feasibility and Efficacy of Diabetic Retinopathy Screening Among Children with Diabetes

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Purpose: To determine the feasibility and efficacy of the use of a non-mydriatic camera to screen for diabetic retinopathy (DR) among children with type 1 or type 2 diabetes mellitus. Recent work shows that only two-thirds of diabetic children receive an annual dilated eye exam, making it the least commonly followed of the American Diabetes Association guidelines for diabetes care in children. Non-mydriatic fundus cameras have been implemented as successful screening tools for DR in adults. These cameras are non-invasive, painless, and produce high-quality fundus photographs capable of detecting most clinically significant cases of DR. However, this approach has not been well studied in children.
Methods: Children with type 1 or 2 diabetes ages 8 to 18 years old who visited the UAB Pediatric Endocrinology Clinic for a routine appointment were invited to enroll. A non-mydriatic camera (Nidek Model AFC-230) was used to obtain fundus images of each eye. Walk-in visual acuity was assessed with a Titmus vision screener. A brief questionnaire was administered to the parent/guardian addressing the child's diabetes and previous eye care utilization. Screening took place over an 8-week period. Fundus images were reviewed and graded by a retina specialist using the UK National Health Classification System for DR.
Results: A total of 236 patients were screened; average age was 14.1 years (SD 2.69), 135 (57.2%) were female, and 158 (67.0%) were Caucasian and 70 (29.7%) African American. Most of the patients had type 1 diabetes (85.6%) and average duration since diabetes diagnosis was 5½ years. Only 57 (24.2%) had 20/20 vision in both eyes and 156 (66.1%) reported having had a dilated eye examination within the previous year. Overall, DR was detected in 9 patients (3.8%), i.e., 7 patients had at least 1 eye graded as having background DR (a single eye for 6 and both eyes for 1); whereas 2 patients were graded as having proliferative DR in both eyes. Overall, 23 (9.7%) patients were referred to an ophthalmologist or other eye care provider.
Conclusions: This study shows the use of a non-mydriatic fundus camera is a feasible and efficacious method to screen for DR in children with diabetes. Screening programs may be beneficial in the management of diabetes in children and prevention of irreversible vision loss.

Poster Number 33

Oxidative Stress Cannot Mimic TXNIP-Induced microRNA Expression in INS-1 Beta-Cells

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Pancreatic beta-cell death via apoptosis represents a critical factor in the pathogenesis of diabetes. Thioredoxin-interacting protein (TXNIP), whose gene is most upregulated in response to glucose in human islets, is a ubiquitously expressed redox protein that has been shown to promote apoptosis in beta-cells. TXNIP is also upregulated in diabetes and induces oxidative stress, which is believed to be one of the main mechanisms by which TXNIP causes its detrimental effects on beta-cells. Recently, we discovered that TXNIP also regulates the expression of microRNAs and a microRNA microarray analysis revealed the following five microRNAs (miR-139-5p, miR-193, miR-204, miR-200c, and miR-141) to be the most upregulated in response to TNXIP overexpression in INS-1 beta-cells. The aim of the present studies was therefore to determine whether this increase in microRNA expression was also due to TXNIP-induced oxidative stress. To address this question, we subjected INS-1 beta-cells to various H_2O_2 and staurosporine concentrations (7-15uM H_2O_2 and 1-100nM staurosporine) and treatment durations (2-24 hours) to induce oxidative stress. RNA was harvested from treated cells and quantitative RT-PCR was performed, testing for alterations in expression of these five microRNAs. However, our results revealed no significant change in microRNA expression in response to oxidative stress, indicating that oxidative stress was not able to mimic the TXNIP effects. Together, our results suggest that TXNIP is inducing microRNA expression by mechanisms other than induction of oxidative stress and thereby provide additional support for the notion that TXNIP has beta-cell effects beyond its role as a redox regulator.

Poster Number 34

Glucose-Mediated Changes in DNA Methylation and Corresponding Gene Expression

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Despite all that is known about hyperglycemia and the subsequent molecular changes in other organ systems, relatively little is known about its role on myocardial tissue in the development of diabetic cardiomyopathy. We have begun the direct assessment of glucose delivery to the intact heart on alterations of gene expression using a combination of an inducible heart specific transgene (glucose transporter 4, GLUT4 or mG4H) and streptozotocin (STZ)-induced diabetes mouse models. Specifically, we have explored one component of glycemic memory, defined as the impact that antecedent glucose concentrations have on persistently increasing the risk of diabetic complications independently of current levels of glycemic control. Our studies in response to diabetes (STZ), transgene-induced uptake of glucose (mG4H), and which of these modifications persist following 2-weeks of transgene silencing now provide a map of cardiac DNA methylation and hydroxymethylation epigenetic marks proposed to mediate glycemic memory. Using bisulfite conversion and genome wide sequencing we have mapped these changes at CpG sites and have begun bioinformatic processing to identify overlap in the gene expression identified by microarray and RNA-seq. These studies show a clear subset of transcripts with altered expression in parallel with significant changes in DNA methylation within the promoter regions of these genes. These studies have also identified candidate mechanisms by which DNA methylation could alter transcript variants through differential methylation at alternate transcription start sites and non-coding RNAs. Ongoing studies in vitro are being conducted to define functional relevance of these modifications as they pertain to transcriptional regulation.

Funding: NIH R00 HL111322
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Poster Number 35

Can Non-Urgent Emergency Department Care Costs for Diabetes be Reduced? Empirical Evidence from a Nationally Representative Sample

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Objective:
Diabetes is an ambulatory care sensitive condition. If well controlled in an ambulatory care setting, diabetes patients should avoid emergency department (ED) use. This study examined cost drivers in non-urgent ED use for diabetes due to ambulatory care system deficiency and the associated cost savings magnitude nationwide.

Methods:
This retrospective cohort study used the 2010-2011 Medical Expenditure Panel Survey data, where individuals aged 18 and older with complete data for all five rounds were selected. This study chose a usual source of care status, perceived convenience to get medical care, and patient evaluation of care quality to measure access to and quality of ambulatory care.
ED use urgency was determined based on modifications of literature criteria. The marginal effect was estimated following the multivariate logit model to analyze the urgent vs. non-urgent ED use in 2011, after controlling for age, sex, race, insurance, rural vs. urban residence in 2010. All predictors were treated as dummy variables.
The lagged time effect was employed to account for the engogeneity between outcomes and predictors. The weights and variance were adjusted using the survey procedures.
Cost savings were calculated among diabetes patients based on significant predictors identified from ambulatory care systems.

Results:
The final study sample consisted of 1,287 adults with at least one ED visit in 2011, which represented a weighted 29,463,684 people in the total population.
Compared to patient perceived high primary care quality, poor and intermediate quality had higher odds of a non-urgent ED visit (OR=2.29 and p=0.025, OR=2.06 and p=0.010, respectively), with a marginal effect (at means) of 13.7% and 11.6% higher predicted probability of non-urgent ED use.
The ambulatory care quality related costs from non-urgent ED care were $36.7 million for diabetes at the national level ($p<0.01$).

Conclusions:
Significant costs can be reduced in non-urgent ED use for diabetes nationwide if ambulatory care quality on diabetes can be improved substantially.

Poster Number 36

TXNIP-Induced microRNA-204 Inhibits Beta Cell Function

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Loss of functional pancreatic beta cell mass is a common characteristic of type 1 and type 2 diabetes. Thioredoxin-interacting protein (TXNIP) plays a critical role in this process. We previously showed that TXNIP is increased in diabetic islets, whereas the genetic deficiency or pharmacological inhibition of TXNIP protects against diabetes by preventing beta cell loss. Our gene expression profiling study demonstrated that 95% of the genes altered by TXNIP overexpression were downregulated even though TXNIP is not known to act as a transcriptional repressor, suggesting that TXNIP might confer its effects via miRNAs. In the present study, we performed miRNA microarray assays comparing TXNIP-overexpressing and LacZ-expressing INS-1 beta cells. By setting 1.6 fold as a threshold, we found that five miRNAs (miR-139-5p, 193, 204, 200c, 141) were upregulated in response to TXNIP overexpression, and confirmed this by real-time RT-PCR. Interestingly, diabetic B6-obese and BTBR-obese mouse islets, in which TXNIP was significantly increased, also showed significantly higher expression of these miRNAs. In contrast, miRNA levels were significantly lower in TXNIP-deficient mouse islets as compared to controls. When studying the potential function of these miRNAs, we found that only miR-204 overexpression significantly inhibited insulin expression. However, miR-204 did so at the transcriptional level rather than targeting insulin directly. Using 3'UTR reporter assays, real-time RT-PCR, immunoblotting and chromatin immunoprecipitation studies, as well as primary human islets we demonstrated that miR-204 instead targets and downregulates the expression of the insulin transcription factor MAFA, which in turn inhibits insulin transcription and results in reduced beta cell insulin content. Taken together, we found that TXNIP and diabetes induce the expression of distinct miRNAs, which in turn control important beta cell functions, such as insulin transcription. The present study thereby sheds new light on the role of miRNAs in TXNIP signaling and diabetes and their effects on beta cell biology.

Poster Number 37

Muscle-Specific TRIB3 Overexpression Produces Weight Gain and Insulin Resistance in Mice, and Exacerbates Glucose-Induced Insulin Resistance in Diabetes

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A component of insulin resistance in diabetes is induced by hyperglycemia itself (glucose toxicity). We previously showed that glucose-induced insulin resistance (GIIR) was dependent upon TRIB3 and involved glucose metabolism via the hexosamine biosynthetic pathway. We also reported role of TRIB3 as a nutrient sensor and metabolic regulator. Currently, we investigated the In Vivo impact of TRIB3 on GIIR and systemic metabolism in Muscle-Specific TRIB3 Overexpressing (MOE) mice.

When compared with wild type (WT), MOE exhibited abnormal expression of genes regulating metabolism in response to short-term fasting and re-feeding, indicative of abnormal fuel metabolism. With 16 weeks' high fat feeding, MOE developed higher body weight (46.5±3.1 *vs.* 40.9±3.5g: $p<.05$) with significantly impaired glucose tolerance (2-hr GTT glucose: 248±27 *vs.* 191±19 mg/dl: $p<.01$). Insulin resistance in MOE was accompanied by decreased AKT phosphorylation in muscle. To determine the role of TRIB3 in GIIR, hyperglycemia was induced by streptozotocin. Serial insulin tolerance tests demonstrated that MOE mice developed a greater degree of insulin resistance after 3 weeks' exposure to hyperglycemia (1-hr ITT glucose: 137±26 *vs.* 90±6 mg/dl: $p<.01$), which was accompanied by a significant decrease in insulin-stimulated glucose oxidation in muscle

In conclusion, our results indicate that: 1) Muscle-specific overexpression of TRIB3 impairs the molecular regulation of fuel metabolism and results in greater weight gain and insulin resistance; 2) Muscle TRIB3 overexpression exacerbates insulin resistance during hyperglycemia, supporting TRIB3 as a critical facilitator of glucose-induced insulin resistance in vivo and pointing to TRIB3 as a novel target for treatment of glucose toxicity.

Poster Number 38

BioAnalytical Redox Biology Core B.A.R.B.

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Please contact us or log into our website for available services. Consultations are better scheduled at least 2-3 weeks before your services.

Poster Number 39

UAB Diabetes Research Center: Intervention and Translational Core

Lead: Cora E. Lewis, Co-Lead: Andrea Cherrington
Core Faculty: Monika M. Safford, James Shikany, Michelle Martin, Robert Oster, Gareth Dutton, Yu-Mei Schoenberger, Ralphenia Pace

Description of the UAB's DRC Intervention and Translational Core specific aims are:

Aim 1 Provide expert consultation for **intervention development** in diabetes prevention and management for example: (e.g., interventions to optimize weight, exercise/physical activity, medication adherence, and/or glycemic control). This expertise will facilitate translation of basic science findings into efficacy-testing interventions ranging from clinical trials to community based trials. (*New equipment: The ITC Core has available to loan 10 new **wActiSleep-BT** wireless sleep and wake monitors in conjunction with the ActiLife software for analysis.)*

Aim 2 Provide expertise on **study design and evaluation, including measurement, quality control, quality assurance, and analytic support**, for research to prevent and manage diabetes, These studies will include but also go beyond traditional clinical trials. For example, group-randomized trials evaluating weight-loss interventions in community settings require specialized expertise in trial design, data capture and transfer, as well as in interventional and analytic tools.

Aim 3 Design and facilitate culturally appropriate **strategies to enhance the recruitment and retention** of minorities and underserved populations in diabetes-related research studies with emphasis on African American and rural.

Poster Number 40

Diabetes Research Center Animal Physiology Core

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- This core provides services for the assessment of body composition, energy balance, cardiovascular parameters, imaging and the development of transgenic animal models. In vivo body composition is measured using dual-energy X-ray absorptiometry, quantitative magnetic resonance and micro-computed tomography. In addition, the core has a digital X-ray machine for the assessment of bone in anesthetized animals or excised bones. Detailed 3-D analyses of trabecular and cortical bone can be made using the Scanco uCT. Total and resting energy expenditure can be measured with indirect calorimetry together with locomotor activity and also body temperature. Glucose homeostasis is assessed with insulin and glucose tolerance tests and with hyperinsulinemic euglucemic clamps.
- Cardiac performance is assessed in vivo using high-resolution echocardiography. Hemodynamics can be assessed using a Miller pressure transducer catheter advanced into the left ventricle. The function of isolated working hearts can also be measured ex vivo. Blood pressure can be monitored in conscious mice and rats using chronic telemetry.
- Bioluminescence imaging can be used to assess dynamic molecular signaling in vivo in real time. Fluorescence imaging can be applied to detect cells and receptors. Radiolabelling of peptides, proteins and viral vectors is provided for in vivo imaging of receptors. Planar, SPECT, SPECT/CT and PET/CT imaging studies of these radiolabelled materials can be conducted in appropriate animal models.
- The Transgenic Mouse Facility provides expert services for the production and storage of transgenic mice including: DNA microinjection in vitro fertilization, embryo and sperm cryopreservation and assisted reproduction and rederivations.

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