

Boshell

RESEARCH PROGRAMS
at AUBURN UNIVERSITY

DIABETES



METABOLIC
DISEASES

6TH ANNUAL RESEARCH DAY

March 1, 2013

Auburn University Hotel
and
Dixon Conference Center



AUBURN
UNIVERSITY

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Preface

Welcome to the *6th Annual Boshell Diabetes and Metabolic Diseases Research Day* at Auburn University! This forum was designed to highlight the outstanding research in the areas of diabetes, obesity, cardiovascular disease, inflammation and other metabolic diseases by investigators in the Boshell Program at Auburn University and other institutions throughout the United States.

The success of this meeting is due to the efforts of many people. Many thanks are extended to members of the planning committee for their endless work: Drs. B. Douglas White and Suresh T. Mathews. We also thank Drs. Terry Brandebourg, Raj Amin, Chris Easley, Chad Foradori, John Quindry and Ramesh Jeganathan for judging the presentations. In addition, we would like to thank the College of Veterinary Medicine and the Office of the Vice President for Research for their financial support of this meeting. Special thanks to Dr. Edward Morrison, Head of the Department of Anatomy, Physiology and Pharmacology for administrative and financial support of the Boshell program.

This conference would not be possible without the work of Debbie Allgood on the conference website, registration and program. Thanks to Hattie Alvis for coordinating the financial aspects of the meeting and Leanne Greene, Karie Dalton and Heath Landrum for web design and conference registration. A special thanks goes to Kathy White for her work in designing the program logo and advertisements. Thanks are also extended to the Auburn-Opelika Tourism Bureau for providing the meeting badges. We greatly appreciate the staff and management of the Auburn University Hotel and Dixon Conference Center. Special thanks to our event planner, Lynn Huggins, for her tireless dedication to the success of this meeting. We would also like to thank the students, mentors and attendees for their participation. You are what make this meeting such a success.

We hope that you find this meeting productive and enjoyable!

Dr. Kevin W. Huggins and Dr. Robert L. Judd
Boshell Diabetes and Metabolic Diseases Research Program

Boshell Diabetes and Metabolic Diseases Research Program



The Boshell Diabetes and Metabolic Diseases Research Program at Auburn University was established through an endowment by the Birmingham-based Diabetes Trust Fund in honor of its founder, Dr. Buris R. Boshell in 2001.

Dr. Boshell was a 1947 graduate of the Auburn Polytechnic Institute (Auburn University) in Agriculture and attended the AU College of Veterinary Medicine for two years before transferring to Harvard Medical School and obtaining his M.D. degree in 1953. Dr. Boshell joined the faculty at the University of Alabama at Birmingham Medical Center in 1959 and became Chief of the Division of

Endocrinology and Metabolism in 1963. During this time, Dr. Boshell established the Diabetes Research and Education Hospital and the Boshell Diabetes and Endocrine Research Center in Birmingham.

Dr. Boshell published more than ninety scientific papers related to diabetes and authored several books including *The Diabetic at Work and Play* (1971) and *Diabetes Mellitus Case Studies* (1976). Dr. Boshell passed away on December 9, 1995.

MISSION STATEMENT

The mission of the Boshell Diabetes and Metabolic Diseases Research Program is to enhance the opportunities for diabetes research at Auburn University by facilitating cross-disciplinary scientific discussion, supporting the study of new ideas, fostering the development of investigators new to the field of diabetes, and expanding the overall base of diabetes investigation at the University. More than thirty-five investigators from across the AU campus are members of the program and actively involved in diabetes research. Specifically, these investigators are addressing many facets of both type 1 and 2 diabetes, with particular focus on the cardiac, neurological and metabolic aspects of the disease.

VISION

The vision of the Boshell Diabetes and Metabolic Diseases Research Program is to improve the life of all people with diabetes through world-class investigation performed at Auburn University into the prevention, cure, and management of diabetes and its complications.

GOALS

In order to accomplish the mission of the program, specific goals have been established:

- Develop effective mechanisms through which diabetes researchers at Auburn University may collaborate with each other and outside institutions.
- Support the exploration of new research ideas through a small grants program.
- Foster the development of new investigators through a travel grant program.
- Obtain external funding to support new multidisciplinary investigation and ongoing program activities.
- Develop relationships with community leaders interested in diabetes investigation to increase public support for diabetes programs.

BOSHELL INVESTIGATORS

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Meeting Schedule

<i>Time</i>	<i>Event</i>	<i>Location</i>
7:30 - 8:30 am	Registration, Poster Setup and Continental Breakfast	Prefunction Foyer
8:30 - 8:45 am	Welcome and Opening Remarks Dr. Carl Pinkert <i>Associate Vice President for Research</i> Dr. Robert Judd <i>Chair, Boshell Diabetes and Metabolic Diseases Research Program</i>	Auditorium
8:45 - 10:15 am	Oral Presentations (Morning) Dr. Suresh Mathews (Moderator) <i>Department of Nutrition, Dietetics and Hospitality Management</i>	Auditorium
10:15 - 10:30 am	Mid-Morning Break	Prefunction Foyer
10:30 - 11:30 am	Plenary Lecture: Dr. Anthony Ferrante <i>Dorothy and Daniel Silberberg Associate Professor of Medicine, College of Medicine, Columbia University</i> “Macrophages: Eating Big in Obesity and Weight Loss”	Auditorium
11:30 - 1:30 pm	Lunch and Poster Presentations: (Presenters need to be at their posters by 12:00 pm)	Ballroom A

Meeting Schedule

<i>Time</i>	<i>Event</i>	<i>Location</i>
1:45 - 2:45 pm	Keynote Address: Dr. Morris White <i>Professor of Pediatrics, Harvard University</i> "Hepatic Insulin Resistance and Metabolic Disease"	Auditorium
2:45 - 3:00 pm	Mid-Afternoon Break	
3:00 - 5:00 pm	Oral Presentations (Afternoon) Dr. Doug White, Moderator <i>Department of Nutrition, Dietetics and Hospitality Management</i>	Auditorium
5:00 - 6:30 pm	Reception	Prefunction Foyer
6:30 - 8:30 pm	Dinner Opening Remarks: Dr. Frank Bartol <i>Professor and Associate Dean, Research and Graduate Studies, College of Veterinary Medicine</i> Introduction: Dr. Kevin Huggins <i>Department of Nutrition, Dietetics and Hospitality Management</i> Kendall Simmons <i>Super Bowl Champion Auburn Great and Diabetes Advocate</i> "Tackling Diabetes: Gaining Control of Diabetes and Your Life"	Ballroom A
8:30	After Dinner Q&A and Autographs	Ballroom A

Plenary Lecture



"Macrophages: Eating Big in Obesity and Weight Loss"

Dr. Anthony Ferrante

*Dorothy and Daniel Silberberg Associate Professor of Medicine
Columbia University*

NOTES:

Keynote Address



"Hepatic Insulin Resistance and Metabolic Disease"

*Dr. Morris White
Professor of Pediatrics
Harvard University*

NOTES:

Dinner Presentation



**"Tackling Diabetes: Gaining Control of Diabetes
and Your Life"**

Kendall Simmons

Super Bowl Champion, Auburn Great and Diabetes Advocate

NOTES:

Oral Presentation Schedule

(Morning)

Auditorium

Moderator: Dr. Suresh Mathews

- 8:45 am **Role of Fructose/Sucrose in Fatty Liver Disease Progression.** Michael W. Greene^{1,2,3}, Christine M. Burrington³, Quan Kaewpoowat⁴, Promporn Suksaranjit⁴, Kunatum Prasadthratsint⁴, and Jian Zhang². ¹Boshell Metabolic Diseases and Diabetes Program and ²College of Human Sciences, Auburn University, Auburn, AL and ³Bassett Research Institute and ⁴Department of Internal Medicine, Bassett Medical Center, Cooperstown, NY
- 9:00 am **Epigenome-wide Association Study of Plasma Adiponectin.** Stella Aslibekyan¹, Marguerite R. Irvin¹, Jin Sha¹, Bertha Hidalgo¹, Degui Zhi², Krista Stanton Thibeault³, Michael Y. Tsai⁴, Paul N. Hopkins⁵, Ingrid B. Borecki⁶, Jose M. Ordovas⁷, Devin M. Absher³, Donna K. Arnett¹. ¹Department of Epidemiology, University of Alabama, Birmingham; ²Department of Biostatistics, University of Alabama, Birmingham; ³HudsonAlpha Institute for Biotechnology; ⁴Division of Epidemiology and Community Health, University of Minnesota; ⁵School of Medicine, University of Utah; ⁶Department of Genetics, Washington University in St Louis; ⁷Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University
- 9:15 am **Obesity Induced Insulin Resistance Contributes to Increased Tumor Growth in a Mouse Model of Human Colon Cancer.** Michael W. Greene^{1,2,3,4}, Erin Gillaspie⁵, Christine M. Burrington³, Ann Marie O'Neill², and Melissa J. Horsman³. ¹Boshell Metabolic Diseases and Diabetes Program, ²College of Human Sciences, and ³AU Research Initiative in Cancer Program, Auburn University, Auburn, AL 36849, USA and ⁴Bassett Research Institute and ⁵Department of Surgery, Bassett Healthcare Network, Cooperstown, NY 13326-1301, USA
- 9:30 am **Metabolically Healthy and Unhealthy Subtypes and Risk of Mortality in Normal Weight, Overweight and Obese Individuals with Chronic Kidney Disease.** Lynae Hanks, PhD, RD,^{1,2} Rikki Tanner, MPH,³ Paul Muntner, PhD,^{1,3} Holly Kramer, MD, MPH,⁵ William M. McClellan, MD, MPH,⁶ David G. Warnock, MD,¹ Suzanne E. Judd, PhD,⁴ and Orlando M. Gutiérrez, MD,^{1,3} *MMSc on behalf of the REGARDS investigators.* Departments of ¹Medicine, ²Nutrition Sciences, ³Epidemiology and ⁴Biostatistics, University of Alabama at Birmingham, Birmingham, AL; ⁵Department of Preventive Medicine, Loyola University, Maywood, IL; ⁶Departments of Epidemiology and Medicine, Emory University, Atlanta, GA
- 9:45 am **Epigenetics of Fasting Insulin and Glucose Concentrations.** Hidalgo B, Irvin MR, Sha J, Kabagambe EK, Zhi D, Aslibekyan S, Absher D, Tiwari H, Arnett, D. Department of Epidemiology, University of Alabama, Birmingham
- 10:00 am **Vasorelaxation Response to Insulin in Equine Lamina Vessel Rings.** Wooldridge AA¹, Waguespack RW¹, Schwartz DD², Venugopal CS³, Eades SC³, Beadle RE³, Auburn University College of Veterinary Medicine, Departments of Clinical Sciences¹ and Anatomy, Physiology, and Pharmacology,² Auburn, AL and Louisiana State University School of Veterinary Medicine³, Baton Rouge, LA

Oral Presentation Abstracts

(Morning)

001**Role of Fructose/Sucrose in Fatty Liver Disease Progression.** Michael W. Greene^{1, 2, 3}, Christine M. Burrington³, Quan Kaewpoowat⁴, Promporn Suksaranjit⁴, Kunatum Prasadthratsint⁴, and Jian Zhang². ¹Boshell

Metabolic Diseases and Diabetes Program and ²College of Human Sciences, Auburn University, Auburn, AL and ³Bassett Research Institute and ⁴Department of Internal Medicine, Bassett Medical Center, Cooperstown, NY

The public health consequences of the obesity epidemic in the United States and Alabama in particular are truly staggering: increased risk of developing cardiovascular disease, certain forms of cancer, type 2 diabetes, and fatty liver disease which can progress to the clinical condition of non-alcoholic steatohepatitis (NASH) which is a precursor for more serious liver diseases, such as cirrhosis and hepatocellular carcinoma. It has been postulated that consumption of sugary drinks are playing a role in the obesity epidemic and possibly the progression of fatty liver disease to NASH. To gain insight into the role of sugary drinks in fatty liver disease progression, we fed C57Bl/6 mice a low fat (LF) or high fat (HF) western diet (WD) without or with fructose and sucrose (F/S) in the drinking water. The LFWD group served as the control group. Measures of obesity, insulin resistance, glucose tolerance, fat and liver gene expression, and liver disease were assessed at 2- and 12-weeks.

Mice in the HFWD + F/S group gained the most weight at 2- and 12-weeks. A 67% increase in liver weight normalized to body weight from 2- to 12-weeks was observed in the HFWD + F/S group compared to <10% in the other groups. Epididymal fat pad weight normalized to body weight (a measure of visceral fat in mice) at 2-weeks was 137% greater in the HFWD + F/S group and ~40% greater in the other treatment groups compared to the control group. The greatest increase (132%) in normalized epididymal fat pad weight from 2- to 12-weeks was observed in the HFWD group. Surprisingly, a 11% decrease was observed in the HFWD + F/S group. Insulin resistance and glucose intolerance at 2- and 12-weeks, as assessed by insulin and glucose tolerance tests, respectively, was greatest in the HFWD + F/S group. In the HFWD group, insulin resistance and glucose intolerance was observed at 12-weeks but not at 2-weeks. Elevated measures of liver disease, alanine aminotransferase and non-alcoholic fatty liver disease score (steatosis, inflammation, and fibrosis), at 12-weeks were greatest in mice from the HFWD + F/S group. Hepatic gene expression at 2-weeks revealed significant changes in genes regulating liver disease for each treatment group compared to the control group. In contrast, significant changes in expression to genes regulating oxidative stress and fibrosis were concentrated in the liver from the HFWD + F/S group at 12-weeks, while genes regulating inflammation overlapped between the HFWD and HFWD + F/S groups. Fat tissue gene expression at 2-weeks revealed significant changes overlapping the treatment groups. Changes in genes regulating inflammation were concentrated in the HFWD and HFWD + F/S groups at 12-weeks while a unique set of genes regulating oxidative stress and lipid metabolism were observed in the HFWD + F/S group.

These results indicate that a sugary drink enhances HFWD-induced obesity, insulin resistance, glucose intolerance, liver and fat tissue gene expression, and leads to fatty liver disease progression in mice.

002

Epigenome-wide Association Study of Plasma Adiponectin. Stella Aslibekyan¹, Marguerite R. Irvin¹, Jin Sha¹, Bertha Hidalgo¹, Degui Zhi², Krista Stanton Thibeault³, Michael Y. Tsai⁴, Paul N. Hopkins⁵, Ingrid B. Borecki⁶, Jose M. Ordovas⁷, Devin M. Absher³, Donna K. Arnett¹. ¹Department of Epidemiology, University of Alabama, Birmingham; ²Department of Biostatistics, University of Alabama, Birmingham; ³HudsonAlpha Institute for Biotechnology; ⁴Division of Epidemiology and Community Health, University of Minnesota; ⁵School of Medicine, University of Utah; ⁶Department of Genetics, Washington University in St Louis; ⁷Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University

Introduction: Adiponectin is an adipose-secreted protein that has been linked to changes in insulin sensitivity, high-density lipoprotein cholesterol levels, and inflammatory patterns. Despite clinical relevance, the determinants of circulating adiponectin levels remain poorly understood, with common genetic polymorphisms as well as environmental variables each accountable for only a modest fraction of outcome variability. Emerging evidence suggests that epigenetic changes such as DNA methylation are associated with adiponectin signaling and merit further evaluation as diabetes risk factors.

Methods: Using data from the Genetics of Lipid Lowering Drugs and Diet Network (n=593), we assayed the methylation status of ~450,000 genome sites and measured serum concentrations of plasma adiponectin. We modeled associations between methylation scores and adiponectin using linear mixed models, adjusted for age, sex, study site, T-cell purity, and pedigree.

Results: We observed the strongest association ($P=1 \times 10^{-6}$) between adiponectin and methylation scores at two CpG sites near *RHCG* on chromosome 15. *RHCG* encodes an ammonium transporter protein, which has implications for the renal effects of insulin resistance and is likely to influence the risk of diabetic complications. Additionally, we found suggestive associations ($P=1 \times 10^{-5}$) with two CpG sites in *CPT1A*, a gene that encodes the carnitine palmitoyltransferase enzyme and has been previously shown to associated robustly with metabolic traits. Finally, we observed biologically relevant associations between adiponectin and differential methylation of a CpG site in *PTPRD* ($P=7 \times 10^{-6}$), a confirmed type 2 diabetes susceptibility locus, and near *ADIPOQ* ($P=1 \times 10^{-5}$), which encodes adiponectin itself.

Conclusions: In this first epigenome-wide study of circulating adiponectin, we identified several biologically plausible CpG sites where differential DNA methylation was suggestively associated with plasma adiponectin levels. Our findings provide support for the role of epigenetic mechanisms in the etiology of metabolic traits.

003**Obesity Induced Insulin Resistance Contributes to Increased Tumor Growth in a Mouse Model of Human Colon Cancer.** Michael W.

Greene^{1,2,3,4}, Erin Gillaspie⁵, Christine M. Burrington³, Ann Marie O'Neill², and Melissa J. Horsman³. ¹Boshell Metabolic Diseases and Diabetes Program, ²College of Human Sciences, and ³AU Research Initiative in Cancer Program, Auburn University, Auburn, AL 36849, USA and ⁴Bassett Research Institute and ⁵Department of Surgery, Bassett Healthcare Network, Cooperstown, NY 13326-1301, USA

Colon cancer is the third most common cancer in the US, with approximately 140,000 new cases each year and close to 50,000 deaths. Strong epidemiological evidence links colon cancer to obesity. Increasingly, there's evidence to suggest that obesity induced insulin resistance is associated with the increased cancer incidence, including colon cancer. In this study, we aimed to establish a new mouse model of obesity and human cancer, assess the effects of obesity on the growth of human colon cancer tumor xenografts, and examine potential mechanisms driving obesity-linked human colon cancer tumor growth.

Homozygous Rag1^{tm1Mom} (Rag1) and C57Bl/6 (B6) mice were used in this study. Mice were divided into 2 groups and fed either a low fat diet (LFD) or a high fat Western style diet (HFD). Mice were kept on the diet for 12 weeks and at 4, 8 and 12 weeks weighed and glucose tolerance and insulin tolerance measured. Insulin sensitivity was also determined by calculating the QUICKI (quantitative insulin sensitivity check index) score. After 4 weeks on the diet, human colon cancer xenografts derived from HT-29 cells were implanted subcutaneously into the mice and tumor growth measured every 2-3 days. After 28 days, mice were sacrificed, tumors harvested, and glucose, insulin, leptin, adiponectin, and free fatty acid (FFA) measured.

Rag1 mice in the HFD group weighed more than those in the LFD group; this difference in weight between the groups increased over time. There was no difference in normalized liver weight between the groups after 12 weeks; however the epididymal fat pads normalized to body weight, a measure of visceral fat, was greater (0.3 vs 0.7) in the HFD group compared to the LFD group. Mice on the HFD displayed progressive impairment of both glucose and insulin tolerance over time compared to the LFD group. Rag1 mice on the HFD showed elevated levels of insulin and glucose after 12 weeks when compared to B6 mice in the HFD group and all mice in the LFD group, validating the Rag1 stain as an obesity and insulin resistance model. Human colon cancer growth rate from HFD mice was significantly greater than that in the LFD group (average 0.023 g/day vs. 0.059 g/day). In agreement, final tumor weights were significantly greater in the HFD fed mice (average 0.57 g vs. 1.02 g). Mice in the HFD group also displayed decreased insulin sensitivity, as indicated by a higher QUICKI score, and showed increased FFA levels. In the HFD group, there was a significant positive correlation ($r^2=0.3$) between tumor weight and insulin levels, a negative correlation ($r^2=0.09$) between QUICKI score and tumor weight and a positive correlation ($r^2 =0.3$) between insulin to FFA level and tumor weight. While leptin levels were significantly higher in the HFD group, there was no correlation with tumor size. Adiponectin levels were inversely correlated to tumor size in both groups (LFD $r^2 = 0.06$; HFD $r^2 = 0.07$).

These results suggest that insulin resistance contributes to increased tumor growth in a novel obesity model of human colon cancer.

O04**Metabolically Healthy and Unhealthy Subtypes and Risk of Mortality in Normal Weight, Overweight and Obese Individuals with Chronic Kidney Disease.**

Lynae Hanks, PhD, RD,^{1,2} Rikki Tanner, MPH,³ Paul Muntner, PhD,^{1,3} Holly Kramer, MD, MPH,⁵ William M. McClellan, MD, MPH,⁶ David G. Warnock, MD,¹ Suzanne E. Judd, PhD,⁴ and Orlando M. Gutiérrez, MD,^{1,3} MMSc on behalf of the REGARDS investigators. Departments of ¹Medicine, ²Nutrition Sciences, ³Epidemiology and ⁴Biostatistics, University of Alabama at Birmingham, Birmingham, AL; ⁵Department of Preventive Medicine, Loyola University, Maywood, IL; ⁶Departments of Epidemiology and Medicine, Emory University, Atlanta, GA

Introduction: Higher body mass index (BMI) is paradoxically associated with lower mortality in persons with chronic kidney disease (CKD), but whether cardiometabolic abnormalities such as insulin resistance, dyslipidemia, and visceral adiposity modulate this association is unclear.

Objective: The objective was to examine the associations of metabolic health with all-cause mortality in normal weight, overweight and obese individuals with CKD.

Methods: Participants with CKD from Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study (n=4,374) were analyzed. The harmonized criteria for metabolic syndrome were used to define metabolic health, and participants were categorized in one of six mutually-exclusive categories defined by combined measures of metabolic health (metabolically healthy, <3 criteria for metabolic syndrome; metabolically unhealthy, ≥3 criteria) and weight status (normal weight, BMI 18.5-24.9 kg/m²; overweight, BMI 25-29.9 kg/m²; obese, BMI ≥30 kg/m²). Cox models were used to estimate the hazard ratio (HR) of death as a function of each category.

Results: A total of 683 deaths were observed over ~4.5 years. The associations of weight/metabolic categories with mortality differed by race (*P*-interaction=0.08). In analyses adjusted for socio-demographic and laboratory factors, compared to metabolically healthy normal weight persons, the HRs of mortality in metabolically healthy overweight and obese persons were 0.57 (95%CI 0.37,0.88) and 0.62 (95%CI 0.38,0.99) respectively among blacks, and 0.87 (95%CI 0.63,1.21) and 1.00 (0.60,1.66) respectively among whites. In contrast, compared to metabolically healthy normal weight persons, the HRs of mortality in metabolically unhealthy overweight and obese persons were 0.77 (95%CI 0.53,1.12) and 0.78 (95%CI 0.55,1.11) respectively among blacks, and 0.79 (95%CI 0.57,1.10) and 1.04 (0.77,1.42) respectively among whites.

Conclusions: Metabolic abnormalities may attenuate survival benefits associated with higher BMI among blacks with CKD. These findings suggest that the metabolic profile of CKD patients may be critical for properly assessing the impact of weight status on health outcomes.

Key Words: chronic kidney disease; metabolic syndrome; obesity; mortality

005

Epigenetics of Fasting Insulin and Glucose Concentrations. Hidalgo B, Irvin MR, Sha J, Kabagambe EK, Zhi D, Aslibekyan S, Absher D, Tiwari H, Arnett, D. Department of Epidemiology, University of Alabama, Birmingham

Background: Despite identification and replication of multiple T2D susceptibility loci in genome-wide association studies (GWAS), much remains to be understood about the genetic background of T2D. Characterization of epigenetic variation across the genome can advance our understanding of genetic susceptibility to T2D.

Methods: As in previous genetic studies among healthy individuals, we conducted an epigenome-wide association study (EWAS) on fasting insulin and glucose among 544 non-diabetic men and women in the Genetics of Lipid Lowering Drugs and Diet Network study. DNA was extracted from CD4+ T-cells isolated from frozen lymphocytes. CpG methylation at approximately 450,000 CpG sites was assayed using the Illumina Infinium HumanMethylation450 Beadchip. We used the LMEKIN package in R to implement a mixed model with methylation beta score (a measure of % methylation) as the dependent variable. In this model, insulin or glucose and covariates (age, sex, study site, and T-cell purity) were entered as fixed effects and family structure as a random effect. A Bonferoni corrected P-value of 1.1×10^{-7} was considered significant.

Results: In adjusted analyses, CpG06500161 was significantly associated with insulin ($P=3.8 \times 10^{-8}$) and CpG01881899 approached genome-wide significance ($P=6.2 \times 10^{-5}$) for insulin. Both CpG sites were identified on chromosome 21. Both CpG sites are found in the ATP-binding cassette sub-family G member 1 gene. Decreased function of ATP-binding cassette (ABC) transporters is associated with impaired insulin secretion and may explain the observed association. None of the CpG sites investigated was associated with fasting glucose after correction for multiple testing.

Conclusions: Our findings suggest that differential methylation of CpG06500161 within the ABCG1 locus may be associated with fasting insulin and merits further evaluation.

O06

Vasorelaxation Response to Insulin in Equine Laminar Vessel Rings.

Wooldridge AA¹, Waguespack RW¹, Schwartz DD², Venugopal CS³, Eades SC³, Beadle RE³, Auburn University College of Veterinary Medicine, Departments of Clinical Sciences¹ and Anatomy, Physiology, and Pharmacology,² Auburn, AL and Louisiana State University School of Veterinary Medicine³, Baton Rouge, LA

Introduction: Hyperinsulinemia causes laminitis experimentally and is a risk factor for naturally occurring laminitis. The mechanism is not understood, but may be due to the effects of insulin on endothelial function. The purpose was to investigate the effects of insulin on laminar vascular function and to impair insulin-induced relaxation *in vitro*. A model to understand the effects of hyperinsulinemia on endothelial function is essential to understand the possible mechanism of hyperinsulinemia induced laminitis.

Materials and Methods: Laminar arteries and veins were isolated after euthanasia from 13 lean horses with baseline insulin concentrations less than 20 μ U/ml. Maximum KCl force was used to determine the type of vessel. Endothelium-dependent responses were evaluated using cumulative concentration response curves to acetylcholine (ACH) and insulin after submaximal contraction with phenylephrine (PE). To experimentally impair insulin induced relaxation, laminar arterial and venous rings were incubated with insulin (10^{-5} M) or bovine serum albumin (BSA, control) for 20 minutes. Vessels were submaximally contracted with phenylephrine, administered a single dose of insulin (10^{-5} M), and relaxation responses recorded for 15 minutes. Data were analyzed using 2-way analysis of variance and $p < 0.05$.

Results: Endothelium-dependent relaxation to ACH was not different between laminar arteries and veins. For insulin dose response curves, veins with less than 2mN KCl induced force relaxed significantly less than the other vessels with 2-6 mN KCl force but were not different from arteries (>6mN KCl force). For the induction of insulin resistance, arteries that were incubated with insulin had a significantly diminished relaxation response ($68 \pm 5.7\%$ of maximum contraction, $n=14$) when compared to incubation with BSA ($48 \pm 5.9\%$ % of maximum, $n=14$). Veins had minimal relaxation response to insulin regardless of incubation (insulin $91.8 \pm 2.3\%$ of maximum, $n=11$; BSA $98.7 \pm 2.2\%$ of maximum, $n=12$).

Discussion and Conclusions: There is a differential response to insulin in different types and sizes of laminar vessels. Laminar veins have minimal relaxation response to a single dose of insulin. Impaired insulin-induced vasorelaxation responses occur in laminar arteries with prior incubation in insulin. A differing physiological response of laminar veins and laminar arteries to insulin-induced relaxation should be investigated further and may be important in the understanding of the link between hyperinsulinemia and laminitis. Impaired vasorelaxation responses can be induced experimentally by incubation with insulin in laminar arteries and may be useful for testing therapeutic interventions or understanding pathophysiology of the disease.

Oral Presentation Schedule

(Afternoon)

Auditorium

Moderator: Dr. Doug White

- 3:00 pm **Superoxide Production by NOD Bone Marrow-Derived Macrophages Mediates Anti-viral Responses to Diabetogenic Encephalomyocarditis Virus Infection.** Ashley R. Burg and Hubert M. Tse, PhD. Department of Microbiology, Comprehensive Diabetes Center, University of Alabama at Birmingham, Birmingham, AL 35294, USA
- 3:15 pm **PKC δ is Activated in the Liver of Obese Zucker Rats and Mediates Diet-induced Whole Body Insulin Resistance and Hepatocyte Cellular Insulin Resistance.** Michael W. Greene^{1,2,3}, Christine M. Burrington³, Mary S. Ruhoff³, Darin T. Lynch³, Niyutchai Chaithongdi⁴ and Yuwen Luo². ¹Boshell Metabolic Diseases and Diabetes Program and ²College of Human Sciences, Auburn University, Auburn, AL 36849, USA, ³Bassett Research Institute, ⁴Department of Internal Medicine, Bassett Medical Center, Bassett Healthcare Network, Cooperstown, NY
- 3:30 pm **Antibodies to Commensal Microbiota are Present in Murine Models of Type 1 Diabetes.** Joseph G Daft^{1,2}, Robin Lorenz^{1,2} M.D, PhD. ²Department of Pathobiology and Comprehensive Diabetes Center, University of Alabama at Birmingham, AL
- 3:45 pm **Angiotensin AT2R Activation Prevents High-Fat Diet-Induced Adiposity Independent of Estrogen in Female Mice.** Sourashish Nag and Tahir Hussain. Department of Pharmacological & Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX 77204
- 4:00 pm **Examining the Role of ROS in CD4 T Cell Effector Responses in T1D.** Lindsey E. Padgett and Hubert M. Tse, PhD. Department of Microbiology, University of Alabama-Birmingham, Birmingham, AL
- 4:15 pm **Meta-analysis of Insulin Glargine and Incidence of Cancer.** Alqahtani N, Qian J, Garza KB, Hansen RA. Pharmacy Care Systems Department, School of Pharmacy, Auburn University, AL
- 4:30 pm **A Critical Role for Phosphorylated Fetuin-A (Ser312) in Modulating Insulin Action in Obesity and Insulin Resistance.** Guang Ren¹, Peter W. Grandjean², Xiaoming He¹, Robby Bowers¹, Carl Okerberg¹, James Papizan¹, Teayoun Kim¹, Rebecca A. Ludvigsen¹, Felipe Araya-Ramirez¹, A. Jack Mahurin³, Jennifer D. Dennis¹, David M. Dean¹, and Suresh T. Mathews¹. ¹Auburn University, Auburn, AL, ²Baylor University, TX, ³Baptist Family Medicine, Montgomery, AL
- 4:45 pm **The Cardioprotective Effects of Adiponectin In Volume Overload-induced Cardiomyopathy.** Lili Wang and Juming Zhong. Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL

Oral Presentation Abstracts

(Afternoon)

007**Superoxide Production by NOD Bone Marrow-Derived Macrophages Mediates Anti-viral Responses to Diabetogenic Encephalomyocarditis Virus Infection.**

Ashley R. Burg and Hubert M. Tse, PhD. Department of Microbiology, Comprehensive Diabetes Center, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Viral infections are implicated to be a key environmental trigger of Type-1 diabetes (T1D), but the mechanism is poorly defined. Understanding how viruses instigate the autoimmune attack against beta-cells is crucial to the development of both preventative and early-intervention therapies. The initial response to viral infection by innate immune cells, such as macrophages, is the production of proinflammatory cytokines (eg. IFN β , TNF α , IL-1 β), and reactive oxygen species (ROS), such as superoxide. To define the importance of ROS synthesis in T1D pathogenesis, we generated Non-Obese Diabetic (NOD) mice unable to synthesize superoxide, due to a mutation in the p47^{phox} (*Ncf1*) subunit of the NADPH oxidase complex. Since NOD.*Ncf1*^{m1J} mice exhibited a high proclivity for T1D-resistance, the innate immune mechanisms associated with T1D protection were elucidated. Bone marrow-derived macrophages (BMM Φ) from NOD.*Ncf1*^{m1J} mice displayed decreased expression of the viral sensors, Toll-like receptor 3 (TLR3) and retinoic acid-inducible gene-1 (RIG-I), resulting in decreased NF- κ B activation, and production of TNF α and IFN β , upon poly(I:C)-stimulation, compared to NOD BMM Φ . Given the major roles of superoxide in both viral infection and T1D, we hypothesize that superoxide is a causal factor in the dynamics of viral-triggered T1D. In the present study, we infected BMM Φ with the diabetogenic Encephalomyocarditis virus (EMCV) to define the role of superoxide production in anti-viral innate immune responses. To this end, we stimulated NOD and NOD.*Ncf1*^{m1J} BMM Φ with an MOI=1 of EMCV strain-B (non-diabetogenic) or strain-D (diabetogenic) for 3, 6, 12, 24 or 48h, then analyzed for production of anti-viral mediators. Interestingly, IL-1 β synthesis, an important driver in T1D progression, was greatly reduced in NOD.*Ncf1*^{m1J} BMM Φ compared to NOD, in response to EMCV-B (2.42 ± 0.02 vs. 20.70 ± 0.77 pg/mL; $p < 0.0001$) and EMCV-D (below detection vs. 3.86 ± 0.22 pg/mL), respectively. Additionally, this effect of superoxide-deficiency was significantly greater ($p < 0.0001$) in response to the diabetogenic EMCV-D, compared to the non-diabetogenic EMCV-B. Similarly, infection with EMCV-D displayed a 2-fold decrease in TNF α synthesis by NOD.*Ncf1*^{m1J} BMM Φ (686.3 ± 11.5 pg/mL) as compared to NOD BMM Φ (1115.0 ± 40.0 pg/mL; $p < 0.0005$); whereas, no difference was seen in EMCV-B-elicited TNF α levels between NOD and NOD.*Ncf1*^{m1J} (1436.0 ± 37.9 and 1205.2 ± 50.4 pg/mL, respectively). Thus, superoxide synthesis by macrophages is necessary to induce an efficient innate immune response towards EMCV infections. Of note, EMCV-D consistently elicited a lower anti-viral response than EMCV-B, posing the idea of a persistent or extended infection, lending to its diabetogenicity. Future studies will define the redox-dependent mechanisms associated with diabetogenic anti-viral responses, and determine if redox modulation of these innate immune responses can prevent viral triggers of T1D.

O08**PKC δ is Activated in the Liver of Obese Zucker Rats and Mediates Diet-induced Whole Body Insulin Resistance and Hepatocyte Cellular Insulin Resistance.**

Michael W. Greene^{1,2,3}, Christine M. Burrington³, Mary S. Ruhoff³, Darin T. Lynch³, Niyutchai Chaithongdi⁴ and Yuwen Luo². ¹Boshell Metabolic Diseases and Diabetes Program and ²College of Human Sciences, Auburn University, Auburn, AL 36849, USA, ³Bassett Research Institute, ⁴Department of Internal Medicine, Bassett Medical Center, Bassett Healthcare Network, Cooperstown, NY

It has been postulated that insulin resistance arises when pathological levels of free fatty acids (FFA) and proinflammatory cytokines disrupt insulin signaling. Protein kinase C delta (PKC δ) is a FFA- and a proinflammatory cytokine-regulated protein kinase that is associated with inhibition of insulin signaling and action. To gain insight into the *in vivo* role of PKC δ in insulin resistance, PKC δ activation was studied in a genetic model of obesity-linked insulin resistance. PKC δ was found to be activated in the liver of obese insulin resistant Zucker rats and in isolated cultured hepatocytes. PKC δ was further studied in PKC δ null mice and their wild-type littermates fed a high-fat or control diet for 10 weeks. PKC δ null mice on a high fat diet had improved insulin sensitivity and hepatic insulin signaling compared to wild-type littermates. Additionally, the deleterious effect of a high fat diet on glucose tolerance in wild-type mice was completely blocked in PKC δ null mice. To directly test the role of PKC δ in cellular insulin resistance, primary hepatocytes from the high-fat diet mice were isolated and stimulated with insulin. Primary hepatocytes from PKC δ null mice had improved insulin-stimulated Akt and FOXO phosphorylation compared to hepatocytes from wild-type littermates. Consistent with this result, TNF alpha mediated inhibition of insulin signaling was blocked in PKC δ knockdown primary hepatocytes. These results indicate that PKC δ plays a role in insulin resistance and are consistent with the hypothesis that PKC δ is a negative regulator of insulin signaling and thus may be a therapeutic target for the treatment of type 2 diabetes.

009

Antibodies to Commensal Microbiota are Present in Murine Models of Type 1 Diabetes. Joseph G Daft^{1,2}, Robin Lorenz^{1,2} M.D, PhD.

²Department of Pathobiology and Comprehensive Diabetes Center, University of Alabama at Birmingham, AL

Type 1 Diabetes (T1D) is defined as the selective immune destruction of insulin producing beta-cells within the islet. Alterations in the intestinal microbiota, changes in intestinal integrity, and an aberrant immune system have been postulated to play key roles in the development of diabetes. Auto-antibodies to insulin, islet associated antigens, and antigens found in the lumen of the intestine, such as cows' milk protein, have been linked to T1D, however, the role of these antibodies in disease progression is still unknown. We hypothesize that a break down in intestinal integrity results in the generation of antibodies to commensal microbial antigens and that these antibodies play a role in the pathogenesis of diabetes.

Non-obese diabetic (NOD) female mice were compared to age and sex matched Non-obese diabetes resistant (NOR) mice. Serum antibodies to CBir1 flagellin (from a cluster XIVa commensal *Clostridium*) were compared between strains. ELISAs showed that both NOD and NOR mice have detectable levels of anti-CBir1 flagellin IgM and IgG at pre-diabetic time points. We postulated that anti-CBir1 antibodies could be involved in the pathogenesis of disease via two mechanisms: (1) cross-reactivity with pancreatic antigens, and/or (2) modulation of the immune response against pancreatic antigens. Through western blotting and immunohistochemistry using an anti-CBir1 flagellin IgM antibody, we have shown that this antibody has the ability to cross react with islet antigens.

To directly test if antibodies to CBir1 flagellin modulates disease progression, NOD and NOR mice are being treated with 2.75% Dextran Sulfate Sodium (DSS) to alter intestinal integrity. This treatment leads to an increase in serum anti-CBir1 flagellin antibody levels as determined by ELISA. However this treatment is non-specific, as it increases systemic immune exposure to all commensal microbiota. To study the direct effect of CBir1 flagellin antibodies, NOD mice have also been injected with anti-CBir1 flagellin IgM and IgG antibodies. These mice are being monitored to determine if this increase in anti-CBir1 flagellin antibodies will alter the incidence of diabetes in both NOD and NOR mice.

O10**Angiotensin AT2R Activation Prevents High-Fat Diet-Induced Adiposity Independent of Estrogen in Female Mice.** Sourashish Nag and Tahir Hussain.

Department of Pharmacological & Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX 77204

Role of renin-angiotensin system (RAS) has been implicated in obesity and metabolic dysfunction. Recently we demonstrated that the genetic deletion of angiotensin AT2 receptor (AT2R) increases adiposity in female mice on high-fat diet (HFD). Further these changes were associated with a parallel decrease in urinary estrogen (17, β -estradiol/E2), a known regulator of adiposity. Aim of the present study was to investigate whether AT2R activation prevents adiposity, in an estrogen-dependent manner. Female C57BL/6 ovary-intact (Ovi) and ovariectomized (Ovx) mice (8-weeks old) were pretreated with AT2R agonist (C21, 0.3 mg/kg, daily i.p.) for 4 days. Also C57BL/6 Ovx mice were supplemented with E2 (5 μ g/day) pellets and/or pretreated with AT2R agonist C21 (0.3 mg/kg, daily i.p.) for 4 days. Thereafter the mice were placed on normal diet (ND) or HFD with concurrent hormone/drugs treatment for next 10 days. Ovi mice on HFD had increased parametrial white adipose tissue (pWAT) weight, plasma insulin, free fatty acid (pFFA), triglyceride (pTAG) and decreased urinary E2 compared to Ovi mice on ND. C21 treatment prevented all HFD-induced changes in Ovi mice. Interestingly, compared to Ovi mice on ND, Ovx mice on ND had increased pWAT weight, plasma leptin, insulin, pFFA, pTAG and decreased urinary E2. These changes were further increased by HFD in Ovx mice. Similar to the effects on Ovi mice on HFD, C21 treatment also prevented all the HFD-induced changes in Ovx mice. As expected, E2 alone or in combination with C21 treatment in Ovx mice prevented HFD-induced increase in pWAT weight, plasma leptin, insulin, pFFA, pTAG and decrease in urinary E2. Notably, C21 treatment in both the Ovi and Ovx mice on HFD had no affect on total kilocalorie intake comparing with controls. We conclude that the pharmacological activation of AT2R prevents adiposity in Ovi and Ovx mice, indicating that the changes produced by AT2R are independent of E2. Thus this study demonstrates that the AT2R may serve as a novel therapeutic target for controlling obesity and associated metabolic disorders in females.

Grant Funding Source: NIH RO1 DK-61578

O11**Examining the Role of ROS in CD4 T Cell Effector Responses in T1D.**

Lindsey E. Padgett and Hubert M. Tse, PhD. Department of Microbiology, University of Alabama-Birmingham, Birmingham, AL

In Type 1 diabetes (T1D), infiltrating leukocytes generate reactive oxygen species (ROS) and pro-inflammatory cytokines that collectively participate in β -cell destruction and enhance T cell autoreactivity. Our laboratory previously demonstrated that superoxide-deficient Non-Obese Diabetic (NOD.*Ncf1*^{m1J}) mice are T1D-resistant partially due to skewed T cell responses. To further dissect the role of ROS in autoreactive T cell responses, we characterized the NOD.BDC-2.5.*Ncf1*^{m1J} mouse, possessing superoxide-deficient, diabetogenic CD4 T cells. NOD.BDC-2.5.*Ncf1*^{m1J} splenocytes stimulated with the BDC-2.5 mimotope or islet cells displayed a 2- and 5-fold increase in IFN- γ (210.0 \pm 7 vs. 96.7 \pm 5 ng/mL; p <0.0001); (31.9 \pm 0.6 vs. 7.4 \pm 0.2 ng/mL; p <0.0001), respectively, in comparison to NOD.BDC-2.5. Exogenous superoxide addition restored Th1 cytokine responses to NOD.BDC-2.5 levels, indicating that a loss of superoxide exacerbated IFN- γ synthesis. Despite elevated Th1 responses, NOD.BDC-2.5.*Ncf1*^{m1J} adoptive transfers were less diabetogenic. Sixty days following transfer, 20% of NOD.BDC-2.5.*Ncf1*^{m1J} (n=8) and 60% of NOD.BDC-2.5-transferred mice (n=8) were hyperglycemic. Reduced diabetogenicity was partially due to decreased islet-infiltrating, TNF- α -synthesizing macrophages and enhanced immunosuppressive cytokine synthesis. Specifically, IL-10 production by NOD.BDC-2.5.*Ncf1*^{m1J} splenocytes was significantly enhanced compared to wild type upon mimotope (511.0 \pm 3.0 vs. 430.0 \pm 40.0 pg/mL; p =0.026) and islet cell (70.0 \pm 3.0 pg/mL vs. 9.0 \pm 23.0 pg/mL; p =0.014) stimulation. Similarly, mimotope- and islet cell-stimulated NOD.BDC-2.5.*Ncf1*^{m1J} splenocytes displayed a 7- and 22-fold increase in TGF- γ (43.7 \pm 1.7 vs. 5.8 \pm 0.6 pg/mL; p <0.0001); (22.8 \pm 1.5 pg/mL vs. undetected; p <0.0001), respectively, compared to wild type. Future experiments will analyze the activation profiles of redox-dependent T cell receptor (TCR) adaptor molecules, as recent data from our laboratory indicates that activated superoxide-deficient NOD.BDC-2.5 CD4 T cells display enhanced phosphorylation of two key tyrosine kinases essential for effective TCR signaling, lymphocyte-specific protein tyrosine kinase (Lck) and linker for activation of T cells (LAT), compared to wild-type. In particular, polyclonal stimulation of NOD.BDC-2.5.*Ncf1*^{m1J} CD4 T cells induced a 1.5- and 1.6-fold increase in the expression of P-Lck (Y505) and P-LAT (Y191), respectively, in comparison to NOD.BDC-2.5 CD4 T cells. Ultimately, dissecting the role of ROS in T cell effector responses may identify novel targets for T1D prevention and therapy.

Diabetes mellitus (DM) can be defined as: a metabolic syndrome that negatively affects insulin secretion or its sensitivity in response to variation in blood glucose levels. There are two types of DM Type 1 and Type 2 and prevalence of both types of diabetes are 10% and 90%, respectively. There are different treatment modalities of DM ranging from life-style changing and diet-control to oral anti-diabetics and insulins. There are three types of insulin; namely: short-acting, intermediate-acting and long-acting insulin. The insulin glargine, however, belongs to long-acting insulin group. Type 2 DM is linked to three types of carcinoma as a result of obesity and insulin resistance. They are colon, pancreas and breast cancer. The current medical practice necessitates including "Cancer" among the list of diabetes complications.

Nevertheless, certain number of studies has discussed the increased possibility of carcinogenicity of insulin preparations, specifically insulin glargine. PubMed database were searched for all observational studies regarding the association between cancer occurrence and the use of insulin glargine using. Data were independently extracted and analyzed using random or fixed effects meta-analysis. Four articles in the journal of Diabetologia were chosen to be included in this meta-analysis. Those retrospective studies were carried out to scrutinize whether or not there is an established risk of cancer development with insulin glargine as well as co-administration of other insulin analogues. They have been performed Germany, Scotland, Sweden and UK. There was no excess risk of cancer incidence from the whole evidence. Randomized trial, German and UK (THIN) studies have shown insignificant correlation between galrgine and increased risk of cancer when compared to human insulin (HR=0.63 (0.36-1.09), 0.86 (0.79-0.94) and 0.81 (0.59-1.11), respectively). Similarly, Swedish as well as Scottish analyses were denying any link of cancer occurrence among galrgine users compared to other insulin consumers. Yet, the Hemkens et al piece of work confirmed the strong relation between cancer and glargine use. A post-hoc analysis of randomized trials showed reassuring findings with respect to the galrgine treated diabetic patients. There has been no significant difference between the two groups in terms of formation of neoplasm, HR=0.90 (0.64-1.26).

As a conclusion, available evidence suggests that there is an increased risk of cancer associated with insulin glargine. However, considerable number of limitations, such as, poor glycaemic control, preceding exposure to OHAs, imbalance between the treatment groups and allocation bias are surrounding the current evidence which create an area of uncertainty via publishing inconclusive findings. Therefore, further research should be conducted to ensure fine tuning the ambiguity of such serious risks.

O13

A Critical Role for Phosphorylated Fetuin-A (Ser312) in Modulating Insulin Action in Obesity and Insulin Resistance. Guang Ren¹, Peter W. Grandjean², Xiaoming He¹, Robby Bowers¹, Carl Okerberg¹, James Papizan¹, Teayoun Kim¹, Rebecca A. Ludvigsen¹, Felipe Araya-Ramirez¹, A. Jack Mahurin³, Jennifer D. Dennis¹, David M. Dean¹, and Suresh T. Mathews¹. ¹Auburn University, Auburn, AL, ²Baylor University, TX, ³Baptist Family Medicine, Montgomery, AL

Fetuin-A is a negative regulator of insulin action, inhibiting insulin receptor tyrosine kinase activity (IR-TKA) and downstream insulin signaling. Elevated serum fetuin-A concentrations are associated with obesity, insulin resistance, impaired glucose tolerance, metabolic syndrome, and fat accumulation in the liver. Several lines of evidence suggest that fetuin-A may be an independent risk factor for type 2 diabetes. Recent evidence indicated a role for fetuin-A in lipid-induced inflammation. Fetuin-A, touted the first hepatokine affecting glucose and lipid metabolism (*Nat Rev Endocrinol*, 2013, *in press*), undergoes several post-translational modifications, including N- and O-glycosylation, and phosphorylation. Fetuin-A, originally identified as phosphorylated protein 63 (pp63), was shown to be secreted by the liver into circulation in the phosphorylated form. Over 75% of fetuin-A phosphorylation is reported to occur on Ser312 and approximately 20-25% on Ser120. Only the phosphorylated form of the protein was earlier shown to be active as an inhibitor of the IR-TKA. However, the majority of fetuin-A in serum appear to be in a dephosphorylated form. Our current understanding of the role of fetuin-A phosphorylation in metabolism and disease states is limited. Therefore, we sought to characterize the role of fetuin-A phosphorylation in insulin action and insulin resistant conditions. We demonstrate that, phosphorylated fetuin-A inhibited insulin-stimulated IR-TKA, Akt activation, glucose uptake, and glycogen synthesis in skeletal muscle cells and adipocytes while Ser312Ala-fetA mutant and Ser120Ala, Ser312Ala-fetA double mutant were devoid of inhibitory activity. Next, we measured fetuin-A phosphorylation status in obese animals and humans. Serum levels of phosphorylated Ser312-fetuin-A were significantly elevated in ZDF rats, and in diet-induced obese mice, and these were shown to be associated with elevations in serum insulin concentrations and HOMA index. Similarly, obese individuals demonstrated significantly elevated serum total fetuin-A and phosphorylated Ser312-fetuin-A, compared to healthy individuals. A single-bout of treadmill walking at 60-70% VO₂max, and expending 500 kcals, significantly decreased total and serum phosphorylated Ser312-fetuin-A in obese individuals, consistent with the improvements in serum insulin concentrations, glucose/insulin ratio, and HOMA index. Further, we observed a significant decrease in AUC_{phosphorylated Ser312-fetuin-A} during an oral glucose tolerance test, 24 hours after a single bout of exercise, consistent with improvements in insulin action. Taken together, these findings implicate a role for phosphorylated fetuin-A as a negative regulator of insulin action and suggest that alterations in phosphorylated fetuin-A concentrations may be associated with changes in insulin sensitivity. Specifically, a role for phosphorylated fetuin-A in the improvement of insulin sensitivity following a single bout of exercise is proposed.

O14

The Cardioprotective Effects of Adiponectin In Volume Overload-induced Cardiomyopathy. Lili Wang and Juming Zhong. Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL

Adiponectin (ADP) is an adipocyte derived protein hormone with respect to metabolic regulation. It has been reported that ADP confers a cardio protective effect during ventricular remodeling and heart failure following pressure overload and myocardial ischemia. However, no study has evaluated the potential relationship of ADP and the volume overload-induced cardiomyopathy. Although both pressure overload and volume overload result in ventricular hypertrophy, the molecular mechanisms inducing the development of heart failure are quite different. The aim of this study was to investigate the roles of ADP in the progression of heart failure induced by volume overload. Aorto-caval fistula (ACF) was performed to create volume overload in SD rats. ACF induced progressive ventricular dilatation and impaired ventricular myocyte contractility and intracellular Ca^{2+} handling. Plasma levels of ADP were reduced with the progression of ACF induced heart failure. Protein expressions of ADP as well as ADP receptor were also reduced in cardiomyocytes isolated from ACF rats. *In vitro* treatment of ADP improved the contractile function and myocardial Ca^{2+} handling in ventricular myocytes isolated from ACF rats, but had no effect on those parameters in control myocytes. *In vivo* supplement of adenovirus-mediated ADP in ACF rats elevated the plasma levels of ADP and prevented cardiac myocyte contractile dysfunction. Taken together, our results indicate that ADP plays an important role in the prevention of volume overload-induced cardiomyopathy.

Poster

Abstracts

P01

Endocrine Disruptors as obesogens and Reproductive Toxicants.

Benson T. Akingbemi and Robert L. Judd. Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL 36849

The obesogen hypothesis postulates that environmental chemicals, which disrupt homeostatic metabolic controls, regulate adipocyte hypertrophy, or stimulate adipogenic pathways and adipocyte hyperplasia, have the capacity to promote obesity. Many environmental contaminants with hormonal activity (endocrine disruptors, ED) are now known to interact with signaling pathways involved in the regulation of lipid metabolism and adipogenesis, thereby contributing to weight regulation. The environmental obesogen hypothesis is relevant to public health because more than 35% of adults and about 17% of children aged 2-19 years are obese in the United States. Of interest to us, a relationship between energy homeostasis and reproduction has been described for a long time. Thus, it is possible that ED-related changes in metabolism affect reproductive activity. However, there is little or no information on the mechanisms by which metabolic dysregulation impacts the reproductive process although several metabolic molecules, e.g., leptin, adiponectin, have binding sites in the male gonad, including Leydig cells. Testicular Leydig cells are the predominant source of androgens, which maintain the male phenotype and support fertility. In this regard, it is concerning that 25% of infants in the United States maintained on soy-based formulas are exposed to high levels of soy isoflavones in the diet (mostly genistein and daidzein) because these compounds have been credited with estrogenic activity. In one study, we determined that developmental exposure to isoflavones decreased estrogen receptor but enhanced adiponectin expression in adipose tissue, and caused an elevation in serum leptin and adiponectin concentrations in adult male rats. Isoflavones also caused a decrease in testosterone secretion and expression of adiponectin and adiponectin receptor type 2 (AdipoR2) in Leydig cells. Relatedly, there have been concerns that early-life exposure to the industrial chemical bisphenol A (BPA) may alter developmental programming and predispose to reproductive problems and obesity in the adult. Exposure of the population to BPA is significant because it is used in the manufacture of polycarbonate plastics, epoxy resins and dental sealants. Recently, we observed that BPA-treated animals maintained on a high fat diet were heavier than their counterparts fed a normal fat diet later in the postnatal period. Furthermore, developmental exposure to BPA increased estrogen sulfotransferase enzyme protein in adipose tissue while decreasing serum adiponectin levels. BPA also caused a decrease in adiponectin and AdipoR2 protein levels in Leydig cells. The possibility that adiponectin directly regulates the steroidogenic machinery was tested by incubation of Leydig cells with recombinant adiponectin, which caused a decrease in androgen biosynthesis that was associated with diminished expression of the cholesterol transporter, steroidogenic acute regulatory protein, in Leydig cells. Taken together, our findings support the view that chemical-induced alterations in metabolic homeostasis have implication for steroid hormone secretion. Moreover, it is likely that changes in adipose tissue metabolism occasioned by exposure to environmental contaminants contribute to deficiencies in reproductive capacity seen in chronic diseases such as diabetes.

P02**Feeding Retinoic Acid Reduces Total Carcass Fat in Growing Broiler****Chickens.** T.D. Brandebourg¹ and C.Y. Hu². ¹Department of Animal Sciences, Auburn University, AL; ² College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, HI

All-trans retinoic acid (RA), the carboxylic form of vitamin A, inhibits fat cell differentiation *in vitro* in multiple mammalian species. However, the ability of RA to limit adipose tissue accretion in meat-producing animals has not been investigated. The objective of this study was to determine the effect of feeding RA on total carcass lipid and tissue concentrations of RA in growing broilers. In the first experiment, 48 one-day-old birds were fed either a commercial starter-grower diet or the commercial diet supplemented with 14 mg/kg RA for six weeks. Retinoic acid decreased weight gain (13%; $P < .001$), feed intake (10%; $.0003$), average daily gain (12%; $.0001$), and carcass weight (13%; $P < .0001$). While RA only tended to decrease abdominal fat mass (12%; $P < .07$), total carcass lipid was significantly decreased by 14% versus control birds ($P < .008$). In the second experiment, 36 one-day-old chicks were randomly assigned to either the control or RA-supplemented diet (14 mg/kg) and six chicks from each treatment were sacrificed on days 28, and 42. Plasma, liver and adipose tissue RA concentrations were determined using HPLC. Liver RA concentrations were three-fold higher in birds consuming the RA-supplemented diet ($P < .02$) versus birds fed the control diet. Retinoic acid supplementation did not significantly affect RA concentration in adipose tissue ($P < .21$). However, RA concentrations were numerically lower in birds fed the RA-supplemented diet on each day measured. Expression of retinoic acid receptors, retinoic acid receptor alpha (RAR α) and retinoid X receptor alpha (RXR α) was verified in adipose tissue by semi-quantitative PCR. These results suggest that feeding RA can reduce carcass adiposity. However this approach may be limited by physiological mechanisms that constrain retinoic acid accumulation in adipose tissue. Better characterization of these mechanisms may reveal targets that could be useful for reducing carcass fat in growing broilers either through selection programs or the administration of exogenous effectors.

P03

Protein Phosphatase PPM1A is Involved in Regulating Pregnane Xenobiotic Receptor-Mediated *CYP3A4* Gene Expression in HepG2 Human Hepatocarcinoma Cells. Patrick Flannery, Kodye Abbott,

Satyanarayana Pondugula. Department of Anatomy, Physiology and Pharmacology, Auburn University, AL

Cytochrome P450 3A4 (CYP3A4) is the most prevalent drug-metabolizing enzyme in human liver hepatocytes that catalyzes the metabolism of more than 50% of clinically used drugs. Human pregnane xenobiotic receptor (hPXR), a ligand-dependent transcription factor, plays a central role in activating the expression of CYP3A4. Expression of CYP3A4 is highly variable depending on the physiological or pathological status of liver. Changes in the expression of CYP3A4 can alter the therapeutic or toxicologic response to a drug and potentially lead to life-threatening adverse drug reactions. However, the molecular mechanisms that contribute to altered CYP3A4 expression are poorly understood. In the current study, we sought to determine whether Mg²⁺/Mn²⁺-dependent phosphatase 1A (PPM1A) regulates hPXR-mediated CYP3A4 expression in HepG2 human liver hepatocarcinoma cells. Overexpression of hPXR and PPM1A was accomplished by transfecting the cells with the respective plasmids, whereas knockdown of endogenous PPM1A expression was achieved by transducing the cells with lentiviral particles carrying the specific shRNA. Transactivation assays and mammalian two-hybrid assays were performed to study *CYP3A4* promoter activity and hPXR-steroid receptor coactivator-1 (SRC-1) interaction, respectively. Overexpression of PPM1A significantly enhanced hPXR-mediated *CYP3A4* promoter activity as well as hPXR interaction with SRC-1. In contrast, knockdown of endogenous PPM1A attenuated both hPXR-mediated *CYP3A4* promoter activity and hPXR interaction with SRC-1. These preliminary results implicate a novel role for PPM1A in regulating hPXR activity and *CYP3A4* expression in liver hepatocytes. In addition, these observations suggest the hypothesis that deregulation of PPM1A expression may contribute to altered hPXR activity and *CYP3A4* expression in hepatocytes.

Acknowledgements: This research was supported by Auburn University Start-up Funds (S.R.P) and Auburn University Animal Health and Disease Research Grant (S.R.P).

P04**Establishing a Lean and Obese Mangalica Pig as a Translational Model for Juvenile Obesity and Metabolic Syndrome.** C.F. Garrett¹, R.H. Amin², C.L. Bratcher¹, E.P. Cambier¹, J.L. Bartosh¹, and T.D. Brandebourg¹.

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Alabama is at the epicenter of an obesity epidemic precipitating increased incidences of type 2 diabetes and heart failure. However, no effective strategies exist for intervention or long-term prevention of obesity in susceptible individuals, thus, better understanding of links between obesity, diabetes, and heart failure has become of paramount importance. To study the underlying mechanisms linking obesity and diabetes, the Mangalica pig was imported to Auburn University for use as a translational model of juvenile obesity given its extreme, early onset, morbidly obese phenotype. Obese or lean groups were created by either allowing *ad libitum* access to feed or restricting energy intake to 65% of *ad libitum* levels. Following 100 days of feeding, obese Mangalicas exhibited 2.5-fold greater subcutaneous (SC) adipose tissue mass but no differences in muscle mass compared to their lean Mangalica counterparts. Preliminary gene expression studies indicate leptin and TNF α mRNA expression levels are 110% and 154% higher in SC of obese versus lean Mangalica suggesting the extreme adiposity of the Mangalica is associated with the development of an inflammatory state. Obese Mangalica pigs exhibit severe fasting hypoglycemia, and altered liver enzyme levels consistent with development of hepatic dysfunction. Obese Mangalica pigs also displayed impaired glucose tolerance compared to their lean counterparts following oral glucose challenge, suggesting development of obesity-induced insulin resistance. Studies to characterize cardiovascular parameters in Mangalica pigs have been initiated at the Auburn University MRI Research Center. Conditions have been established to image left ventricle thickness and ongoing studies are being conducted to characterize the effect that emerging obesity has upon ventricular wall thickening, systolic and diastolic ejection volumes and vessel wall thickness in order to assess obesity-induced cardiovascular phenotypes. The melanocortin-4 receptor (MC4R) gene has been cloned from Mangalica pigs and allelic variation has been analyzed across 19 unrelated pigs to date. Mutations in MC4R have been associated with increased adiposity and feed intake in rodent models. Taken as a whole, these data provide evidence that obese Mangalica pigs indeed have a metabolic phenotype that mimics juvenile obesity-like characteristics, as evidenced by impaired glucose homeostasis and a proinflammatory shift in gene expression that is consistent with the development of frank diabetes.

P05

Oxidative Stress in Hearts of Mice Feed a High Fat Diet. Gorman Teresa, Schwartz Dean, Judd Robert, Wanders Desiree. Department of Anatomy, Physiology, Pharmacology, College of Veterinary Medicine, Auburn, AL

Evidence suggests that excess superoxide generation leading to oxidative stress and/or the reduced capacity of organisms to regulate oxidative/redox environments play a major role in the initiation and progression of obesity related diseases. Oxidative stress contributes to the progression of numerous diseases throughout the body, including the cardiovascular system. The purpose of our study was to examine the effects of niacin on antioxidant gene expression and activity in a mouse model of obesity. Niacin, also known as vitamin B3, is a drug that has been used for over 50 years and is known for its lipid-lowering effects, helping to reduce symptoms of high cholesterol, high blood pressure, and diabetes.

Mice were fed high fat (60% kcal as fat) or normal diets for 11 weeks (10% kcal fat) as part of a separate study. Niacin was added to the drinking water to half of the mice for the last 5 weeks. At the end of the study, hearts were rapidly frozen in liquid nitrogen for the determination of sirtuin (sirt), glutathione peroxidase (Gpx-1), superoxide dismutase (SOD), and catalase (CAT) gene expression and superoxide dismutase activity and protein carbonylation. Mice fed the high fat diet had significantly increased body weights. There was no change in the expression of SOD-1, GPx, and CAT gene expression as determined by real-time pcr in the hearts of any of the groups compared to normal diet. There was also no change in SOD activity or protein carbonylation in the hearts of high fat and normal diet mice. A significant increase in the gene expression of sirt1 and sirt5 was seen in the high fat diet compared to normal diet. Niacin alone also caused an increase in sirt1 gene expression. Expression of sirt1 and sirt5 in the hearts of the niacin plus high fat diet were not significantly different from normal diet.

Mice on a high fat diet for 11 weeks did not show alterations in antioxidant gene expression, SOD activity or protein carbonylation. Increases in sirt1 and sirt5 may be related to these protective effects on oxidative stress in the high fat feed mice.

P06

Rescue of Intracellularly Retained Human Melanocortin-4 Receptor Mutants. Hui Huang and Ya-Xiong Tao. Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL 36849

The melanocortin-4 receptor (MC4R) is a G protein-coupled receptor (GPCR) primarily expressed in the central nervous system. It is a critical regulator of energy homeostasis, regulating both food intake and energy expenditure. Mutations of *MC4R* gene have been identified as the most common cause of monogenic obesity. Up to now, more than 150 naturally occurring mutations or common alleles of the *MC4R* have been identified. Most of the inactivating mutants are defective in intracellular forward trafficking. Some of these mutants can activate G proteins once expressed on the cell surface. In this study, we investigated whether small molecule MC4R ligands could act as pharmacological chaperones promoting the proper folding of intracellularly retained MC4R mutants in Neuro2A cells. Three MC4R ligands including two antagonists (Ipsen 5i and ML00253764) and one agonist (THIQ) were studied. All three ligands are specific for MC4R and can cross the blood brain barrier. Fourteen human MC4R mutations were studied, including ten (N62S, I69R, P78L, C84R, G98R, Y157S, W174C, P260Q, F261S, and C271Y) that are retained intracellularly, and four (Δ 88-92, D90N, I102S, and N274S) that are expressed normally on the cell surface but defective in ligand binding (Δ 88-92) or signaling (D90N), or retains partial (I102S) or normal (N274S) function. Neuro2A cells transiently transfected with the empty vector, WT or mutant receptors were treated with the small molecules for 24 h, and then the maximal cAMP production stimulated by NDP-MSH were measured using radioimmunoassay. Different concentrations of Ipsen 5i or THIQ were used to treat intracellularly retained MC4R mutants, and the optimal concentration, 10^{-6} M Ipsen 5i and 10^{-5} M THIQ, were identified. With the treatment of 10^{-6} M Ipsen 5i, seven mutants (N62S, I69R, P78L, C84R, W174C, P260Q, and C271Y) rescued by this small molecule were functional in cAMP production; for most of these mutants, the maximal cAMP production was increased to similar level as that of the WT. With 10^{-5} M THIQ treatment, six mutants (N62S, P78L, C84R, W174C, P260Q, and C271Y) restored function in cAMP production. With 10^{-5} M ML00253764 treatment, four mutants (N62S, C84R, W174C, and C271Y) restored function in cAMP production. None of these three small molecules had effect on the four control mutants. In summary, we identified three small molecule ligands that could act as pharmacological chaperones, rescuing intracellularly retained MC4R mutants in neuronal cells. These results will be useful in research towards personalized medicine for obese patients carrying *MC4R* mutations.

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The AU Transgenic Facility is a core facility available to the university community located within the College of Veterinary Medicine. The AUTF mission is to provide Auburn University investigators with genetically engineered mice and related technologies used in basic science research. Transgenic mice are extremely valuable in studies of gene function. The process by which a single cell, the zygote, develops into the wide range of cell types in the body is the result of very specific and coordinated gene expression. Transgenic technology is also very useful for production of animal models of human diseases, to study the processes by which genes cause disease, and in understanding mechanisms by which a change in gene structure or expression may cause cancer or AIDS. Other uses for transgenic animals include drug and product testing, development of gene therapies, and production of drugs or other biologically important molecules (“molecular pharming”).

The AUTF offers the following services:

- **DNA Microinjection:** 150 ova are injected per “project”. Comprehensive service including genotyping and a 3 transgenic mouse guarantee (with putatively non-lethal transgenes) is provided. At this time, B6SJLF2, ICR, C57BL/6 or FVB genetic backgrounds can be accommodated and 12 or more offspring will be generated for analysis by the investigator, or a 2nd round of injection will be performed. Other strains can be accommodated.
- **ES Cell Transfer:** 20-40 blastocysts are injected with an investigator’s ES cell clone per “project”; 12 or more offspring or 3 (>50%) chimeras will either be delivered or the cell line will be reinjected.
- **Gene Targeting:** A comprehensive gene-targeting service includes electroporation of an investigator’s vector, clone selection, propagation, analysis (Southern or PCR), and chimera production.
- **Line Rederivation** (using donor males in IVF): Costs are based on superovulating 12 females, with fertilized ova transferred to a maximum of 3 synchronized recipients. Health surveillance data will be obtained prior to delivery.
- **Embryo Cryopreservation** (using donor males in IVF): Fifteen females are superovulated, with resultant fertilized ova stored for up to one year. Embryo recovery, culture, and transfers within one year to a maximum of 3 recipients are included in this service.
- **Sperm Cryopreservation** (using 2-4 donor males): Mouse spermatozoa will be harvested and cryopreserved. Chargeback for any test IVF protocols is not included.

For further information, please contact Dr. Irwin (mhi0001@auburn.edu)

P08

Induction of CXCR7 Switches SDF-1 Signaling and Phagocytic Function in Macrophages: a Potential Role in Atherosclerosis. Wanshu Ma, Yiwei

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Objective: The discovery of CXCR7 as a new receptor for SDF-1 places many previously described SDF-1 functions attributed to CXCR4 in question, though whether CXCR7 acts as a signaling or “decoy” receptor has been in debate. It is known that CXCR7 is not expressed in normal blood leukocytes; however, the potential role of leukocyte CXCR7 in disease states has not been addressed.

Methods and Results: We show that CXCR7 was detected in macrophage-positive area of aortic atheroma of ApoE-null mice, but not in healthy aorta. During monocyte-to-macrophage differentiation, CXCR7 was up-regulated at mRNA and protein levels, with more expression in M1 than in M2 phenotype. In addition, CXCR7 induction was associated with a SDF-1 signaling switch from pro-survival ERK and AKT pathways in monocytes to pro-inflammatory JNK and p38 pathways in macrophages. The latter effect was mimicked by a CXCR7-selective agonist TC14012 and abolished by siRNA knockdown of CXCR7. Furthermore, CXCR7 activation increased macrophage phagocytic activity, which was suppressed by CXCR7 siRNA silencing or by inhibiting either the JNK or p38 pathways.

Conclusions: CXCR7 is induced during monocyte-to-macrophage differentiation, which is required for SDF-1 signaling to JNK and p38 pathways, leading to enhanced macrophage phagocytosis, thus possibly contributing to atherogenesis.

P09

PPAR Delta Mediated Cardioprotection Against Hypertrophy in the Diabetic Heart Requires Frataxin. Shravanthi Mouli, Gayani Nanayakkara and Rajesh Amin. PPAR and Metabolic Research Lab, Dept. of Pharmacal Sciences, Harrison School of Pharmacy, Auburn University, AL

Type 2 diabetes is at epidemic proportions today with the major form of fatality is associated from heart failure. The progression of the diabetic heart to failure centrally involves myocardial energy dysregulation and the compensatory cardiac hypertrophy. Derangements in cardiac mitochondrial energetics contribute significantly to the development of diabetic cardiomyopathy and the ensuing heart failure. More specifically, in the failing diabetic heart, attenuated tricarboxylic acid (TCA) cycle activity has been observed. The nuclear class of hormone receptors, peroxisomal proliferator activating receptors (PPAR)-delta is known to improve cardiac bioenergetics, thus protects the heart against cardiac hypertrophy. However the direct molecular signaling targets for cardioprotection by PPAR δ is not well known. We have recently found that ligand activation of PPAR δ results in an increase in the mitochondrial iron-sulfur cluster regulating protein frataxin. This nuclear encoded mitochondrial protein is a key player in the regulation of iron metabolism and storage within the mitochondria. Furthermore frataxin is centrally involved in regulation of energy by mediating the activity of aconitase, which is an important enzyme in the TCA cycle. Friedreich's ataxia (FRDA), an autosomal recessive disease results from an expansion of GAA tri-nucleotide repeat in the intron 1 of the frataxin gene. The majority of FRDA patients develop diabetes and cardiac hypertrophy which manifests into heart failure. Further, we observed the development of cardiac hypertrophy in diabetic (db/db) mice; interestingly these hearts also exhibited reduced levels of frataxin protein levels. Therefore, we hypothesize that therapeutically improving frataxin expression by PPAR δ will protect against development of cardiac hypertrophy in the diabetic heart. Our preliminary data supports our hypothesis by demonstrating that PPAR δ agonist improved mRNA and protein expression of frataxin in H9C2 cardiomyocytes and fibroblasts from human FRDA patients. To better understand these results, we explored various promoter regions of frataxin for specific PPAR recognition elements by utilizing *in-silico* studies and promoter activity assays. Lastly we found that PPAR δ agonist protects cardiomyocytes against doxorubicin induced cardiac hypertrophy. Our future work will focus to elucidate cardioprotective mechanisms of PPAR δ activation in the diabetic frataxin deficient mouse model against the development of cardiac hypertrophy and the ensuing heart failure.

P10**PPAR γ Activation Improves the Molecular and Functional Components of I_{to} Remodeling by Angiotensin II.** Gayani Nanayakkara¹, Nilmini

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Diabetic cardiomyopathy is known to manifest cardiac arrhythmias and is the major cause of mortality among diabetics. These life threatening cardiac arrhythmias and electrophysiological abnormalities in diabetic myocardium is evident by prolonged QT interval in electrocardiogram (ECG). Attenuation of outward potassium current (I_{to}) prolong the QT interval in the mouse myocardium. Previous reports demonstrate that diabetes impair I_{to} by attenuating the expression of pore forming subunits of the channel which causes a reduction in potassium current amplitude. Angiotensin II (AT II) can profoundly affect the expression of molecular components of I_{to} . Hyperglycemic conditions have been shown to increase cardiac derived AT II expression, and thus may be responsible for the attenuated I_{to} in the diabetic myocardium. Therefore, therapies that improve I_{to} may exert a profound protection against development of life threatening cardiac arrhythmias in the diabetics. In the current study we demonstrate that peroxisomes proliferator activated receptor-gamma (PPAR γ) activation by pharmacological agents exert a profound protection against AT II mediated attenuation of I_{to} . We observed that PPAR γ activation significantly suppresses AT II induced NADPH oxidase activity and ensuing reactive oxygen species (ROS) production in cardiomyocytes. This antioxidant effect of PPAR γ activation significantly improved the membrane association of molecular components of I_{to} in isolated cardiomyocytes as well as in diabetic (db/db) mouse heart. Furthermore, ECG recordings from db/db mice treated with PPAR γ agonists demonstrated improved QT intervals. Interestingly, by quantitative PCR, we also observed a reduction in expression of AT II receptors in db/db hearts treated with PPAR γ agonists. Therefore, short term PPAR γ activation in the diabetic heart offers protection against AT II induced alteration in the I_{to} and ECG in diabetic patients.

P11

Modulation of Autoimmune Diabetes by Antibodies Specific for N-acetyl-D-glucosamine. J. Stewart New, Brian L.P. Dizon, John F. Kearney. Immunology program, University of Alabama, Birmingham, AL

The increasing incidence of autoimmune disease in developed societies has been linked with decreased exposure to environmental antigens, implying that antigen exposure modifies immune system development. Vaccination with Group A Streptococci (GAS) produces a strong antibody response to N-acetyl-D-glucosamine (GlcNAc). This GlcNAc moiety is conserved in mammals, and GlcNAcylated proteins are enriched in pancreatic β -cells. Anti-GlcNAc antibodies generated against GAS bind GlcNAc epitopes in human and murine β -cells. Developmental remodeling of the pancreas is accompanied by significant β -cell apoptosis, which may serve as an initial source of autoantigen priming in Type 1 Diabetes (T1D). We therefore hypothesize that anti-GlcNAc IgM generated during GAS infection mediates non-inflammatory clearance of apoptotic β -cell antigens. Using the Min6 insulinoma cells, we demonstrate that β -cell apoptosis results in surface exposure of GlcNAc residues reactive with anti-GAS Abs. During DC priming with irradiated β -cells anti-GlcNAc Abs suppressed CD4 T cell activation and cytokine production. We show that neonatal immunization with GAS, but not Group C Streptococci, reduces the incidence of diabetes in female NOD-mice. Furthermore, passive transfer of anti-GlcNAc Abs protects NOD.Rag1ko mice from diabetes onset following adoptive transfer of diabetogenic BDC2.5 T-cells. Our data suggest that anti-GlcNAc Abs generated by vaccination with GAS can suppress development of T1D.

P12**Antioxidant-Mediated Reversal of Oxidative Damage from Rotenone-induced Mitochondrial Complex I Inhibition in the Mouse Hippocampus.**

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Mitochondrial dysfunction is known to be a major factor in many age-related diseases with dementia, as well as in age related memory decline. However, the specific role played by complex I (CI) of the mitochondrial respiratory chain in the physiological processes regulating memory is not well understood. To address this issue, rotenone-induced oxidative damage on excitatory synaptic physiology and the beneficial effects of an antioxidant were evaluated in the mouse hippocampus; a primary brain region regulating memory formation. Rotenone (400 mg/kg/day) was administered orally to female ICR mice (3-4 mo of age) for one week. A separate group of mice were co-administered a synthetic lipoylcarnitine antioxidant (PMX-500F). Control mice were administered vehicle only (0.5% carboxymethyl cellulose solution). Electrophysiological recordings were performed in fresh brain slices, and whole hippocampal homogenates were used for western blot analysis. Long term potentiation (LTP) was reduced by rotenone exposure ($P < 0.05$). Potentiation during theta burst stimulation (TBS) was similar among the treatment groups ($P > 0.05$). Neurotransmitter release, which increased after TBS, was lower in the rotenone treated mice ($P < 0.05$). In rotenone-treated mouse hippocampi, these changes were accompanied by reduced basal synaptic transmission ($P < 0.05$), increased BAX translocation to mitochondria ($P < 0.05$), decreased BAD phosphorylation ($P < 0.05$) and decreased ERK1/2 phosphorylation ($P < 0.05$). Co-treatment with PMX-500F normalized these effects to control levels ($P > 0.05$). These results show that an antioxidant can provide a protective effect against rotenone-induced impairment in the mouse hippocampus, in both excitatory synaptic physiology and by activation of proapoptotic processes.

P13**Residual Feed Intake Studies in Cattle Reveal a Potential Role for Gonadotropin Releasing Hormone (GnRH) in Regulating Feed Efficiency.** S.D. Perkins, C.N. Key, C.F. Garrett, C.D. Foradori, C.L.

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Residual feed intake (RFI) is a heritable feed efficiency measure. Mechanisms underlying variation in feed efficiency are currently poorly understood while the relationship between RFI and carcass composition is unknown. To address these issues, forty-eight Angus-sired steers were trained to the Calan Gate (Northwood, NH) system at the Beef Evaluation Unit at Auburn University to facilitate measurement of individual feed intake. Daily feed intake and RFI were assessed during a 70 day feeding trial. The test diet was 50% sorghum-sudan silage, 50% grain (2.9 Mcal ME/kg DM). Feed intake was recorded daily while body weights and hip heights were recorded at 14 day intervals. Ultrasound measurements of rib eye muscle area (REA) and subcutaneous backfat (BF) were recorded initially and prior to slaughter. Upon completion of the feeding trial, RFI was calculated for each animal as the difference between actual feed intake and the expected intake to create two divergent cohorts consisting of High (H) and Low (L) RFI individuals. Steers were humanely harvested and subcutaneous adipose tissue (SC), trigeminal ganglion (TG) and hypothalamic tissue (HT) samples were collected, immediately frozen and stored at -80 °C in order to facilitate targeted gene expression and microarray studies into the mechanisms underlying variation in RFI. After chilling for 24 hours post harvest, carcass characteristics were measured. Carcass and growth data were analyzed using a mixed model with RFI level (L, H) as the independent variable (SAS, 2002). Means were separated using lsmeans at a significance level of $P < .05$. The lsmeans for RFI were -1.3 and 1.5 respectively for the L and H cohorts ($P < .001$) and were greater than two standard deviations apart indicating two divergent cohorts were indeed created. As expected feed intake was higher for the H individuals versus the L steers ($P < .001$) while on test gain was not different between the two groups. There were no differences in intramuscular fat, subcutaneous fat thickness, or loin muscle area (REA) between L and H cohorts suggesting there is no relationship between RFI and body composition. Targeted gene expression studies in the arcuate nucleus indicate that neuropeptide-Y (NPY), relaxin-3 (RLN3), melanocortin 4 receptor (MC4R), and GnRH mRNA expression was 64%, 59%, 58%, 86% lower respectively in the arcuate nucleus of low RFI steers while Pro-opiomelanocortin (POMC) expression was 350% higher in these more efficient animals. Expression levels of agouti-related protein (AGRP), relaxin/insulin-like family peptide receptor 1 (RXFP1), and melanocortin 3 receptor (MC3R) mRNA were similar between low and high RFI animals. Pituitary expression of gonadotropin beta subunits (FSH β , LH β) was significantly correlated to hypothalamic GnRH levels suggesting changes in gene expression in the arcuate nucleus indeed had functional consequences. Interestingly, these expression profiles suggest GnRH may play a role in regulating feed efficiency. Overall, these data support the hypothesis that differences in orexigenic neuropeptide expression in the arcuate nucleus underlie variation feed intake and feed efficiency.

P14

Resveratrol Protects the Oxidative Damage in the Brain of ob/ob Mice.

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Resveratrol is a polyphenolic phytoalexin and known to exert anti-diabetic actions. Several studies have demonstrated a link between diabetes and Alzheimer's disease. The present study evaluates the neuroprotective action of resveratrol on obese (ob/ob) mice induced oxidative stress. Resveratrol was administered orally at the dose of 25 mg kg⁻¹ body weight daily for 3 weeks to lean and obese (ob/ob) mice. The lipid peroxide was significantly increased in brain of obese mice. The enzymic antioxidants like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and non-enzymic antioxidants like tocopherol, ascorbic acid and glutathione were decreased in obese mice brain. Administration of resveratrol decreased the lipid peroxide levels and up-regulated the antioxidant activities in obese mice brain. These findings suggest the neuroprotective effect of resveratrol by improving the oxidative damage in brain tissue of obese mice.

P15

Culture and Characterization of Late Outgrowth Endothelial Colony Forming Cells in Adult Horses. Salter M.S.¹, Seeto W.J.², Lipke E.A.²,

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Introduction: Endothelial progenitor cells (EPCs), derived from bone marrow stem cells, circulate in peripheral blood and function in vascular homeostasis and repair. EPCs are being used as biomarkers for metabolic and cardiovascular diseases in humans by looking at number and function of cells. Similarities exist between human metabolic disease and equine metabolic syndrome, (EMS), with chronic laminitis and poor vascularization of the hoof a common outcome. This study sought to culture a subgroup of EPC's, late outgrowth endothelial colony forming cells (LOCs), from healthy horses. Cultured equine cells were characterized using phenotypic assays of vascular tube formation in Matrigel™ and acetylated low density lipoprotein (Di-Ac-LDL) uptake and functional assays using two cell surface markers, Von Willebrand Factor and CD14.

Materials and Methods: A 5 mL peripheral blood sample from 10 horses and 20 mL bone marrow sample from 2 horses were collected. Samples were cultured in endothelial growth medium with a whole blood method, (peripheral samples) or density gradient centrifugation, (Ficoll Paque™ plus), method (bone marrow). Colonies were counted, harvested, expanded and used for characterization assays. Vascular tube formation in Matrigel™ was assessed after 5 and 24 hours in culture and number of branch points recorded. For the Di-Ac-LDL uptake assay, cells from one horse were cultured with fluorescent labeled Di-Ac-LDL for 6 hours, counterstained with 4', 6-diamidino-2-phenylindole and imaged. Human LOCs served as a positive control and 3t3 fibroblast cells served as the negative control for both assays. Standard immunoblotting procedures for Von Willebrand Factor and CD14 were performed.

Results: All data are expressed as mean ± standard deviation. Two out of ten horses produced colonies from peripheral blood samples, (5 col/mL ± 1.7) and bone marrow samples from both horses produced colonies, (1.9 col/mL ± 1.3). Cells had characteristic single layer cobblestone morphology and significant outgrowth upon expansion. Equine and human LOCs formed vascular tubes. Equine cells from both peripheral and bone marrow blood samples showed vascular tube and branch formation (39.29 ± 29.22). Equine and human LOCs showed an uptake of Di-Ac-LDL whereas 3t3 fibroblast cells did not uptake Di-Ac-LDL or form vascular tubes. Equine EPCs were positive for Von Willebrand Factor and negative for CD14 cell surface markers.

Discussion and Conclusions: Based on the results from this study, LOCs can be isolated and cultured from peripheral and bone marrow blood samples of healthy horses. Information from this study will aid in future research investigating additional sampling and characterization methods. The biology of EPCs in the horse may be useful in understanding the pathogenesis of EMS. Additional areas of interest are combining equine LOCs with bioengineered materials to aid in additional therapeutic treatment.

P16

AURIC: The Auburn University Research Initiative in Cancer. Bruce F. Smith, V.M.D., Ph.D., Director, Auburn University Research Initiative in Cancer, Department of Pathobiology and Scott-Ritchey Research Center, Auburn University, AL

The Auburn University Research Initiative in Cancer (AURIC) is a new initiative at Auburn designed to foster an environment of excellence in cancer research in order to better both human and animal health. AURIC works through a variety of approaches to improve investigator access to needed resources for cancer research. This poster describes what AURIC offers to students and faculty, as well as how to become a member of the program.

P17

Interaction of IRS-1 with Nerve Growth Factor Receptor TrkA. Geetha Thangiah[†], Shraddha D. Rege[†], Salome E. Mathews[†], Susan Meakin[¶], and Ramesh B. Jeganathan[†]. [†]Department of Nutrition, Dietetics and Hotel Management, Auburn University, Auburn, AL 36849; [¶] Molecular Brain Research Group, Laboratory of Neural Signaling, Roberts Research Institute, University of Western Ontario, London, ON, Canada, N6A 5K8

Diabetes has an impact on central nervous system with impairment in learning, memory and problem solving ability. Neuronal loss and cerebral cortex degeneration has been reported in diabetic patients. Diabetes has been associated with an increased risk of developing AD. Nerve growth factor (NGF) plays a crucial role in differentiation, survival, maintenance of sensory and sympathetic neurons. Two cell surface receptors for NGF have been identified: TrkA and p75. Here we show that tyrosine phosphorylation sites in TrkA sequence are similar to insulin receptor. Binding of NGF to TrkA induces autophosphorylation, and interaction of IRS-1. The interaction of IRS-1 with TrkA requires the kinase activity of TrkA. Moreover, the activation of Akt and MAPK is dependent upon TrkA kinase domain. These results suggest that TrkA receptor may be involved in insulin signaling.

P18

Suppressive effects of omega-3 Stearidonic fatty acid on human prostate cancer cell viability. Caitlin Hellmich Trebelhorn¹, John C. Dennis¹, T. Samuel², Satya Pondugula¹, Elaine Coleman¹, Edward

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Prostate cancer (PCa) is a leading cause of cancer deaths among men in the United States. The marine-based omega-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid [EPA; 20:5 (n-3)] and docosahexaenoic acid [DHA; 22:6 (n-3)], have antitumorigenic properties but are inefficiently converted from their primary metabolic precursor, alpha-linolenic acid (ALA; 18:3 (n-3)). Stearidonic acid [SDA; 18:4 (n-3)], a plant derived omega-3 abundant in echium and borage oils, is an EPA and DHA precursor that humans can metabolize making it an alternative source to marine-based PUFAs. We treated cultures of three human prostatic cell lines with SDA to determine its effect on cell viability, androgen receptor (AR) expression, and Nuclear Factor Kappa B (NF-kB) activity. We found that SDA at 50 micromole/L and higher concentrations killed androgen-dependent LNCaP and androgen-independent PC3 cancer cell lines but not RWPE1 normal prostate epithelial cells. The cell death was significantly higher in PC3 compared with LNCaP cells. Immunocytochemistry showed that SDA treated LNCaP cells down regulated testosterone-induced AR expression. Likewise, SDA treatment decreased TNF-alpha-induced NF-kB activity in LNCaP cells as determined by a luciferase colorimetric assay. These results suggest that SDA could be used as a cytotoxic dietary-supplement for PCa treatment particularly in the difficult to treat androgen-independent PCa.

Key words: Stearidonic acid (SDA), androgen receptor (AR), Nuclear Factor Kappa B (NF-kB), prostate cancer (PCa) Research supported by Merial and the Animal Health and Disease Research Program (CVM-AHDR)

P19

Niacin Increases Adiponectin and Decreases Markers of Adipose Tissue Inflammation in Obese Mice.

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The goal of this study is to determine the effects of niacin on adiponectin and markers of adipose tissue inflammation in an obese mouse model. Male C57BL/6 mice were placed on a control or HFD and were maintained on such diets for the duration of the study. After 6 weeks on the control or high fat diets, vehicle or niacin treatments were initiated for 5 weeks of treatment. Identical studies were conducted concurrently in mice that lack the niacin receptor (PUMA-G^{-/-} mice). Niacin increased serum concentrations of the anti-inflammatory adipokine, adiponectin by 21% in obese wild-type mice, but had no effect on lean wild-type or lean or obese PUMA-G^{-/-} mice. Niacin increased adiponectin gene and protein expression in the obese wild-type mice only. The increases in adiponectin serum concentrations, gene and protein expression occurred independently of changes in expression of PPAR γ C/EBP α or SREBP-1c (key transcription factors known to positively regulate adiponectin gene transcription) in the adipose tissue. Further, niacin had no effect on adipose tissue expression of ERp44, Ero1-L α , or DsbA-L (key ER chaperones involved in adiponectin production and secretion). However, niacin treatment attenuated HFD-induced increases in adipose tissue gene expression of MCP-1 and IL-1 β in the wild-type obese mice. Niacin also reduced the expression of the pro-inflammatory M1 macrophage marker CD11c in obese wild-type mice. In conclusion, niacin treatment attenuates obesity-induced adipose tissue inflammation through increased adiponectin and anti-inflammatory cytokine expression and reduced pro-inflammatory cytokine expression in a niacin receptor-dependent manner.

P20

Dietary Methionine Restriction Attenuates the Development of Age-Associated Inflammation. Desiree Wanders, Nancy T. Van, Thomas W. Gettys. Laboratory of Nutrient Sensing and Adipocyte Signaling, Pennington Biomedical Research Center, Baton Rouge, LA 70808

Introduction and Objective: Dietary methionine restriction (MR) extends lifespan by delaying all causes of death, reduces body weight and adiposity and improves insulin sensitivity in rodents, in the face of hyperphagia. Nearly all age-associated pathologies share an inflammatory etiology. The objective of the current investigation was to examine the tissue-specific effects of dietary MR initiated in juvenile and adult animals on age-induced inflammation. **Methods:** *Experiment 1:* 47 male juvenile (4 wk of age) F344 rats were placed on Control (0.86% methionine) or MR (0.17% methionine) diets for 3 (n=8 CON, n=8 MR), 9 (n=8 CON, n=8 MR), or 20 (n=7 CON, n=8 MR) months. *Experiment 2:* 40 male adult (5 mo of age) F344 rats were placed on CON or MR diets for 3 (n=10 CON, n=10 MR) or 6 (n=10 CON, n=10 MR) months. Following dietary regimens, animals were sacrificed and retroperitoneal, epididymal, and inguinal white adipose tissue (RPWAT, EWAT, and IWAT, respectively), and liver were collected and examined for markers of inflammation through quantitative RT-PCR. **Results:** MR limited body weight and fat accretion in juvenile and adult animals. In juvenile animals, the most pronounced anti-inflammatory effects of MR occurred at 20 months in the RPWAT, followed by liver, EWAT, and IWAT. While we do not have corresponding 20 month MR data initiated in adulthood, we demonstrate that 6 months of dietary MR initiated in adult rats produced the most profound anti-inflammatory effects in the EWAT, followed by liver, RPWAT and IWAT. Interestingly, it appears that rather than reducing expression of inflammatory cytokines or immune cell markers, MR “clamps” the inflammatory state of the tissues, while tissues of Control animals become more inflamed as the aging process progresses. **Conclusions:** Dietary MR attenuates age-induced inflammation in juvenile and adult rats, as evidenced by a MR-induced stasis in inflammatory status of various tissues analyzed, while inflammatory state of tissues from Control animals progressed with age.

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A β Impairs TrkA Polyubiquitination and Activation of MAPK and Akt.

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Amyloid β protein (A β or Abeta) is a peptide (36–43 amino acids) derived by the β - and γ -secretase cleavage of amyloid precursor protein (APP). It is well known as a component of amyloid plaques in association with Alzheimer's disease (AD). A number of evidence suggest that A β plays a central role in the development of AD pathology. Here, we show that A β impairs TrkA polyubiquitination. A β leads to disassociation of TrkA with p75^{NTR} and p62/TRAF6 complex, subsequently impairing TrkA ubiquitination in the PC12 cells and human hippocampal brain tissues. TrkA signaling events, including Ras/MAPK and PI3K/Akt pathways, is deactivated with A β . These findings demonstrate that A β blocked the association of TrkA with p75^{NTR}, leading to inactivation of TrkA signaling pathway.



Jim Fyffe Diabetes Research Fund

The late Jim Fyffe, "Voice of the Tigers" for 23 years, continues to influence Auburn fans and alumni through a diabetes research fund bearing his name. Fyffe suffered from diabetes and died suddenly of a brain aneurysm in May 2003.

The Jim Fyffe Diabetes Research Fund at Auburn University honors the longtime broadcaster by supporting graduate students conducting diabetes research and by providing funding for diabetes research projects. The fund was established by Auburn University, Auburn Network and Jim Fyffe's widow, Rose Fyffe.

"In talking with the university and his family, we felt this would be a great way to honor Jim and help others who are searching for a cure to this disease," said Jon Cole, vice president of the Auburn Network.

Donations go toward diabetes research in the Boshell Diabetes and Metabolic Diseases Research Program. The program is funded through an endowment established in 2001 by the National Diabetes Association, formerly the Diabetes Trust Fund, in honor of its founder, the late Dr. Buris R. Boshell, a noted diabetes researcher who attended the veterinary college before enrolling in medical school.

"Jim Fyffe's life and untimely death have greatly increased public awareness of the devastating reality of diabetes," said Dr. Robert Judd, the AU Boshell chair. "I was always impressed with Jim's love for Auburn and for his devotion to finding a cure for diabetes."

Donations can be made to the Jim Fyffe Diabetes Research Fund and sent to the A.U. Foundation, 317 S. College St., Auburn, AL 36849.

