18th Annual Research Symposium

Answering the Call for Progress Against Diabetes

Friday, November 12, 2021
Virtual Conference

OU Health | Harold Hamm Diabetes Center

TABLE OF CONTENTS

Harold Hamm Diabetes Center Research Symposium 2021

Program Schedule

Friday, November 12, 2021

8:15 – 8:30 AM *Welcome*

Ann Louise Olson, Ph.D.
Professor of Biochemistry and Molecular Biology
Edith Kinney Gaylord Foundation Presidential Professor
Presidential Associates Presidential Professor
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

Jed Friedman, Ph.D.
Director, Harold Hamm Diabetes Center
Associate Vice-Provost for Diabetes Programs
Chickasaw Nation Endowed Chair
Professor of Physiology, Biochemistry & Molecular Biology
Professor of Pediatrics, Division of Endocrinology and Metabolism
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

ORAL PRESENTATIONS

SESSION 1
Moderator: David Sparling, M.D., Ph.D.

8:30 – 9:00 AM **Karen Jonscher, Ph.D.**
Associate Professor of Biochemistry & Molecular Biology
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

9:00 – 9:30 AM *Pathology of Pediatric Nonalcoholic Fatty Liver Disease*
Kevin Short, Ph.D., FACSM
Associate Professor of Pediatric Diabetes and Endocrinology
CHF Choctaw Nation Chair in Pediatric Endocrinology and Diabetes
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

9:30 – 10:15 AM *Maladaptive Regeneration — The Reawakening of Developmental Pathways in Obesity*
Utpal Pajvani, M.D., Ph.D.
Herbert Irving Associate Professor of Medicine
Division of Endocrinology
Columbia University

10:15 – 10:30 AM *Panel Q&A*

10:30 – 10:45 AM Break

10:45 – 12:45 PM *Student presentations and judging*

SESSION 2
Moderator: Ankur Rughani, M.D.

1:00 – 1:30 PM *Diabetes Related Complications in Youth Onset Type 2 Diabetes: Outcomes of the TODAY study*
Jeanie Tryggestad, M.D.
Associate Professor of Pediatric Diabetes and Endocrinology
Paul and Ruth Jonas Chair
Pediatric Diabetes and Endocrinology Section
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

1:30 – 2:00 PM *Targeting Nutrient Signaling in the Aging Heart*
Ann Chiao, Ph.D.
Assistant Member
Aging & Metabolism Research Program
Oklahoma Medical Research Foundation

2:00 – 2:45 PM *RNA Regulation in Islet Function and Diabetes*
Lori Sussel, Ph.D.
Professor of Pediatrics and Cell & Developmental Biology
Sissel and Findlow Stem Cell Chair
Barbara Davis Center for Diabetes
Associate Vice Chancellor for Basic Science Research
University of Colorado, Denver

2:45 – 3:00 PM *Panel Q&A*

3:00 – 3:15 PM Break

SESSION 3
Moderator: Shaoning Jiang, Ph.D.

3:15 – 3:45 PM *Diabetes Health Disparity, Genetics, and Opportunities for Translational Research*
Dharambir K. Sanghera, Ph.D., F.A.H.A.
Professor of Pediatrics Genetics
Dr. Geoffrey Altshuler Endowed Research Chair in Genetics
Director, Molecular Genetic Epidemiology Laboratory
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

3:45 – 4:15 PM *The Olive Baboon as a Non-Human Primate Model of Deciphering Mechanisms Through Which Diet and Obesity Epigenetically Program the Developing Fetus*
Dean A. Myers, Ph.D.
John W. Records Chair in Maternal Fetal Medicine
Vice Chair for Basic Research
Department of Obstetrics and Gynecology
Member, Harold Hamm Diabetes Center
Associate Vice President for Health Sciences Research
University of Oklahoma Health Sciences Center

4:15 – 5:00 PM *Diabetes Affects Cytotrophoblast, the Master Cell of the Placenta*
Kent Thornburg, Ph.D.
M. Lowell Edwards Chair
Professor of Medicine
Director, Center for Develop. Health, Knight Cardiovascular Institute
Director, Bob and Charlee Moore Institute for Nutrition & Wellness
Oregon Health & Science University

5:00 – 5:15 PM *Panel Q&A*

5:15 PM *Awards Presentation*
Ann Louise Olson, Ph.D.
Professor of Biochemistry and Molecular Biology
Edith Kinney Gaylord Foundation Presidential Professor
Presidential Associates Presidential Professor
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

VISITING SPEAKERS BIOGRAPHICAL INFORMATION

Utpal B. Pajvani, M.D., Ph.D.
Herbert Irving Associate Professor
Division of Endocrinology
Department of Medicine
Columbia University

Dr. Utpal Pajvani, Herbert Irving Associate Professor of Medicine in the Division of Endocrinology at Columbia University, is a physician-scientist with clinical and research focus in Type 2 Diabetes and related metabolic diseases. He graduated from MIT with a degree in Biology, then earned M.D. and Ph.D. degrees from the Albert Einstein College of Medicine. Dr. Pajvani completed Internal Medicine residency and fellowship training in Endocrinology, Diabetes & Metabolism at the Columbia University Medical Center.

Dr. Pajvani has been on the faculty of Columbia University since 2011. He is a teaching attending on the inpatient and outpatient Endocrinology and General Medicine services of the New York Presbyterian Hospital, and sees patients at the Naomi Berrie Diabetes Center at Columbia University. Dr. Pajvani's research focuses on the role of developmental pathways in the regulation of Type 2 Diabetes and Non-Alcoholic Fatty Liver Disease, and the use of existing therapeutic agents in other scientific areas in novel applications to ameliorate obesity-induced complications including cancer. He has received intramural and NIH support for his research, and has mentored medical and graduate students as well as postdoctoral and clinical fellows.

Dr. Utpal Pajvani's current research focuses on the role of developmental pathways in the regulation of Type 2 Diabetes, and the use of existing therapeutic agents in other scientific areas in novel applications to ameliorate obesity-induced insulin resistance.

Lori Sussel, Ph.D.
Professor of Pediatrics and Cell & Developmental Biology
Sissel and Findlow Stem Cell Chair
Barbara Davis Center for Diabetes
Associate Vice Chancellor for Basic Science Research
University of Colorado, Denver

Dr. Sussel received her graduate degree from Columbia University Medical School and pursued a postdoctoral fellowship at the University of California, San Francisco, where she studied the transcriptional regulation of CNS and pancreas development. This research led Dr. Sussel to her first faculty position at the Barbara Davis Diabetes Center at the University of Colorado where her research program focused on characterizing the conserved regulatory pathways that controlled pancreas development and islet function. In 2007, she moved to the Naomi Berrie Diabetes Center at Columbia University Medical School where she rose through the ranks to become a Tenured Professor of Genetics and Developmental Biology. During her time at Columbia, Dr. Sussel made major contributions to the understanding of the islet dysfunction that occurs during the course of diabetes. Furthermore, she pioneered the new field of long non-coding RNAs and their regulation of islet development and function. In 2016, Dr. Sussel returned to Colorado at assume the Research Director position of the Barbara Davis Center for Diabetes Research. At the University of Colorado Anschutz Medical Campus (UC-AMC) she is continuing to investigate the regulation of islet cell development, differentiation and function during normal and diabetic conditions. In addition to her research, Dr. Sussel is committed to graduate and postgraduate training and the need to promote diversity, equity and inclusion in science. In addition, she is working to establish an internationally recognized research division at the BDC focusing on all aspects of T1D research and discovery. In 2020, UC-AMC was awarded an NIH Diabetes Research Center (DRC) grant, with Dr. Sussel as Director and lead PI. Also in 2020, Dr. Sussel also became Associate Vice Chancellor for Basic Science Research at the University of Colorado to promote to the needs and perspectives of basic science researchers on a medical campus.

Kent Thornburg, Ph.D.
M. Lowell Edwards Chair
Professor of Medicine
Director, Center for Developmental Health, Knight Cardiovascular Institute
Director, Bob and Charlee Moore Institute for Nutrition & Wellness
Oregon Health & Science University

Kent L. Thornburg, PhD, is the M. Lowell Edwards Chair of Cardiovascular Research, Professor of Medicine, in the Knight Cardiovascular Institute at Oregon Health & Science University. He holds joint professorships in the Departments of Physiology & Pharmacology, Biomedical Engineering and Obstetrics & Gynecology. He directs the Center for Developmental Health in the Knight Cardiovascular Institute and the OHSU Bob and Charlee Moore Institute for Nutrition & Wellness. He has expertise in cardiac and pulmonary physiology, placentology, and developmental programming. He studies the physiological adaptations to pregnancy and the roles of maternal diet and body composition in regulating placental and fetal growth and lifelong health. He collaborates with scientists in England, New Zealand, Switzerland, Finland, Australia and India. He oversees clinical studies on pregnancy in rural Oregon and Alaska.

Kent Thornburg trained in developmental physiology, placentology and heart development in zoology at Oregon State University for the Master of Science and Doctor of Philosophy degrees. He studied cardiovascular physiology at OHSU as an NIH Fellow. He then completed studies at Washington University and the University of Oregon in electron microscopy and physics.

Kent Thornburg is an elected fellow of the American Physiological Society and has served as Editor of the international journal, Placenta, as consulting editor for Pediatric Research; he currently sits on the editorial board of the American Journal of Physiology. He serves regularly on advisory panels at the National Institutes of Health, the American Heart Association and the Children's Heart Foundation and serves on the scientific advisory board of the Preeclampsia Foundation. He has been regularly involved as director of T32 research training for the Knight Cardiovascular Institute and has held grants from four institutes at NIH. He recently co-chaired the task force to determine the 10 year vision of the developmental origins of health and disease for the National Institute of Child Health and Human Development.

JUDGES

SHORT PRESENTATIONS

Steven D. Chernausek, M.D.
Professor of Pediatrics
CHF Edith Kinney Gaylord Chair
Adjunct Professor Physiology
Director, Pediatric Metabolic Research Program
Section Chief, Diabetes and Endocrinology
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

David A. Fields, Ph.D.
CMRI Chickasaw Nation Chair in Diabetes Research
Associate Professor
Section of Diabetes and Endocrinology
Department of Pediatrics
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

Jed Friedman, Ph.D.
Director, Harold Hamm Diabetes Center
Associate Vice-Provost for Diabetes Programs
Chickasaw Nation Endowed Chair
Professor of Physiology, Biochemistry & Molecular Biology
Professor of Pediatrics, Division of Endocrinology and Metabolism
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

Tiangang Li, Ph.D.
Harold Hamm Endowed Chair
Associate Professor
Department of Physiology
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

Kevin Short, Ph.D., FACSM
Associate Professor of Pediatric Diabetes and Endocrinology
CHF Choctaw Nation Chair in Pediatric Endocrinology and Diabetes
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

Archana Unnikrishnan, Ph.D.
Assistant Professor
Department of Biochemistry and Molecular Biology
Member, Harold Hamm Diabetes Center/Chickasaw Nations Scholar
Oklahoma Center for Geroscience & Brain Aging.
University of Oklahoma Health Sciences Center

ORAL PRESENTATION MODERATORS

Shaoning Jiang, Ph.D.
Assistant Professor
Section of Diabetes & Endocrinology
Department of Pediatrics
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

Ankur Rughani, M.D.
Fellow
Section of Diabetes and Endocrinology
Department of Pediatrics
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

David Sparling, M.D., Ph.D.
Associate Section Chief
Section of Pediatric Diabetes and Endocrinology
CHF Paul and Ann Milburn Chair in Pediatric Diabetes
Member, Harold Hamm Diabetes Center
University of Oklahoma Health Sciences Center

NON-AWARD ELIGIBLE ABSTRACTS

NITRATE EXPOSURE REPROGRAMS HEPATIC AMINO ACID AND NUTRIENT SENSING PATHWAYS: A METABOLOMIC AND TRANSCRIPTOMIC INVESTIGATION IN ZEBRAFISH (CANIO RERIO)

Norman G. Hord

OU Health, Harold Hamm Diabetes Center, Department of Nutritional Sciences, College of Allied Health, University of Oklahoma Health Sciences Center

Dietary nitrate (NO_3^-), consumed primarily from leafy green and root vegetables, lowers blood pressure and improves aerobic exercise performance. During exercise, the accelerated demands of the working muscle require increased demands on the liver to mobilize energy stores and recycle metabolites. We measured mRNA expression of zebrafish genes involved in energy metabolism and utilized untargeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis to determine the effect of nitrate treatment and exercise on the liver genome and metabolome of zebrafish (Danio rerio) at the Sinnhuber Aquatic Research Laboratory at Oregon State University. In the absence of exercise, nitrate treatment upregulated expression of genes central to nutrient sensing (peroxisome proliferator-activated receptor gamma coactivator 1- alpha; pgc1a) and protein metabolism (mammalian target of rapamycin; mtor). Upregulation of these genes was associated with a greater abundance of metabolites involved in endogenous NO metabolism, dopamine biosynthesis, branched chain amino acid metabolism, and lipid metabolism in nitrate-treated livers at rest, compared to rested-control liver. The main novel findings of this study were that sub-chronic nitrate treated zebrafish had a greater abundance of arginine in the liver, likely modulating endogenous NO metabolism and contributing to enhanced exercise performance.

BONE TRABECULAR SCORE IN ADOLESCENTS WITH TYPE 1 DIABETES: RELATION TO URINARY PENTOSIDINE

Sowmya Krishnan, Shelly Gulati, Huaiwen Wang, Steven D Chernausek

Department of Pediatrics, Section of Diabetes and Endocrinology
University of Oklahoma Health Science Center

Adolescence is a time of accelerated bone mineral deposition and bone health at this time is particularly sensitive to adverse metabolic aberrations like diabetes. While bone mineral density (BMD) measurements have been the gold standard to assess bone health, BMD tends to underestimate fracture risk in patients with diabetes. Trabecular bone score (TBS) generated from a novel software using DXA spinal measurements is a measure of spinal bone microarchitecture and is a better predictor of fractures in adults with type 1 diabetes. TBS measures in adolescents with diabetes have not been reported.

Factors known to adversely affect bone health in patients with diabetes include hyperglycemia (poorly controlled patients with diabetes have a higher risk of fractures). Hyperglycemia leads to an increase in advanced glycation end products that promote collagen cross links, contributing to bone fragility. Diabetes also results in an increase in circulating sclerostin (a protein produced from osteocytes) that is a potent inhibitor of WNT signaling, a pathway critical for osteoblastogenesis. It is presently unclear if these factors are altered in adolescents with T1D and how they impact bone health. In this study we examined TBS measures in adolescents with and without T1D and explored their relationships with HbA1C, urinary pentosidine (a measure of collagen glycosylation), and serum sclerostin.

This was a cross-sectional study of adolescents with T1D (diagnosed at least two years prior to study enrollment) and healthy control participants. Those with a medical illness or receiving medications affecting bone health, including oral contraceptives, were excluded. A total of 19 participants with T1D (17.4 ± 1.5 years) and 13 controls (17.5 ± 1.5 years) were studied. Groups did not differ for height, BMI, sex distribution. As expected, mean HbA1C was higher in those with T1D (7.42 ± 1.1 vs 5.1 ± 0.5; $p<0.001$). There was no difference in whole body, lumbar spine and dual femur BMD between the two groups. TBS did not differ between patients with T1D and control participants (1.52 ± 0.14 vs 1.58 ± 0.11; $p=0.23$ respectively). Urinary pentosidine (1.0 ± 0.87 vs 1.2±0.97; p=0.5) and serum sclerostin (26.62 ±15.57 vs 31.31 ± 22.80; $p=0.5$) also did not differ between adolescents with and without diabetes respectively. While there was no correlation of any of the BMD measures with urinary pentosidine, HbA1C or serum sclerostin, TBS had a significant negative correlation with urinary pentosidine (r:-0.37; $p=0.04$). We conclude 1) Adolescents with well-controlled T1D show no evidence for diminished bone health by TBS or BMD. 2) Increase in collagen glycosylation adversely impacts bone microarchitecture and this could be one of the mechanisms of increased bone fragility in patients with diabetes.

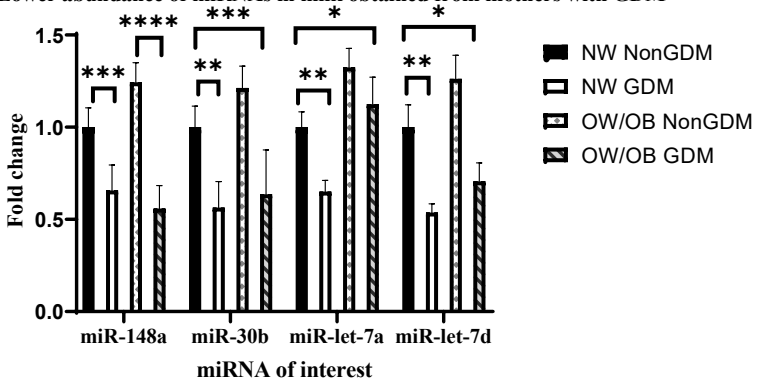
GESTATIONAL DIABETES ALTERS HUMAN MILK EXOSOMAL MICRORNA COMPOSITION

Kruti Shah, David Fields, Nathan Pezant, Shelly Gulati, Ellen Demerath, Jeanie Tryggestad

Department of Pediatrics, Section of Pediatric Diabetes and Endocrinology
Harold Hamm Diabetes Center, University of Oklahoma Health Sciences Center
Oklahoma Medical Research Foundation, Department of Genes and Human Disease
Division of Epidemiology and Community Health, University of Minnesota

Human milk (HM) is a distinctive biological fluid that is enriched with a variety of factors including microRNAs (miRNAs) which potentially provide both short and long-term benefit to the infant. miRNAs are small, non-coding RNAs that bind to complementary sequences within the 3′ untranslated region of mRNAs modulating protein production. The abundance of miRNAs in HM can be affected by a variety of maternal factors such as obesity which we have previously demonstrated. Limited data exists on miRNA abundance in breast milk of mothers with GDM. Therefore, the objectives of this study were: 1) Compare the abundance of exosomal miRNAs in HM collected at 1-month post-partum from women with and without GDM; and 2) Test relationships between HM miRNA abundances at 1-month post-partum and infant growth and body composition during the first 6-months of life. Four miRNAs (miR-148a-3p, miR-30b-5p, miR-let-7a-5p and miR-let-7d-5p) known to be involved in insulin signaling and adipogenesis pathways, were examined in HM. Milk samples were collected from a cohort of 94 mothers (62 mothers without GDM and 32 mothers with GDM) at 1-month post-partum. Participants were grouped by pre-pregnancy BMI group [obese (OB)-pre-pregnancy BMI ≥ 30 kg/m^2, overweight (OW)- pre-pregnancy BMI-25 to <30 kg/m^2 and normal weight (NW)-pre-pregnancy BMI-18.5 to <25 kg/m^2]. For each of these BMI strata, we included all women with GDM and then randomly selected twice as many women without GDM from the same BMI stratum, for a 1:2 case: control ratio in each stratum. For statistical analysis, the two higher BMI groups were collapsed, for a total of four groups: NW and OW/OB non-GDM, and NW and OW/OB GDM. Maternal dietary intake data was collected during the third trimester of pregnancy and at one and three months postpartum using the Diet History Questionnaire II (DHQ II). Maternal diet quality during pregnancy and lactation was estimated using the healthy eating index score (HEI)–2015. At 1 and 3 months, infant weight, % body fat, fat mass and fat free mass were measured using PEA POD and at 6 months, these were measured using dual X-ray energy absorptiometry. miRNA abundance was measured by real-time PCR. Linear regression was used to determine the difference in miRNA abundance in women with and without GDM and to test the association of miRNA abundance with infant growth and body composition measures from 1 to 6 months. The abundances of miR-148a, 30b, let-7a and let-7d were reduced in milk from mothers with GDM (**Figure 1.**) after adjusting for potential cofounders such as maternal age, pre-pregnancy BMI, gestational weight gain, post-partum weight loss, diet quality score, maternal education, income, gestational age and infant sex. Independent of GDM status, higher maternal diet quality was associated with increased abundance of each of the measured miRNAs. miR-148a was associated with decreased infant weight at 1 and 6 months and adiposity (% body fat and fat mass) at 1 month. miR-30b was associated with increased infant weight and adiposity (fat mass) at 1 month of age. Our findings suggest fine tuning between miRNAs in HM to modulate early infant growth and body composition. Given maternal diet quality was associated with increased abundances of miRNAs involved in adipogenesis and insulin signaling pathways, improved infant outcome may be achieved by focusing on healthier diet while breastfeeding. HM miRNAs may be a future therapeutic target to mitigate the risk of metabolic disease in offspring of women with GDM.

Figure 1. Lower abundance of miRNAs in milk obtained from mothers with GDM



Mean ± SEM. * =p <0.05; **=p <0.01; ***=p<0.001

SHORT PRESENTATIONS JUDGING SCHEDULE

Presentations will be via Zoom and in order as listed in the groups below

GROUP A

#	Time	Presenter	Title
1	10:45 – 11:00 am	Neeharika Bade	SEROLOGICAL MARKERS USEFUL FOR DETECTING FIBROSIS IN NASH POPULATION
2	11:00 – 11:15 am	Lijie Gu	PHARMACOLOGICAL INHIBITION OF CULLIN NEDDYLATION IMPROVES HEPATIC INSULIN SENSITIVITY
3	11:15 – 11:30 am	Sabira Jazir	BLOCKING NECROPTOSIS REDUCES INFLAMMATION AND TUMOR INCIDENCE IN A MOUSE MODEL OF DIET-INDUCED HEPATOCELLULAR CARCINOMA
4	11:30 – 11:45 am	Ashok Mandala	EARLY LIFE EXPOSURE TO MICROBIOTA-DEPENDENT INDOLES CONFERS LONG-TERM PROTECTION AGAINST NAFLD
5	11:45 – 12:00 pm	Nikhil Patil	DISSECTING THE MECHANISM OF CINNABARINIC ACID MEDIATED CYTOPROTECTION AGAINST NON-ALCOHOLIC FATTY LIVER DISEASE
6	12:00 – 12:15 pm	Christina Sciarrillo	ABBREVIATED FAT TOLERANCE TEST AS A SCREENING TOOL FOR NON-ALCOHOLIC FATTY LIVER DISEASE IN CHILDREN
7	12:15 – 12:30 pm	Norman Hord*	NITRATE EXPOSURE REPROGRAMS HEPATIC AMINO ACID AND NUTRIENT SENSING PATHWAYS: A METABOLOMIC AND TRANSCRIPTOMIC INVESTIGATION IN ZEBRAFISH (CANIO RERIO)

Judges: **non-award eligible presentation*

Steven Chernausek, M.D.

David Fields, Ph.D.

GROUP B

#	Time	Presenter	Title
8	10:45 – 11:00 am	Olivia Brooks	MICRORNA-126 INCREASES ADIPOSE SIGNALING AND DECREASES PROLIFERATION IN MESENCHYMAL STEM CELLS
9	11:00 – 11:15 am	April Teague	MICRORNA-126 IMPACTS INSULIN SIGNALING AND PROLIFERATION IN VASCULAR SMOOTH MUSCLE CELLS
10	11:15 – 11:30 am	Shiwali Goyal	TARGETED SEQUENCING OF GWAS-DERIVED CANDIDATE GENES OF T2DM IN ASIAN INDIAN ENDOGAMOUS ETHNIC GROUPS: FINDINGS FROM THE INDIGENIUS CONSORTIUM
11	11:30 – 11:45 am	Mary Ellen Jensen	miR-130b/301b NEGATIVELY REGULATES BEIGE ADIPOGENESIS IN-VITRO AND IN-VIVO
12	11:45 – 12:00 pm	Madhusmita Rout	THE ROLE OF APOC3 GENETIC VARIATION, SERUM TRIGLYCERIDES AND RISK OF CARDIOMETABOLIC DISORDER WITH DEVELOPMENT OF CORONARY ARTERY DISEASE IN ASIAN INDIANS, EUROPEANS, AND OTHER ETHNIC GROUPS
13	12:00 – 12:15 pm	Ankur Rughani	IMPACT OF MATERNAL DIABETES ON MICRORNA 146B EXPRESSION IN FETAL TISSUE
14	12:15 – 12:30 pm	Kameron Sugino	HIGHER COMPLEX-CARBOHYDRATE DIET DURING PREGNANCY INCREASES THE NATURAL PROBIOTIC BIFIDOBACTERIACEA IN GUT MICROBIOME IN WOMEN WITH GESTATIONAL DIABETES MELLITUS

Judges:

Tiangang Li, Ph.D.
Archana Unnikrishnan, Ph.D.

GROUP C

#	Time	Presenter	Title
15	10:45 – 11:00 am	Ahmadreza Homayouni	A NOVEL FEATURE SELECTION METHOD FOR NON-IMAGE-BASED DIABETIC RETINOPATHY PREDICTION
16	11:00 – 11:15 am	Kaushalya Jayathilake	INCREASE INFLAMMATORY CYTOKINES AND ACUTE PHASE PROTEINS IN A TISSUE-SPECIFIC MANNER DURING EQUINE INSULIN DYSREGULATION
17	11:15 – 11:30 am	Beibei Liu	MECHANISM OF BDNF-MEDIATED NEUROPROTECTION IN DIABETIC RETINOPATHY
18	11:30 – 11:45 am	Keaton Minor	NAD REDOX IMBALANCE ALTERS NAD SYNTHESIS PATHWAYS IN DIABETIC CARDIOMYOPATHY
19	11:45 – 12:00 pm	Hina Nizami	SARM1 NAD HYDROLASE DEFICIENCY PROTECTS HEART AGAINST METABOLIC CARDIOMYOPATHY IN MICE
20	12:00 – 12:15 pm	Sowmya Krishnan*	BONE TRABECULAR SCORE IN ADOLESCENTS WITH TYPE 1 DIABETES: RELATION TO URINARY PENTOSIDINE
21	12:15 – 12:30 pm	Kruti Shah*	GESTATIONAL DIABETES ALTERS HUMAN MILK EXOSOMAL MICRORNA COMPOSITION

Judges:

Jed Friedman, Ph.D.

Kevin Short, Ph.D.

**non-award eligible presentation*

SHORT PRESENTATIONS

All abstracts - listed alphabetically by author

1. **SEROLOGICAL MARKERS USEFUL FOR DETECTING FIBROSIS IN NASH POPULATION**
   BADE, NEEHARIKA; Sirish Palle, Kevin Short
2. **MICRORNA-126 INCREASES ADIPOSE SIGNALING AND DECREASES PROLIFERATION IN MESENCHYMAL STEM CELLS**
   BROOKS, OLIVIA; Shaoning Jiang, April Teague, Jeanie Tryggestad
3. **MICRORNA-126 IMPACTS INSULIN SIGNALING AND PROLIFERATION IN VASCULAR SMOOTH MUSCLE CELLS**
   BROOKS, OLIVIA; Estefania Fematt-Garcia, April Teague, Jeanie Tryggestad
4. **TARGETED SEQUENCING OF GWAS-DERIVED CANDIDATE GENES OF T2DM IN ASIAN INDIAN ENDOGAMOUS ETHNIC GROUPS: FINDINGS FROM THE INDIGENIUS CONSORTIUM**
   GOYAL, SHIWALI; Vettriselvi Venkatesan, Cynthia Bejar, Juan Lopez-Alvarenga, Rector Arya, Teena Koshy, Umarani Ravichandran, Surendra Sharma, Sailesh Lodha, Amaresh Reddy Ponnala, Krishna Kumar Sharma Sr., Mahaboob Vali Shaik, Roy Resendez, Deepika Ramu, Priyanka Venugopal, Parthasarathy R, Noelta S, Juliet Ezeilo, Srinivas Mummidi, Chidambaram Natesan, John Blangero, Krishna Medicherla, Sadagopan Thanikachalam Sr., Thyagarajan Sadras Panchatcharam, Dileep Kumar K, Rajeev Gupta, Solomon Franklin Paul, Ravindranath Duggirala, Dharambir Sanghera
5. **PHARMACOLOGICAL INHIBITION OF CULLIN NEDDYLATION IMPROVES HEPATIC INSULIN SENSITIVITY**
   GU, LIJIE; Cheng Chen, David Matye, Mohammad Hasan, Yung Dai Clayton, Tiangang Li
6. **A NOVEL FEATURE SELECTION METHOD FOR NON-IMAGE-BASED DIABETIC RETINOPATHY PREDICTION**
   HOMAYOUNI, AHMADREZA; Tieming Liu, Thanh Thieu, Zhuqi Miao, William Paiva
7. **NITRATE EXPOSURE REPROGRAMS HEPATIC AMINO ACID AND NUTRIENT SENSING PATHWAYS: A METABOLOMIC AND TRANSCRIPTOMIC INVESTIGATION IN ZEBRAFISH (CANIO RERIO)**
   HORD, NORM

8. **INCREASE INFLAMMATORY CYTOKINES AND ACUTE PHASE PROTEINS IN A TISSUE-SPECIFIC MANNER DURING EQUINE INSULIN DYSREGULATION**
   JAYATHILAKE, KAUSHALYA; Melody de Laat, M. Furr, R. Carlos, Véronique Lacombe
9. **BLOCKING NECROPTOSIS REDUCES INFLAMMATION AND TUMOR INCIDENCE IN A MOUSE MODEL OF DIET-INDUCED HEPATOCELLULAR CARCINOMA**
   JAZIR, SABIRA; Evan Nicklas, Ramasamy Selvarani, Arlan Richardson, Deepa Sathyaseelan
10. **miR-130b/301b NEGATIVELY REGULATES BEIGE ADIPOGENESIS IN-VITRO AND IN-VIVO**
    JENSEN, MARY ELLEN; Wenyi Luo, Jacob Friedman, Steven Chernausek, Shaoning Jiang
11. **BONE TRABECULAR SCORE IN ADOLESCENTS WITH TYPE 1 DIABETES: RELATION TO URINARY PENTOSIDINE**
    KRISHNAN, SOWMYA; Shelly Gulati, Huaiwen Wang, Steven D Chernausek
12. **MECHANISM OF BDNF-MEDIATED NEUROPROTECTION IN DIABETIC RETINOPATHY**
    LIU, BEIBEI; Meili Zhu, Yun-Zheng Le
13. **EARLY LIFE EXPOSURE TO MICROBIOTA-DEPENDENT INDOLES CONFERS LONG-TERM PROTECTION AGAINST NAFLD**
    MANDALA, ASHOK; Rachel Janssen, April Teague, Wanke Zhao, Jacob Friedman, Karen Jonscher
14. **NAD REDOX IMBALANCE ALTERS NAD SYNTHESIS PATHWAYS IN DIABETIC CARDIOMYOPATHY**
    MINOR, KEATON; Christine Light, Xiaojian Shi, Haiwei Gu, Chi Fung Lee
15. **SARM1 NAD HYDROLASE DEFICIENCY PROTECTS HEART AGAINST METABOLIC CARDIOMYOPATHY IN MICE**
    NIZAMI, HINA; Christine Light, Chi Fung Lee
16. **DISSECTING THE MECHANISM OF CINNABARINIC ACID MEDIATED CYTOPROTECTION AGAINST NON-ALCOHOLIC FATTY LIVER DISEASE**
    PATIL, NIKHIL; Iulia Rus, Aditya Joshi

17. **THE ROLE OF APOC3 GENETIC VARIATION, SERUM TRIGLYCERIDES AND RISK OF CARDIOMETABOLIC DISORDER WITH DEVELOPMENT OF CORONARY ARTERY DISEASE IN ASIAN INDIANS, EUROPEANS, AND OTHER ETHNIC GROUPS**

    ROUT, MADHUSMITA; Shiwali Goyal, Yosuke Tanigawa, Weihua Zhang, Jin-Fang Chai, Marcio Almeida, Xueling Sim, Megan Lerner, Juliane Chainakul, Jonathan Garcia Ramiu, Chanel Seraphin, Blair Apple, April Vaughan, James Muniu, Juan Peralta, Donna Lehman, Sarju Ralhan, Gurpreet Wander, Jai Rup Singh, Narinder Mehra, Evgeny Sidorov, Marvin Peyton, Piers Blackett, Joanne Curran, E Shyong Tai, Rob van Dam, Ching-Yu Cheng, Ravindranath Duggirala, John Blangero, John Chambers, Charumathi Sabanayagam, Jaspal Kooner, Manuel Rivas, Christopher Aston, Dharambir Sanghera

18. **IMPACT OF MATERNAL DIABETES ON MICRORNA 146B EXPRESSION IN FETAL TISSUE**

    RUGHANI, ANKUR; Jeanie Tryggestad

19. **ABBREVIATED FAT TOLERANCE TEST AS A SCREENING TOOL FOR NON-ALCOHOLIC FATTY LIVER DISEASE IN CHILDREN**

    SCIARRILLO, CHRISTINA; Bryant Keirns, Destinee Elliott, Stephen Clarke, Edralin Lucas, Misty Hawkins, Sirish Palle, Kevin Short, Sam Emerson

20. **GESTATIONAL DIABETES ALTERS HUMAN MILK EXOSOMAL MICRORNA COMPOSITION**

    SHAH, KRUTI; David Fields, Nathan Pezant, Shelly Gulati, Ellen Demerath, Jeanie Tryggestad

21. **HIGHER COMPLEX-CARBOHYDRATE DIET DURING PREGNANCY INCREASES THE NATURAL PROBIOTIC BIFIDOBACTERIACEA IN GUT MICROBIOME IN WOMEN WITH GESTATIONAL DIABETES MELLITUS**

    SUGINO, KAMERON; Teri Hernandez, Lynn Barbour, Daniel Frank, Jacob Friedman

AWARD ELIGIBLE ABSTRACTS

SEROLOGICAL MARKERS USEFUL FOR DETECTING FIBROSIS IN NASH POPULATION

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Nonalcoholic liver disease (NAFLD) is a metabolic disease that encompasses a spectrum of progressive pathological conditions, ranging from nonalcoholic steatosis to steatohepatitis (NASH), advanced fibrosis and cirrhosis. Pediatric NAFLD is becoming the leading cause of chronic liver disease world-wide, affecting 10%-15% of children and adolescents in Western countries. The development of noninvasive biomarkers of disease is a major focus of interest in nonalcoholic fatty liver disease (NAFLD). Very few have been tested in children. Through our study we intend to bridge the gap in this knowledge of the usefulness of biomarkers in Pediatrics. In a cross-sectional analysis which included a total of 77 samples (25- normal weight controls,27- obese controls and 25 with biopsy proven NAFLD), we measured the levels of Cytokeratin (CK)18 and CK18 fragments which are markers of apoptosis, YKL –40, Collagen 4, Laminin, Pro collagen 3 (P3NP), Hyaluronan which are markers of fibrosis and an acute phase protein, Alpha 2 macroglobulin. From our study, we were able to demonstrate that CK18, CK 18 fragments, collagen 4, YKL-40, P3NP were significantly elevated in the children with suspected NAFLD compared to obese controls and normal weight controls. These results suggest that CK18, CK 18 fragments, collagen 4, YKL-40, P3NP are elevated in children with suspected NAFLD and should be further investigated as potential diagnostic markers of severity of NAFLD.

MICRORNA-126 INCREASES ADIPOSE SIGNALING AND DECREASES PROLIFERATION IN MESENCHYMAL STEM CELLS

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In utero, mesenchymal stems cells (MSC) develop into adipose and other tissues. Exposure to DM *in utero* appears to affect early programming of MSC development. miRNA-126-3p is increased in HUVEC and in circulation of infants exposed to the diabetic milieu. We hypothesize that increased miRNA-126-3p in DM-exposed infants affects cellular metabolism by increasing senescence, decreasing proliferation, and increasing adipocyte differentiation capacity of MSCs. To test this hypothesis, MSCs were isolated from the umbilical veins of infants exposed to diabetes *in utero* (DM) or unexposed (control) born via elective C-section.

To examine MSC senescence and CD13 abundance, DM and control MSCs were stained with CD13 or isotype-control antibody, then stained with propidium. Flow cytometry revealed a 16.85% increase in CD13 in DM MSCs as compared with control MSC, indicating a higher propensity for adipocyte differentiation. Cell cycle data revealed that compared with Control MSC, 21.26% more DM cells were in G1 phase while 50.99% fewer DM were in S phase.

To examine abundance of LRP6, a target protein of miRNA-126-3p, DM and control MSC were transfected for 48h with NTC, miRNA-126-3p, and anti-miRNA-126-3p. They were then incubated for 10min in the presence or absence of 10ng Insulin Growth Factor (IGF1). Western Blotting revealed a 38.0% reduction in LRP6 following transfection with mi-RNA-126, as well as a 49.0% reduction in GSK3B downstream from LRP6.

MTT assay examined miRNA-126-3p effects on MSC proliferation. DM and Control MSC were transfected for 48h with NTC, miRNA-26-3p, or anti-miRNA-126-3p. They were then incubated for 24h in the presence or absence of 10ng IGF1. Proliferation of stimulated MSCs decreased by 25.57% with miRNA-126-3p transfection, and proliferation recovered by 40.38% with anti-126-3p transfection.

These data suggest that miRNA-126-3p and DM exposure alters cellular metabolism and increase adipocyte differentiation in MSCs. The alterations in miRNA abundance may potentially program the infant toward future metabolic disease later in life.

MICRORNA-126 IMPACTS INSULIN SIGNALING AND PROLIFERATION IN VASCULAR SMOOTH MUSCLE CELLS

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Human umbilical vein endothelial cells (HUVEC) are a rich source of micro-RNAs such as miRNA-126-3p which is increased in HUVEC exposed to the diabetic milieu of pregnancy. Micro-RNAs are secreted into the extracellular space, potentially influencing other cells. Smooth Muscle Cells (SMC) take up micro-RNAs when cocultured with endothelial cells. miRNA-126-3p targets mRNA within the insulin signaling cascade, reducing related protein abundance. We hypothesize that miRNA-126-3p decreases the abundance of its reported protein targets, IRS1 and PI3K p85b, and decreases overall SMC proliferation. To test this hypothesis, HUVEC and SMC were isolated from the umbilical veins of infants born via elective C-section from women with and without diabetes.

To examine miRNA-126-3p abundance, SMC were cocultured for 48h in media preconditioned with HUVEC that were either exposed to diabetes *in utero* (DM) or not (control), or unconditioned media. RT-PCR revealed a three-fold increase in miRNA-126-3p abundance in SMCs in Control HUVEC compared with unconditioned media, and a five-fold increase in DM HUVEC compared with unconditioned media.

To examine protein abundance, SMC were cocultured for 48h in media preconditioned with DM or control HUVEC, or unconditioned media. SMC were then transfected for 48h with a non-coding control mimic (NTC) or with miRNA-126-3p, and then incubated for 10 min in the presence or absence of 10nM insulin. For the coculture model, one-way ANOVA compared protein abundance among SMC exposed to DM or control HUVEC or unconditioned media. For the transfection model, two-way ANOVA assessed the differences in expression of each protein by miRNA and insulin treatment. Abundance of all proteins was compared to the NTC-transfected, unstimulated SMC. When cultured with conditioned HUVEC media, SMC IRS1 decreased by 14% +/- 9% ($p = 0.064$) and 35% +/- 6% ($p < 0.001$) in control and DM, respectively, compared to unconditioned media. PI3K p85b decreased by 21% +/- 4% ($p = 0.001$) and 15% +/- 6% ($p = 0.015$) in control and DM, respectively, compared to unconditioned media. In the transfection model, IRS1 decreased to 61% +/- 38% ($p = 0.061$ for effect of miR) and PI3K p85b decreased to 36% +/- 24% ($p = 0.001$ for effect of miRNA) of control levels after transfection with miRNA-126-3p. Phosphorylation of T308-Akt, an insulin signaling target downstream of IRS1 and PI3K, decreased 43% ($p = 0.012$ for effect of miR and $p < 0.001$ for effect of insulin), and insulin-stimulated phosphorylation of T308-Akt was similarly decreased by miRNA-126-3p.

MTT assay examined the effects of miRNA-126-3p on SMC proliferation. SMC were first transfected for 48h with NTC, miRNA-126-3p, or anti-miRNA-126-3, then incubated for 24h in the presence and absence of 10ng/mL of insulin growth factor (IGF1). Stimulated SMCs transfected with miRNA-126-3p displayed a 48.0% reduction in proliferation as compared with NTC, followed by a 97.34% recovery in proliferation of SMCs transfected with anti-miRNA-126-3p.

These data suggest that miRNA-126-3p decreases abundance of insulin signaling pathway proteins in SMCs, altering insulin sensitivity and decreasing proliferation thus potentially providing a mechanism for the *in utero* programming of the diabetic milieu toward future cardiometabolic disease in the infant.

TARGETED SEQUENCING OF GWAS-DERIVED CANDIDATE GENES OF T2DM IN ASIAN INDIAN ENDOGAMOUS ETHNIC GROUPS: FINDINGS FROM THE INDIGENIUS CONSORTIUM

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South Asians (SA), people for the Indian subcontinent comprise one-quarter of the global population and have a 6 times higher risk of developing Type II Diabetes Mellitus (T2DM) than Europeans. However, underlying causes of this disparity are currently unknown and are difficult to be explained by conventional risk factors of T2DM. Here, we aimed to evaluate genetic determinants of T2DM using family-based cohorts from four distinct Endogamous Ethnic Groups (EEGs) representing two Northern (Punjab [Sikhs] and Rajasthan [Agarwals]) and two Southern (Tamil Nadu [Chettiars] and Andhra Pradesh [Reddys]) states of India, and to examine whether genetic variants found through targeted sequencing of the previously established 10 SA T2DM risk loci (including the one from the Sikh population) are relevant to other EEGs. All are part of the INDIGENIUS consortium, supported by an Indo-U.S. Collaborative Research Partnership on T2DM.

Targeted sequencing of 10 SA-specific GWAS loci (*HMG20A*, *AP3S2*, *ST6GAL1*, *GRB14*, *VPS26A*, *HNF4A*, *SGCG*, *KCNJ11*/*ABCC8*, *IGFBP2*, and *TMEM163*, containing 48 genes and intergenic regions) was performed on 32 multiplex families of Sikh EEG and validation studies were performed on additional ~4500 individuals of Punjabi ancestry; all individuals were part of the Asian Indian Diabetic Heart Study/Sikh Diabetes Study (AIDHS/SDS). Our data revealed many common and rare variants associated with T2DM and other cardio-metabolic traits. The common variants from the known candidate genes i.e. rs1470579 in *IGF2BP2* and rs7903146 in *TCF7L2* showed robustly significant association signals with T2DM in SA meta-analysis with an OR=0.88 [0.84-0.92; $p=5.65x10^{-11}$] and OR=1.32 [1.28-1.36; $p=1.08x10^{-44}$], respectively. Interestingly, our study also identified some rare variants that were population-specific (rs370655945 *SACS*, rs373044286 *HK1,* and rs148713144 *DDX50*) and not observed in EXAC or 1000Genomes, but were present in multiple members affected with T2DM in few pedigrees. The most prominent signal was observed in the *SACS* (rs370655945) to be robustly associated with T2DM (OR=0.71 95%CI [0.59-0.83]; $p=4.24x10^{-08}$). These results show the potential for identifying sub-forms of T2DM and gain insight into the molecular mechanisms underlying T2DM using a pedigree-based design. **Funding**: National Institute of Diabetes and Digestive and Kidney Diseases (R21DK105913), Indian Council of Medical Research (55/6/2/Indo-US/2014-NCD-II). The AIDHS/SDS was also supported by National Institute of Health grants KO1TW006087, funded by the Fogarty International Center, and R01DK082766, funded by the National Institute of Diabetes and Digestive and Kidney Diseases.

PHARMACOLOGICAL INHIBITION OF CULLIN NEDDYLATION IMPROVES HEPATIC INSULIN SENSITIVITY

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Background and significance. Hepatic insulin resistance is a hallmark feature of non-alcoholic fatty liver disease (NAFLD) and type-2 diabetes (T2D) and significantly contributes to systemic insulin resistance. Abnormal activation of nutrient and stress-sensing kinases leads to serine/threonine phosphorylation of insulin receptor substrate (IRS) and subsequent IRS proteasome degradation, which is a key underlying cause of hepatic insulin resistance. Recently, members of the cullin-RING E3 ligases (CRLs) have emerged as mediators of IRS protein turnover. CRLs are activated upon cullin neddylation, a process of covalent conjugation of a ubiquitin-like protein called Nedd8 to a cullin scaffold. **Aim.** To investigate the role of CRLs in regulating hepatic insulin signaling in NAFLD. **Approach.** Neddylation inhibitor MLN4924 was used to study its effect on insulin sensitivity in liver cells and mice. siRNA-mediated screening was used to identify the Cul members that mediates IRS protein turnover in liver cells. Co-immunoprecipitation was performed to study Cullin and IRS interaction. Glucose homeostasis and insulin sensitivity were studied in mice with AAV-mediated liver specific knockdown of Cul1 or Cul3. **Results.** Neddylation inhibition delays CRL-mediated IRS protein turnover to prolong insulin action in cultured liver cells. Consistently, acute MLN4924 treatment rapidly decreases hepatic glucose production without causing hypoglycemia in mice, while chronic MLN4924 treatment completely normalizes hyperglycemia in obese mice independent of obesity. In vitro siRNA based screening showed that knockdown of Cul1 or Cul3, but not other cullin family members, stabilized IRS protein and increased insulin sensitivity in AML12 cells. Consistently, Co-immunoprecipitation demonstrated IRS interaction with Cul1 and Cul3. AAV-mediated-liver specific knockdown of Cul1 in mice improved insulin sensitivity, lowered fasting glucose, and prevented Western diet-induced obesity and NAFLD. In contrast, AAV-mediated liver Cul3 knockdown only decreased fasting glucose in chow-fed mice but not Western diet-fed mice. **Conclusion:** These findings suggest that pharmacological inhibition of cullin neddylation is a novel therapeutic strategy for hepatic insulin sensitization in NAFLD and T2D. This effect may be at least in part mediated by hepatic Cul1 and Cul3. However, the metabolic regulatory roles of hepatic Cul1 and Cul3 remain to be further defined in future studies.

A NOVEL FEATURE SELECTION METHOD FOR NON-IMAGE-BASED DIABETIC RETINOPATHY PREDICTION

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Background and Objective: Two barriers to diabetic retinopathy (DR) early detection are the low patient compliance rate to the recommended annual ophthalmic examinations and a lack of specialists in rural areas. Non-image-based clinical prediction models provide a potentially cost-effective way to detect DR in the early stages. However, diabetic retinopathy prediction models currently available include a large number of independent factors, making them challenging to utilize in a clinical setting. This study reduces the number of laboratory variables to create a more cost-effective diabetic retinopathy prediction model without sacrificing accuracy. The result of this work will help physicians use a small set of available variables to identify high-risk diabetic patients who are prone to develop DR. Medical doctors thus can intervene at the proper time to prevent vision loss.

Methods: We employed a retrospective case/control cohort study based on data extracted from a large electronic health records (EHR) database. The univariate association was used to evaluate patient demographics and results from 20 lab tests, 3 demographics, and 2 comorbidity variables in relation to incidence of diabetic retinopathy. A novel feature selection method based on an ablation study was proposed to identify top predictive features and then evaluated using 10-fold cross-validation. Our ablation-based feature selection is the inverse of the well-known recursive feature elimination procedure, and compared to batch-selection based on feature importance, our method decouples feature correlation. It thus provides a better medical explanation to individual features.

About 35,000 DR patients were included in the case-cohort, while the control cohort consisted of a similar number of non-DR diabetic patients, matched by demographic and healthcare utilization attributes. Using the entire set of features (25 variables), the XGBoost classifier achieves an AUC (Area Under Curve) of 98.50%, and creatinine is the feature with the highest gain value. After removing creatinine, we train an XGBoost classifier on the remaining training set and evaluate its performance on the testing set. The AUC of the existing model is 98.4%, and neuropathy is announced the champion among the current feature set. We keep repeating this process until the AUC drops below the prespecified threshold.

Results: The AUC of the reduced model was 96.61%, which is close to the accuracy of the full model with 20 laboratory, 3 demographic, and 2 comorbidity variables (AUC=98.50%). In our reduced model, six laboratory, one demographic, and two comorbidity predictors are essential to detect DR.

Conclusion: Healthcare providers are given the most informative set of features that are easy to obtain. This study can significantly decrease the currently high noncompliance rate with annual routine ophthalmology exams and increase the chance of early detection of diabetic retinopathy development. Thus, it will eventually lead to saving diabetics' eyesight. The future work of this study will focus on the validation of the proposed model with data from other sources.

INCREASE INFLAMMATORY CYTOKINES AND ACUTE PHASE PROTEINS IN A TISSUE-SPECIFIC MANNER DURING EQUINE INSULIN DYSREGULATION

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Similar to human diabetes, equine metabolic syndrome (EMS) causes insulin dysregulation leading to debilitating sequela including laminitis. Endocrinopathic laminitis is pathologically similar to the multi-organ dysfunction and peripheral neuropathy found in human patients with metabolic syndrome. The pathophysiological mechanisms underlying EMS and laminitis are not well elucidated. Interestingly, in contrast with humans, increase prevalence of cardiac diseases has not been yet reported during EMS. Therefore, using two unique equine models, we hypothesized that insulin dysregulation induces an increased expression of inflammatory proteins in a tissue specific manner.

Following a 48-hour prolonged euglycemic-hyperinsulinemic clamp (pEHC, n=4) or an electrolyte solution (control, n=4), striated muscle and lamellae biopsies were obtained. All hyperinsulinemic horses developed laminitis despite being previously healthy. Two other groups of horses (n=3-5/group) were categorized as insulin resistant (IR) or insulin sensitive (IS), using a frequently sampled intra-venous glucose tolerance test Biopsies from visceral and subcutaneous adipose tissues and skeletal muscle were collected in both groups Protein expression was quantified via Western blotting and statistical analysis was performed using one-tailed t test or Mann Whitney test.

HSP90 protein expression was significantly higher in lamellae of pEHC group, as well as in visceral adipose tissue and skeletal muscle of IR horses ($p=0.039$, $p=0.046$, $p=0.017$ vs. controls, respectively), but not in subcutaneous adipose and cardiac tissue. IL1β protein expression was higher in lamellae from pEHC horses and visceral adipose from IR horses ($p=0.015$, $p=0.0009$ vs. controls, respectively). IL6 protein expression was significantly higher in lamellae in pEHC horses ($p=0.005$), but not in adipose tissue. TNFα protein expression was significantly higher in lamellae of pEHC horses ($p=0.0001$). Alpha-2 Macroglobulin (A2M) protein expression was higher in lamellae of pEHC group and in visceral adipose of IR horses ($p=0.018$, $p=0.039$ vs. control, respectively) but not in subcutaneous adipose tissue. Fibrinogen protein expression was significantly higher in the lamellae of pEHC horses and in subcutaneous adipose tissue of IR horses. Protein expression of A2M, fibrinogen, IL1β, IL6 and TNFα expression was unchanged in striated muscles among the groups.

Upregulation of inflammatory cytokines and acute phase proteins in lamellae and adipose tissue during equine insulin dysregulation may reveal novel biomarkers and potential therapeutic targets for EMS. Further, the lack of increase of inflammatory proteins in the heart of both equine models of insulin dysregulation could underscore potential cardioprotective mechanisms in this species.

BLOCKING NECROPTOSIS REDUCES INFLAMMATION AND TUMOR INCIDENCE IN A MOUSE MODEL OF DIET-INDUCED HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) ranks as the fourth cancer-related cause of death worldwide. Non-resolving chronic inflammation is proposed to be a major contributor in the development and progression of HCC. Necroptosis is a novel programmed cell death pathway that plays a major role in inflammation through the release of damage-associated molecular patterns (DAMPs). Necroptosis is initiated when necroptotic stimuli sequentially activate receptor-interacting serine/threonine-protein kinase (RIPK1), RIPK3, and mixed lineage kinase domain like pseudokinase (MLKL) through phosphorylation leading to membrane disruption and DAMPs release. DAMPs in turn activate immune cells to produce proinflammatory cytokines (e.g. TNFα) leading to a positive feedback loop resulting in chronic non-resolving inflammation. Diabetes is considered a major risk factor for HCC development. In diabetes, nonalcoholic fatty liver disease (NAFLD) progresses to nonalcoholic steatohepatitis (NASH), fibrosis, and ultimately to HCC. Based on this, we hypothesized that inflammation arising from hepatic necroptosis plays a major role in the progression of NAFLD to HCC and that preventing necroptosis will reduce inflammation and thereby the progression of NAFLD to HCC. To test our hypothesis, we used *Ripk3*$^{-/-}$ and *Mlkl*$^{-/-}$ mice which are deficient in the key necroptotic proteins. Mice were fed either a normal chow diet as control or choline-deficient L-amino acid-defined high fat (60%) diet (CD-HFD) which induces HCC in 6-months. After 6-months of CD-HFD feeding, 100% of control mice developed liver tumors, whereas *Ripk3*$^{-/-}$ and *Mlkl*$^{-/-}$ mice showed a significant reduction in tumor incidence. As immune cells contribute to inflammation, we assessed changes in immune cell population in liver by flow cytometry. A significant increase in immune cell infiltration (CD45 positive cells), total monocyte and macrophage (F4/80 positive cells) and inflammatory monocytic (Ly6C^{hi} CCR2^{+} cells) populations were observed in the livers of control mice fed CD-HFD. Blocking necroptosis significantly reduced immune cell infiltration in the livers of *Ripk3*$^{-/-}$ *and Mlkl*$^{-/-}$ mice fed CD-HFD. Thus, our findings show for the first time that blocking necroptosis could reduce immune cell infiltration and tumor incidence in a mouse model of diet-induced HCC.

miR-130b/301b NEGATIVELY REGULATES BEIGE ADIPOGENESIS IN-VITRO AND IN-VIVO

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Adipose tissue is a highly dynamic organ with essential roles in energy homeostasis and in the development of obesity. In contrast to white adipocytes which store energy, thermogenic brown or beige adipocytes dissipate energy in the form of heat and thereby counteract obesity. MicroRNAs are small noncoding RNAs that influence gene expression post-transcriptionally and regulate cell development and function. Previous studies by our group and others have shown upregulation of the miR-130b/301b microRNA cluster in metabolic disorders, such as obesity. Here we investigated the roles of miR-130b/301b in regulating beige-ing of subcutaneous fat cells. Progenitor cells from mouse subcutaneous white adipose tissue (iWAT) show reduced expression of miR-130b/301b during induction of brown adipogenesis, while forced overexpression of miR-130b-3p or miR-301b-3p results in decreased UCP1 and mitochondrial respiration, suggesting that a decline in endogenous miR-130b-3p or miR-301b-3p is required for adipocyte precursors to develop the brown phenotype. Mechanistically, miR-130b/301b directly targets PGC-1α and AMPKα1, key regulators of brown adipogenesis and mitochondrial biogenesis. *In vivo*, circulating exosomal miR-130b/301b levels were increased in mice with pre-weaning exposure to a high fat diet (HFD) as compared to control Chow diet. Lack of miR-130b/301b resulted in increased expression of genes linked to adipocyte thermogenesis in subcutaneous adipose tissue of weaning mice exposed to maternal HFD. Together, these data identify the miR-130b/301b cluster as a new regulator of beige adipogenesis involving PGC-1α and AMPK signaling, and therefore a potential therapeutic target against adult and childhood obesity.

MECHANISM OF BDNF-MEDIATED NEUROPROTECTION IN DIABETIC RETINOPATHY

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To study Müller cell (MC)-mediated neuroprotection in diabetic retinopathy (DR), we previously demonstrated that disruption of vascular endothelial growth factor receptor-2 (VEGFR2) in MCs caused an accelerated loss of retinal MCs, neurons, and major neuroprotectant, brain-derived neurotrophic factor (BDNF) in diabetes (*Diabetes*, 64: 3554). To investigate the mechanism(s) of VEGF-/BDNF-mediated MC viability, we asked the question if VEGF supports MC directly by improving the survival of MCs, indirectly by upregulating the production/secretion of BDNF, or both, we examined the levels of secreted BDNF by ELISA and the number of MCs by live cell assay in cultures of rat MC line (rMC1) in diabetes-like condition (high glucose, HG) or normal condition (normal glucose, NG). Under diabetic condition, VEGF stimulated MC viability and BDNF secretion in a dose-dependent manner. BDNF significantly increased the MC viability in a dose-dependent manner. Targeting the canonical BDNF receptor, tropomyosin receptor kinase B (TRK-B), by siRNA knockdown in rMC1 caused a significant downregulation of activated form of AKT and ERK, classical cell survival and proliferation mediators. In conclusion, our finding suggests that VEGF is a master regulator of MC viability under diabetic conditions, which elevates BDNF secretion in rMC1. BDNF further supports MC viability through AKT survival and ERK proliferation pathways. Since a substantial portion of patients with long-term anti-VEGF treatment appears to have retinal thinning, a pathological characteristic neuronal degeneration that might be due to blocking VEGF signaling. Hence, supporting MC viability with BDNF may be a feasible strategy for neuroprotection during anti-VEGF treatment for DR and hypoxic retinal diseases.

EARLY LIFE EXPOSURE TO MICROBIOTA-DEPENDENT INDOLES CONFERS LONG-TERM PROTECTION AGAINST NAFLD

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Nonalcoholic fatty liver disease (NAFLD) is the most common progressive liver disease, affecting 40% of obese youth and 10% of the general pediatric population. Early life exposure to maternal Western-style diet (WSD) plays a vital role in developmental programming in offspring, inducing inflammation and altering gut microbial species and their metabolites. The microbiota, acting via secreted factors related to tryptophan (Trp) metabolism, regulate inflammatory processes, but their cellular targets in the pathogenesis of NAFLD are unclear. Trp metabolites such as indoles act via the aryl hydrocarbon receptor (AHR), a transcription factor expressed in liver and immune cells, which promotes epithelial barrier protection and protects against infection and damage caused by hyper-inflammatory responses, but the signaling pathways remain poorly understood. We have found that offspring whose mothers received WSD (WSD-O) during gestation and lactation have reduced serum levels of the Trp metabolites; indole and I3A, associated with infiltration of pro-inflammatory macrophages into the liver. We hypothesized that maternal supplementation with indole or I3A during pregnancy and lactation would blunt the development of WSD-induced NAFLD in the offspring. We found that body weight was significantly reduced in offspring from WSD-O treated with indoles at 16 wks despite consuming a WSD ($p<0.01$) vs WSD-O, whereas I3A-O treated offspring were unchanged. Histological examination of the liver at revealed that both maternal indole and I3A significantly reduced hepatic steatosis in offspring. Indices of NAFLD, including hepatic triglyceride levels, genes involved in *de-novo* lipogenesis (*Srebp1c*, *Fasn*, *Scd-1*, and *Acc1; p<0.05*) and expression of pro-fibrotic markers (*Acta2*, *Tgfb1*, *Timp1*, and *Col1a1; p<0.05*) were decreased in both indole-O and I3A-O offspring, expression levels of pro-inflammatory genes (*Tlr4*, *Ly6c* and *Mcp-1*) were reduced in indole-O treated mice, while I3A-O specifically increased expression of *Il-10*, an anti-inflammatory gene, suggesting these metabolites act via distinct mechanisms. To test their response in-vitro, we treated RAW264.7 macrophages with lipopolysaccharide (LPS; 10ng/ml for 6h) and HepG2 human hepatoma cells with free fatty acid (FFA; 500μM for 24h). Pre-treatment of cells with indole or I3A significantly reduced expression of pro-inflammatory *Il1b, Tnfα, Nlrp3*, and *Mcp1*, and genes involved in *de-novo* ceramide synthesis (*Sptlc2* and *Degs1; p<0.05).* Interestingly, addition of exogenous ceramide to macrophages had no effect on the anti-inflammatory effect of indole, but blunted the effect of I3A, indicating a crucial role for ceramide in pharmacological actions of I3A but not indole. Together, these findings suggest that early supplementation of microbiota-derived agonists of AHR, in vivo and in vitro, protects against inflammation in macrophages and hepatocytes and ameliorates NAFLD. Future studies will probe the role for ceramide metabolism in this process.

NAD REDOX IMBALANCE ALTERS NAD SYNTHESIS PATHWAYS IN DIABETIC CARDIOMYOPATHY

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Heart failure occurs at nearly twice the rate in diabetic patients as compared to normal subjects. NAD redox imbalance or depletion have emerged as hallmarks of diabetes and heart disease. Recently, we have shown that NAD redox imbalance exacerbates the progression of diabetic cardiomyopathy (DCM). However, how the NAD metabolic pathways change and contribute to the NAD depletion seen in DCM is not well understood. We aimed to investigate how NAD metabolic pathways change in response to NAD redox imbalance and DCM. We used two mouse models with an altered NAD redox state. 1. Cardiac-specific NDUFS4-KO (cKO) mice which have complex I deficiency, decreased NAD/NADH levels, but normal baseline cardiac function and energetics. 2. Cardiac-specific expression of nicotinamide phosphoribosyltransfase (NAMPT) in mice elevates cardiac NAD levels. We used streptozotocin (STZ) injections to induce chronic diabetic stress, and measured systolic (fractional shortening) and diastolic (E'/A', e/E' ratio) function through echocardiography. As expected, we found that the cKO groups had exacerbated systolic and diastolic dysfunction as compared to control mice when subjected to diabetic stress. Using qPCR, we measured expression levels of genes related to NAD metabolism and found no changes in expression levels in genes for NAD consumption (sirtuins, PARPs and hydrolases) and for the three NAD synthesis pathways, except Nicotinamide Riboside Kinase 2 (NMRK2). NMRK2 mediates NAD synthesis in the Preiss-Handler pathway and the Salvage pathway. NMRK2 phosphorylates nicotinamide riboside (NR) to NMN, which is subsequently synthesized into NAD. Interestingly, when measuring the NAD metabolite levels in the heart tissues, we noticed an increase in the substrate of NMRK2, NR, and a decrease in the products, NMN/NAMN, coinciding with decreased NAD levels in diabetic cKO hearts. The results show that upregulation of NMRK2 is associated with worse cardiac function and lower NAD/NADH ratio in diabetic cKO hearts.

In order to raise NAD levels and NAD/NADH ratio, we overexpressed NAMPT in control and cKO hearts. We found that NAMPT overexpression protected both control and cKO mice from DCM. Importantly, the cardioprotection by NAMPT expression was associated with a decrease in the expression of NMRK2. Downregulation of NMRK2 was also observed in NAMPT expressions of control or cKO mice without stress. In summary, our data showed that NMRK2 gene expression negatively correlates with cardiac function, NAD levels and NAD/NADH ratio in diabetic hearts. Further understanding on how NMRK2 is transcriptionally regulated and how NMRK2 impacts the NAD metabolome to mediate disease progression is of future importance.

SARM1 NAD HYDROLASE DEFICIENCY PROTECTS HEART AGAINST METABOLIC CARDIOMYOPATHY IN MICE

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Heart disease continues to be the leading cause of death and is twice as common in patients with Type 1 (T1D) or Type 2 diabetes (T2D) than in non-diabetics. However, pathogenic mechanisms of metabolic cardiomyopathy remain elusive. We and others have previously shown that NAD^+ depletion is associated with suppressed NAD^+ synthesis pathway in cardiac dysfunction induced by pressure overload and metabolic stress. However, how NAD^+ consumption mechanisms lead to NAD^+ depletion remain to be understood. SARM1 is a novel intracellular NAD^+ hydrolase whose deficiency is shown to attenuate diabetic neuropathy, suggesting its possible link with metabolic stress. Herein, we explored the role of SARM1 as a novel pathogenic regulator of metabolic cardiomyopathy in mice. SARM1-KO mice (KO) showed down-regulation of SARM1 mRNA and proteins in the heart, but did not show change in baseline cardiac function. Male WT and SARM1-KO mice were challenged with vehicle or STZ to induce T1D. Fasting glucose levels were similarly elevated in WT and SARM1-KO mice after 16-week diabetic stress. Chronic diabetes promoted progressive cardiac systolic (fractional shortening) and diastolic dysfunction (E'/A', e/E' and IVRT), and elevated cardiac nppb expression. SARM1-KO diabetic mice hearts had elevated NAD^+ levels, suppressed up-regulated nppb levels, and improved cardiac function compared to WT diabetic mice. Chronic diabetes increased cardiac fibrosis and up-regulated expression of pro-fibrotic genes, ctgf and Inhbb, all of which were suppressed by SARM1 deficiency.

Another set of male WT and KO mice were fed with high-fat diet (HFD) for 16 weeks. HFD feeding similarly increased body weight of both WT and KO mice, compared to mice fed with normal chow. HFD feeding did not affect systolic function in WT and KO mice. However, HFD promoted diastolic dysfunction and hypertrophy, which were alleviated by SARM1 deficiency. Taken together, our data suggest a protective effect of SARM1 deficiency on metabolic cardiomyopathy, though mechanistic details need further investigation.

DISSECTING THE MECHANISM OF CINNABARINIC ACID MEDIATED CYTOPROTECTION AGAINST NON-ALCOHOLIC FATTY LIVER DISEASE

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Nonalcoholic fatty liver disease (NAFLD) is a chronic condition characterized by excess accumulation of lipids in liver and can lead to a range of progressive liver disorders like non-alcoholic steatohepatitis, liver cirrhosis, and hepatocellular carcinoma. While lifestyle and diet modifications have proven to be effective in NAFLD treatment, they are not sustainable in long-term and currently there are no approved pharmacological therapies to treat NAFLD. We have recently identified a tryptophan catabolite, cinnabarinic acid (CA) as a novel endogenous Aryl hydrocarbon Receptor (AhR) agonist capable of conferring cytoprotection against plethora of ER/oxidative stressors. In this study, CA was tested for hepatoprotection against palmitic and oleic acid-induced steatosis, apoptosis and inflammation in HepG2 and AML12 cell lines mimicking NAFLD model. CA treatment significantly reduced steatosis, attenuated expression of free fatty acid transporters, as well as genes involved in *de novo* lipogenesis and triglyceride synthesis. Moreover, expression of the pro-inflammatory cytokines – TNFα, TGFβ, MCP1 were significantly downregulated upon CA treatment. Finally, immunoblotting revealed decreased hepatocyte apoptosis and stimulated phosphorylation of AMPKα potentially promoting fatty acid oxidation. Our results clearly demonstrate that CA confers hepatoprotection against steatosis and inflammation through control of hepatic lipid uptake and *de novo* lipogenesis pathways. This work was supported by HHDC-PHF seed grant.

THE ROLE OF APOC3 GENETIC VARIATION, SERUM TRIGLYCERIDES AND RISK OF CARDIOMETABOLIC DISORDER WITH DEVELOPMENT OF CORONARY ARTERY DISEASE IN ASIAN INDIANS, EUROPEANS, AND OTHER ETHNIC GROUPS

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Hypertriglyceridemia has emerged as a critical coronary artery disease (CAD) risk factor. Rare loss-of function (LoF) variants in apolipoprotein C-III have been reported to reduce triglycerides (TG) and are cardioprotective in American Indians and Europeans. However, there is a lack of data in other Europeans and non- Europeans. Also, whether genetically increased plasma TG due to ApoC-III is causally associated with increased CAD risk is still unclear and inconsistent. The objectives of this study were to verify the cardioprotective role of earlier reported six LoF variants of APOC3 in South Asians and other multi-ethnic cohorts and to evaluate the causal association of TG raising common variants for increasing CAD risk.

We performed gene-centric and Mendelian randomization analyses and evaluated the role of genetic variation encompassing APOC3 for affecting circulating TG and the risk for developing CAD. Circulating concentrations of ApoC-III were quantified by enzyme-linked immunosorbent assay (ELISA) to understand its effect in developing CAD. One rare LoF variant (rs138326449) with a 37% reduction in TG was associated with lowered risk for CAD in Europeans ($p = 0.007$), but we could not confirm this association in Asian Indians ($p = 0.641$). Our data could not validate the cardioprotective role of other five LoF variants analysed. A common variant rs5128 in the APOC3 was strongly associated with elevated TG levels showing a p-value $2.8 \times 10-424$. Measures of plasma ApoC-III in a small subset of Sikhs revealed a 37% increase in ApoC-III concentrations among homozygous mutant carriers than the wild-type carriers of rs5128. A genetically instrumented per 1SD increment of plasma TG level of 15 mg/dL would cause a mild increase (3%) in the risk for CAD ($p = 0.042$).

Our results highlight the challenges of inclusion of rare variant information in clinical risk assessment and the generalizability of implementation of ApoC-III inhibition for treating atherosclerotic disease. More studies would be needed to confirm whether genetically raised TG and ApoC-III concentrations would increase CAD risk. **Funding:** The Sikh Diabetes Study/ Asian Indian Diabetic Heart Study was supported by NIH grants-R01DK082766; R01DK118427 (NIDDK) and NOT-HG-11-009 (NHGRI) and grants from Presbyterian Health Foundation and Harald Hamm Diabetes Center of Oklahoma. Sequencing services were provided through the RS&G Service by the Northwest Genomics Center at the University of Washington, Department of Genome Sciences, under US Federal Government contract number HHSN268201100037C from the National Heart, Lung, and Blood Institute of the NIH.

IMPACT OF MATERNAL DIABETES ON MICRORNA 146B EXPRESSION IN FETAL TISSUE

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Exposure to diabetes mellitus (DM) *in utero* increases future cardiovascular risk (CVD) in the offspring. However, the mechanism underlying increased CVD risk is unknown. Human umbilical vein endothelial cells (HUVEC) have been used as a model for CVD risk in diabetes as chronic inflammation accelerates endothelial dysfunction contributing to macrovascular disease. MicroRNAs (miRNA), small noncoding RNAs that repress mRNA translation, have been proposed as an epigenetic mechanism that alter metabolic programming. Our group used an unbiased sequencing approach to assess the impact of *in utero* DM exposure on miRNA expression in HUVEC from infants exposed to the diabetic milieu of pregnancy and infants born to mothers with normal glycemia matched for infant sex, ethnicity, and gestational age. miRNA sequencing revealed that miR-146b had decreased expression in DM-exposed infants. RT-PCR confirmed a 25% decrease in miR-146b (p 0.03) from the HUVEC of DM-exposed infants (N = 23) compared to controls (N = 39). In order to explore the component(s) of the diabetic milieu potentially altering miRNA-146b abundance, HUVECs from control and DM-exposed infants were cultured in 25mM mannitol (hyperosmolar), 25mM glucose (hyperglycemia), 4-hydroxynonenal 5uM and 10uM (oxidative stress), TNF-alpha 30ng/mL (inflammation), and palmitic acid 0.5mM and 1.0 mM (hyperlipidemia). As compared to the non-DM controls, exposure to TNF-alpha increased miRNA-146b expression 3-fold in non-DM HUVECs and over 4-fold in DM-exposed HUVECs, while exposure to palmitic acid 1.0 mM increased miR-146b exposure about 1.9-fold in non-DM HUVECs and almost 2.5-fold in DM-exposed HUVECs. Furthermore, western blot analysis of DM-exposed HUVECs cultured in TNF-alpha and palmitic acid revealed decreased expression of both TRAF6 and sortilin, both of which have been associated with CVD, and consistent with increased expression of miR-146b. While individually the conditions did not result in a decrease in miR-146b abundance, the combination of these factors may contribute to the decrease observed *in vivo*. An increase in sortilin abundance has been associated with increased CVD risk. miRNA-146b abundance through its target sortilin may play a role in the development of excess CVD risk after *in utero* DM exposure.

ABBREVIATED FAT TOLERANCE TEST AS A SCREENING TOOL FOR NON-ALCOHOLIC FATTY LIVER DISEASE IN CHILDREN

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Background: The standard for early screening of NAFLD in children is circulating liver enzymes, but those measures lack clinical specificity and sensitivity for NAFLD. As an alternative diagnostic approach we tested the hypothesis that the postprandial triglyceride (TG) response to an abbreviated fat tolerance test (AFTT) could discriminate pediatric patients with NAFLD from obese and normal weight peers. **Methods:** In this ongoing study, 13 normal weight controls (6M/7F; age: 16.6 ± 2.2 y; BMI%: 49 ± 24 %), 11 controls with obesity (4M/7F; age: 16.9 ± 2.2 y; BMI%: 98 ± 1 %), and 8 patients with NAFLD (6M/2F; age: 14.8 ± 1.6 y; BMI%: 99 ± 0 %) completed an AFTT. Following an overnight fast, participants consumed a high-fat meal (73% fat; 9 kcal/kg) and TG were measured at baseline and 4h post-meal. We previously showed that this abbreviated protocol is a valid surrogate for traditional postprandial TG testing (serial hourly blood sampling for 6h postprandial period). Liver steatosis was measured as the controlled attenuation parameter (CAP) with Fibroscan. **Results:** The NAFLD group displayed higher fasting TG (128 ± 45 mg/dL) than normal weight (82 ± 34 mg/dL; $p = 0.03$) but NAFLD did not differ from obese (114 ± 46 mg/dL). NAFLD exhibited higher 4h postprandial TG and ΔTG (206 ± 69 mg/dL; 78 ± 31 mg/dL, respectively) than normal weight (110 ± 46 mg/dL, $p = 0.002$; 28 ± 38 mg/dL, $p = 0.02$) but did not differ from obese (156 ± 71 mg/dL, $p = 0.20$; 39 ± 43 mg/dL, $p = 0.10$); normal weight and obese controls did not differ in fasting ($p = 0.08$) or 4h postprandial TG ($p = 0.09$). The NAFLD and obese groups did not differ in liver steatosis (337 and 292 dB respectively, $p = 0.09$); both groups were higher than normal weight (209.7 dB, p's < 0.05). **Conclusion:** The preliminary findings suggest that the postprandial TG response in NAFLD is greater in children with NAFLD compared to normal weight peers without NAFLD, but does not differ between children with NAFLD and children with obesity.

HIGHER COMPLEX-CARBOHYDRATE DIET DURING PREGNANCY INCREASES THE NATURAL PROBIOTIC BIFIDOBACTERIACEA IN GUT MICROBIOME IN WOMEN WITH GESTATIONAL DIABETES MELLITUS

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Conventional dietary approaches for controlling gestational diabetes mellitus (GDM) result in a carbohydrate-restricted, higher fat diet to help control postprandial glucose levels and mitigate glucose-mediated fetal macrosomia. However, a high fat maternal diet may impair glucose uptake by skeletal muscle and promote an altered microbiome in the mother, which may be transferred to the infant. The Choosing Healthy Options in Carbohydrate Energy (CHOICE) study, was a randomized, controlled trial (RCT) and provided women with diet-controlled GDM with the CHOICE diet (60% **complex** carbohydrate/25% fat/15% protein) versus a conventional GDM diet (CONV, 40% carbohydrate/45% fat/15%) from the time of GDM diagnosis to delivery. Fecal samples for microbiome sequencing using shotgun metagenomics were taken from 20 CHOICE and 17 CONV mothers at baseline (mean of 31 wks) and at 37 wks. All meals and snacks were provided, the diets were eucaloric, and fiber content did not differ. Microbiome taxonomy and protein functional annotation of the assembled reads were analyzed by diet. We found no differences in alpha or beta diversity of the microbiome between the two diets over the course of pregnancy. At the family-level, women on the CHOICE diet had significantly higher levels of *Bifidobacteriaceae* after 6 weeks on CHOICE compared to women on the CONV diet. *Bifidobacterium adolescentis* was the main Bifidobacterium species driving the relationship between diet and *Bifidobacteriaceae* abundance. Functional annotation of these genes revealed women on the CHOICE diet had 105 microbial gene abundances altered, while women on the CONV diet had only 31 abundances altered between 31 and 37 weeks. Within the CHOICE group, there was an increase in abundance of genes involved with metabolic pathways such as glycolysis/gluconeogenesis and the pentose phosphate pathway, but these same pathways had decreased gene abundances in the microbiome of the women on the CONV diet. In summary, we found that a eucaloric fixed diet higher in complex carbohydrates and lower in fat (CHOICE) maintained normoglycemia and suppressed postprandial FFAs while altering the maternal microbiome, including an increase in the natural probiotic *Bifidobacteriaceae*. Given the various beneficial effects associated with *Bifidobacteriaceae*, a higher complex carbohydrate/lower fat diet may confer a possible benefit to mothers with GDM. Changes in metabolic pathways based on gene protein abundance suggest this may increase healthy gut bacterial metabolism.

PUBLISHED ABSTRACTS

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Published abstracts - listed alphabetically by author

1. **CHEMOGENETIC STIMULATION OF PROOPIOMELANOCORTIN PROJECTIONS TO THE NUCLEUS ACCUMBENS MODULATE ACQUISITION AND MAINTENANCE OF OPERANT SUCROSE PELLET SELF-ADMINISTRATION IN MICE**
   ELIASON, NICOLE; Amanda Sharpe
2. **ASSESSMENT OF POLYGENIC RISK SCORE FOR PREDICTING T2D RISK: RESULTS OF THE AIDHS/SDS**
   GOYAL, SHIWALI; Dharambir Sanghera
3. **PULMONARY GLUCOSE DYSREGULATION LEADS TO INCREASED INFLUENZA VIRAL REPLICATION**
   ROCHOWSKI, MATTHEW; Campolo A, Allen S, Lin Liu, Lacombe VA
4. **A WELL-FORMULATED KETOGENIC DIET MITIGATES VISUAL AND MOTOR DEFICITS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS**
   ZYLA KATARZYNA; Dorothy Walton, Kendra Plafker, Susan Kovats, Steve Brush, Martin-Paul Agbaga, Scott M Plafker

CHEMOGENETIC STIMULATION OF PROOPIOMELANOCORTIN PROJECTIONS TO THE NUCLEUS ACCUMBENS MODULATE ACQUISITION AND MAINTENANCE OF OPERANT SUCROSE PELLET SELF-ADMINISTRATION IN MICE

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Obesity is a crisis in the United States, with many co-morbid diseases that can drastically decrease quality of life. While diet is a major focus for therapeutic intervention, the need to understand underlying appetitive neurocircuitry persists. The hypothalamic arcuate nucleus serves as an integration point for circulating nutritional cues and mediates down-stream appetitive behaviors. Proopiomelanocortin (POMC) projections within the hypothalamus are well-known for their anorexigenic activity, but little is understood about the role of projections to the nucleus accumbens (NAcc). The NAcc is best known for its role in reward-based learning, but the contribution of POMC projections to NAcc are controversial since the two major POMC-derived peptides (◎-endorphin and ◎-MSH) have opposite effects on feeding. Our objective here was to determine the effect of stimulating POMC projections in the NAcc on acquisition and maintenance of operant food self-administration. Adult POMC-Cre mice were microinjected into the NAcc with a Cre-dependent retrograde adeno-associated viral vector expressing Gq Designer Receptor Exclusively Activated by Designer Drugs (DREADD). Mice were trained to self-administer palatable 20-mg sucrose pellets in daily operant sessions. Acquisition of self-administration (fixed ratio 30) and baseline self-administration were measured daily for 2 weeks. Throughout behavioral testing, mice received daily injections of either JHU37152 (DREADD agonist) or saline (i.p.) 15 min prior to the session. JHU produced a significant increase in rate of acquisition and accuracy compared to the saline group. Furthermore, JHU reduced consumption across groups following a saline period, with amplified effects in mice previously exposed to JHU. These results suggest a complex role of POMC peptides within the NAcc that may increase reward learning while decreasing consumption.

ASSESSMENT OF POLYGENIC RISK SCORE FOR PREDICTING T2D RISK: RESULTS OF THE AIDHS/SDS

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Genome-wide polygenic risk score (PRS) is considered a good predictor of disease risk. However, its utility as an independent risk predictor in complex diseases such as type 2 diabetes (T2D) remains inconclusive. Epidemiological studies reported that people from the Indian subcontinent suffer from a greater risk of cardiovascular disease-associated mortality than other global populations. Overwhelming data support a strong influence of genetic factors and their interaction with lifestyle and environmental factors. Populations from the Indian subcontinent are underrepresented in genetic studies despite Asian Indians contributing more than 25% of the world population and have up to six times more risk for T2D than European whites. Here we have evaluated the performance of multilocus PRS in T2D risk-prediction in comparison to the Clinical Risk Score (CRS, modified from the Joint British Society Score) using 4,588 individuals (2,566 cases and 2,022 controls) from the Asian Indian Diabetes Heart Study/Sikh Diabetes Study (AIDHS/SDS). The PRS was constructed from 63 significant variants associated with T2D in Asian Indians [PRS (AI)]. For comparison, we used PRS (EU), which was comprised of 59 known SNPs to be associated with T2D in Europeans.

Logistic regression analyses were performed using age, gender, BMI, and five principal components as covariates to determine the risk prediction for T2D using PRS (AI), PRS (EU), and CRS. PRS (AI) for T2D yielded an adjusted odds ratio (OR) 1.86 95% CI (1.73-2.00), $p=2.39x10^{-62}$ in the training set, and OR 1.51 95% CI (1.43-1.59), $p=5.64x10^{-55}$ for the test set. PRS (EU) gave an adjusted OR=1.70 (1.32-2.17; $p=3x10^{-5}$) and OR=1.74 (1.42-2.13; $p=8.55x10^{-8}$) in the training and test set respectively. In comparison, the composite analysis of CRS revealed a higher OR both in training, and the test sets, respectively [OR 2.63 95%CI (2.36-2.92), $p=1.58x10^{-69}$ and OR 2.71 95%CI (2.50-2.95), $p=1.66x10^{-123}$]. Next, to test the discriminative accuracy of PRS and CRS, we constructed the receiver operating curve (ROC) and estimated the area under the curve (AUC). An adjusted AUC of 0.76 and an AUC of 0.82 was observed for PRS (AI) and CRS, respectively. On the other hand, the observed AUC for PRS (EU) (derived using European genes), was lower (0.67) than the AUC of 0.76 using PRS (AI).

Even though the PRS(AI) was an independent predictor of the T2D risk, the CRS still has outperformed the PRS in this study. Nevertheless, as more risk loci with population-specific effects are being identified by sequencing, combining the PRS approach with CRS would enhance the predictive power of these analyses. Ultimately, these efforts will help identify and stratify individuals at higher risk for developing T2D. **Funding:** The Sikh Diabetes Study/ Asian Indian Diabetic Heart Study was supported by NIH grants-R01DK082766; R01DK118427 (NIDDK), and grants from the Presbyterian Health Foundation.

PULMONARY GLUCOSE DYSREGULATION LEADS TO INCREASED INFLUENZA VIRAL REPLICATION

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Diabetes is characterized by sustained hyperglycemia due to either lack of insulin production (Type 1) or lack of insulin action (Type 2). Hyperglycemia is an independent risk factor for the development of severe respiratory infections, including influenza infections. However, the regulation of glucose metabolism in the lung (a major organ to utilize glucose) during diabetes or infection has received little attention. We hypothesize that hyperglycemia predisposes diabetic subjects to excess glucose in the airway, allowing for greater viral replication. To test this hypothesis, subsets of type 1 (T1Dx) and type 2 (T2Dx) diabetic mice were treated with insulin or metformin, respectively, to restore euglycemia, then intranasally infected with influenza. Glucose concentrations of the bronchoalveolar lavage fluid (BALF) were measured with a glucose oxidase assay. *In vitro*, human bronchial epithelial cells (HBECs) were incubated with varying glucose concentrations, 2-deoxyglucose, insulin, or AMPK modulators, then infected. Viral loads were determined by immunofluorescence and/or qrtPCR for viral protein hemagglutinin. *In vivo*, diabetic and infected mice demonstrated increased glucose concentrations in the BALF. Viral load was significantly greater in lung homogenates of both T1Dx and T2Dx mice, which was rescued by insulin or metformin, respectively. *In vitro*, HBECs incubated in high [glucose] had a significantly greater percentage of cells infected than those incubated in normal [glucose]. Conversely, cells treated with 2-deoxyglucose demonstrated reduced viral replication. Activation of glycolysis using insulin or AICAR (an AMPK activator) increased viral replication, while inhibition of AMPK (using compound c) decreased viral replication. These novel findings suggest that: 1) hyperglycemia increases BALF [glucose], thus H1N1 replication capacity in diabetic lung, and 2) influenza viral replication is dependent on the activation of host-cell glycolysis. Better understanding of the mechanisms altering glucose metabolism during viral infection may lead to the discovery of novel therapeutic targets for diabetic patients infected with influenza.

A WELL-FORMULATED KETOGENIC DIET MITIGATES VISUAL AND MOTOR DEFICITS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Purpose: 50% of multiple sclerosis (MS) patients suffer from optic neuritis during disease progression. Symptomatic relief from steroids is the primary treatment but only reduces symptom severity in a subset of patients. Ketogenic diets (KD) are gaining popularity as therapeutic interventions based on documented successes in reducing motor deficits in small cohorts of MS patients. Our study investigated the efficacy of the KD to ameliorate optic neuritis in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS.

Methods: C57BL/6 mice were fed either the KD (77.1% fat from MCT, flaxseed, canola oils, 22.4% protein, 0.5% carbohydrate (CHO)) or a control diet (10.4% fat, 20.4% protein, 69.3% CHO) and 2 weeks later, immunized against the myelin oligodendrocyte glycoprotein (MOG) peptide antigen. Mice were scored daily for motor deficits and every 2-3 days for visual acuity by optokinetic tracking. Glucose and ketone levels in blood were measured longitudinally. Post-mortem, retinal ganglion cells (RGCs) were quantified and immune profiles from splenoctyes were characterized.

Results: The KD largely conferred resistance to EAE symptom onset and preserved RGCs and visual acuity. Curiously, EAE mice fed a KD exhibited splenomegaly and enlarged lymph nodes with a corresponding increase in immature erythrocytes. Additionally, males displayed an increase in candidate suppressor monocytes while females exhibited an increase in transitional B cells in the spleen. The KD also reduced the ratio of pro-inflammatory omega-6 fatty acids to anti-inflammatory omega-3 fatty acids in the plasma, red blood cells, and liver.

Conclusions: These findings support a model in which reducing dietary carbohydrates using a KD promotes a systemic anti-inflammatory milieu that mitigates autoimmune-induced demyelinating visual and motor deficits, further supporting ongoing clinical trials using such a strategy to treat MS patients.

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