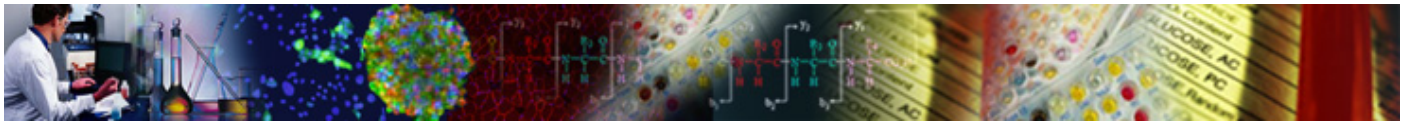
Summer | 2013

DIABETES RESEARCH CENTERS



NIDDK Medical Student Research Symposium

Student Life Center

Vanderbilt University

Nashville, TN

July 31 – August 1, 2013

Funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

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Description of the Programs

The National Institutes of Health (NIH) through the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) supports programs to encourage physicians to enter careers involving research. Two NIDDK-supported summer programs, the **NIDDK Medical Student Research Program in Diabetes** and the **Vanderbilt O'Brien Summer Kidney Research Program,** are concluding with the fifth annual NIDDK Medical Student Research Symposium at Vanderbilt University on July 31 – August 1, 2013.

These programs offer first- and second-year medical students the opportunity to conduct mentored research. The research opportunities range from basic laboratory studies to clinical studies in humans. Participating students also participate in a core curriculum of seminars on research approaches as well as a variety of clinical and research topics in diabetes and kidney disease. These programs help students gain an improved understanding of career opportunities in biomedical research. At the conclusion of the summer, each student presents his/her work at the national research symposium, which provides a venue for the students to meet, share their research, and to learn about career pathways and opportunities in NIDDK-related professions.

The **NIDDK Medical Student Research Program in Diabetes** began in 2009. For the summer of 2013 program, 114 students from 68 medical schools were chosen to conduct research under the direction of an established scientist in the areas of diabetes, metabolism, obesity and digestive disease at an institution with one of 16 NIDDK-funded Diabetes Research Centers. Some students were supported by the Diabetic Complications Consortium.

The **Vanderbilt O'Brien Medical Student Research Program in Kidney Diseases**, funded by the NIDDK and coordinated through the Vanderbilt O'Brien Kidney Physiology and Disease Center, began in 2011. For the summer of 2013 program, 17 first-year medical students from 13 medical schools were selected to participate in this program. These students have been conducting research on aspects of kidney injury and associated diseases under the supervision of NIDDK-funded investigators in 17 different laboratories and clinical research programs at Vanderbilt University.

Symposium Agenda for

NIDDK Medical Student Research Program Symposium
Student Life Center, Vanderbilt University
July 31 – August 1, 2013

Tuesday, July 30

7:00 pm **Opening reception** in Centennial Ballroom, Holiday Inn Vanderbilt

Wednesday, July 31

8 – 8:55 am Poster set up and continental breakfast in the Student Life Center

9:00 **Welcome and introduction**
Alvin C. Powers, MD
Joe C. Davis Chair in Biomedical Science
Professor of Medicine and Molecular Physiology & Biophysics
Chief, Division of Diabetes, Endocrinology & Metabolism
Director, Vanderbilt Diabetes Center
Vanderbilt University

9:15 – 10:00 **Presentation – Ballroom C**
Steven Kahn, MD
Professor of Medicine, Division of Metabolism, Endocrinology & Nutrition
Director, Diabetes Research Center
University of Washington
Seattle VA Puget Sound Health Care System

10:00 – 11:30 **Poster session 1 (moderated)**

11:45 – 1:00 **Lunch with visiting professors in small groups**

1:00 – 1:45 **Presentation – Ballroom C**
Monica Peek, MD, MPH
Assistant Professor
Division of General Internal Medicine, Department of Medicine
Associate Director, Chicago Center for Diabetes Translation Research
University of Chicago

1:45 – 3:15 **Poster Session A (open)**
1:45 – 2:30 *Odd* numbered posters present
Even numbered posters visit
2:30 – 3:15 *Even* numbered posters present
Odd numbered posters visit

3:15 **Reassemble for Program Information – Ballroom C**

3:30 - 5:00 **Poster Session 2 (moderated)**

5:15 **Reception**

5:45 **Buffet dinner** in the Board of Trust Room

Thursday, August 1

9:00 am **Welcome – Ballroom C**
Mark de Caestecker, MB, BS, PhD
Associate Professor of Surgery, Medicine, Division of Nephrology and Associate Professor of Cell & Developmental Biology
Vanderbilt University Medical Center

9:05 – 9:45 **Presentation – Ballroom C**
Kerri Cavanaugh, MD, MHS
Assistant Professor of Medicine
Medical Director, Vanderbilt Campus Dialysis Clinic
Vanderbilt University Medical Center

9:45 – 11:30 **Poster Session 3 (moderated)**

11:45 – 1:00 **Lunch** with visiting professors in small groups

1:00 – 1:45 **Presentation – Ballroom C**
Art Castle, PhD
Director, Metabolomics and Informatics Program, Division of Diabetes, Endocrinology & Metabolic Diseases
National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health
And
Alvin C. Powers, MD
Joe C. Davis Chair in Biomedical Science
Professor of Medicine and Molecular Physiology & Biophysics
Chief, Division of Diabetes, Endocrinology & Metabolism
Director, Vanderbilt Diabetes Center
Vanderbilt University Medical Center

2:00 – 3:30 **Poster Session B (open)**
2:00 – 2:45 *Even* numbered posters present
Odd number posters visit
2:45 – 3:30 *Odd* numbered posters present
Even number posters visit

3:30 – 4:00 **Career discussion with student panel**
L. McLean House, Class of 2015, University of Tennessee Health Sciences Ctr
Sarah E. Scott, Class of 2014, Vanderbilt University School of Medicine
R. Taylor Sundby, Class of 2015, Vanderbilt School of Medicine

4:00 – 4:15 **Concluding remarks**

6:15 Meet the bus in front of the Holiday Inn Vanderbilt for transport to restaurant
6:30 Dinner in downtown Nashville
9:00 Approximate end to dinner; students on own for return to hotel

Friday, August 2
Students depart on own

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Medical Schools of Students Participating in the 2013 NIDDK Medical Student Research Program in Diabetes

Albert Einstein College of Medicine

Arizona College of Osteopathic Medicine

Baylor College of Medicine

Boston University School of Medicine

Brody School of Medicine, East Carolina University

Creighton University School of Medicine

Drexel University College of Medicine

Eastern Virginia Medical School

Florida Atlantic University, Charles E. Schmidt College of Medicine

Georgetown University School of Medicine

Harvard Medical School

Howard University College of Medicine

Indiana University School of Medicine

Jefferson Medical College

Kansas City University of Medicine and Biosciences

Keck School of Medicine of the University of Southern California

Louisiana State University School of Medicine, New Orleans

Medical College of Georgia

Medical University of South Carolina

Meharry Medical College

Michigan State University College of Human Medicine

Morehouse School of Medicine

National University of Ireland, Galway

New York Medical College

Pennsylvania State University College of Medicine

Perelman School of Medicine at the University of Pennsylvania

Ponce School of Medicine

Rosalid Franklin University, Chicago Medical School

Rush Medical College

Sapienza University of Rome, Medicine

Saint Louis University School of Medicine

State University of New York, Downstate

Texas A&M Health Science Center

Tulane University School of Medicine

UMDNJ - New Jersey Medical School

UMDNJ- School of Osteopathic Medicine

University Alabama, Birmingham

University at Buffalo School of Medicine

University of Arizona College of Medicine, Phoenix

University of Arkansas for Medical Sciences

University of California, San Diego School of Medicine

University of California, San Francisco School of Medicine

University of Central Florida College of Medicine

University of Hawaii

University of Illinois at Chicago

University of Kansas School of Medicine

University of Maryland School of Medicine

University of Massachusetts Medical School

University of Michigan Medical School

University of Oklahoma College of Medicine

University of Pennsylvania

University of Puerto Rico School of Medicine

University of Rochester Medical Center

University of South Alabama

University of South Carolina School of Medicine

University of Tennessee Health Sciences Center

University of Texas Medical Branch

University of Vermont College of Medicine

University of Virginia School of Medicine

University Rochester Medical Center

Wake Forest University School of Medicine

Warren Alpert Medical School of Brown University

Washington University

Wayne State University School of Medicine

Weill Cornell Medical College of Cornell University

Western University of Health Sciences

Medical Schools of the Students Participating in the 2013 Medical Student Research Training Program in Nephrology and Hypertension

Chicago Medical School at Rosalind Franklin University

Creighton University School of Medicine

Drexel University College of Medicine

Indiana University School of Medicine

Louisiana State University School of Medicine

Princeton University

UMDNJ, Robert Wood Johnson Medical School

University at Buffalo School of Medicine

University of Kansas School of Medicine

University of Oklahoma College of Medicine

University of Tennessee Health Science Center

University of Texas at Southwestern Medical Center

University of Toledo College of Medicine

Medical Schools of Students Participating in the 2013 NIDDK Medical Student Research Program in Digestive Disease

Florida State University College of Medicine

Louisiana State University - New Orleans

Loyola University Chicago Stritch School of Medicine

Thomas Jefferson University

University of Central Florida College of Medicine

University of Utah

Visiting Professors/Faculty for the NIDDK Medical Student Research Symposium

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Biographies of NIDDK Visiting Professors and Faculty

Art L. Castle, PhD, Director, Metabolomics and Informatics Program, Division of Diabetes, Endocrinology and Metabolic Diseases, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health
Arthur L. Castle has a PhD in Physiology from University of Texas, Austin. He did his postdoctoral research training study glucose transporters at NIDDK/NIH before working as a Manager in Bioinformatics for Gene Logic Inc. His research experience includes diabetes, metabolism, genomics, bioinformatics, and computer simulation/modeling. Dr. Castle joined the Division of Diabetes, Endocrinology and Metabolic Diseases, NIDDK, NIH, DHHS in 2004, where he is current the program director for fellowships ("F") awards, institutional training grants, metabolomics and informatics.

Kerri L. Cavanaugh MD, MHS received her bachelor's degree in Chemistry and Psychology from Dartmouth College in 1995, her medical degree from Yale in 1999 and completed an Internal Medicine Residency and fellowship in Nephrology in 2005. During this time she also served as Chief Resident of Internal Medicine at Johns Hopkins Hospital. In 2006, she graduated from the Johns Hopkins Bloomberg School of Public Health with a Masters in Health Science degree with a focus in clinical epidemiology. She joined the faculty at Vanderbilt in Internal Medicine Division of Nephrology and the Vanderbilt Center for Health Services Research in 2006, she is currently an Assistant Professor of Medicine and serves as the Medical Director for the Vanderbilt Campus Dialysis Clinic. Dr. Cavanaugh's primary research interest is to identify mechanisms and interventions to facilitate patient-provider health communication about complex chronic kidney disease care to improve patient understanding, self-efficacy, participation in self-care and health outcomes. She has experience and expertise in survey design, validation and administration, development of educational interventional materials, protocol and training development, and also execution of trials to examine educational/behavioral interventions. She is currently examining methods to enhance anemia management by promoting shared-decision making and safe use of erythropoiesis stimulating agents. She has been funded by the NIH and the National Kidney Foundation.

Mark de Caestecker, MB, BS, PhD, received his undergraduate degree from Cambridge University and medical degree (M.B., B.S.) from The Middlesex Hospital (now University College) in London, England. After training in internal medicine at the Middlesex and Charring Cross Hospitals in London, Dr. de Caestecker trained in Nephrology, obtained his PhD in Medicine at the University of Manchester, and underwent post-doctoral training on a Wellcome Trust Clinical Scientist Fellowship at the NIH under the mentorship of Dr. Anita Roberts. He is currently an Associate Professor in the Division of Nephrology at Vanderbilt, and conducts NIH funded research on regenerative therapies in acute and chronic kidney injury, and on hereditary vascular diseases. He currently serves on the Respiratory and Integrative Biology and Translational Research study section. Dr. de Caestecker is Director of the Vanderbilt-HHMI Certificate Program in Molecular Medicine, and Director of the Vanderbilt O'Brien Kidney Center Education program. He has directed the short-term summer research-training program in nephrology for the last three years.

Steven E. Kahn, MB, ChB grew up in South Africa where he received his medical education at the University of Cape Town. After doing required rotational training in obstetrics and gynecology and in medicine, he immigrated to the United States where he did an internal medicine residency at Albert Einstein Medical Center in Philadelphia. Thereafter, he undertook his fellowship training in endocrinology and metabolism at the University of Washington in Seattle, where he has been on the faculty since. He is currently Professor of Medicine in the Division of Metabolism, Endocrinology and

Nutrition and has held leadership roles at the University, including being Associate Chief of Staff for Research and Development at VA Puget Sound Health Care System. He currently directs the Diabetes Research Center at the University of Washington. Dr. Kahn has a robust research program involving both clinical and basic investigation of the pathogenesis of type 2 diabetes, and which is funded by the NIH, the Department of Veterans Affairs and the American Diabetes Association. His clinical studies have included participation in the landmark Diabetes Prevention Program that demonstrated that diabetes can be prevented in individuals who are at high risk for the disease and in ADOPT, which compared the approach to initial therapy for patients with early type 2 diabetes. He is also leading the Restoring Insulin Secretion (RISE) Study, a multicenter clinical trial which was recently launched by NIH to study novel ways to prevent the loss of insulin secretion and thus slow the progression of hyperglycemia in prediabetes and type 2 diabetes. His basic studies have focused on the role of amyloid as a destructive process in the islet. Another important component of his academic life is the training of medical students and fellows. These individuals have come from the United States and abroad and have had very positive impacts on his program and have used the knowledge gained working with him in establishing their own successful programs.

Monica Peek, MD, MPH, FACP, is an Assistant Professor in the Division of General Internal Medicine at the University of Chicago where she provides clinical care, teaches and does health services research in the area of health disparities. She received her medical degree and master's degree in public health from the Johns Hopkins University, and completed her residency training at Stanford University Hospital. She then worked for the National Health Service Corps for two years at a community health center for the medically underserved in Ohio before relocating to Chicago.

Dr. Peek is the Associate Director of the Chicago Center for Diabetes Translation Research and a member of the Robert Wood Johnson Foundation (RWJF) program office *Finding Answers: Disparities Research for Change*. She has been funded by RWJF and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to explore racial differences in patient/provider communication and to pilot patient-empowerment interventions to enhance such communication among racial/ethnic minorities with diabetes. She is an inaugural faculty fellow of the Bucksbaum Institute for Clinical Excellence. Dr. Peek is the Principal Investigator of grants from the Merck Company Foundation and NIH/NIDDK to improve diabetes care and outcomes among residents on the South Side of Chicago, a predominantly African-American working class community with significant disparities in diabetes health outcomes such as lower extremity amputations. Dr. Peek was part of the NIDDK strategic planning committee whose 2010 report 'Advances and Emerging Opportunities in Diabetes Research' set forth the diabetes research agenda for the next 5-10 years.

Dr. Peek also does research on the development and evaluation of community-based, culturally-tailored interventions to promote healthy behaviors and preventive care, including women's health (i.e. breast cancer screening), physical activity and diabetes self-management.

Alvin C. Powers, MD, the Joe C. Davis Chair in Biologic Science and Professor of Medicine, Molecular Physiology and Biophysics at Vanderbilt University, is the Director of the Vanderbilt Diabetes Center, the Chief of the Vanderbilt Division of Diabetes, Endocrinology, and Metabolism, and the Director of the Vanderbilt Diabetes Research and Training Center, a NIH-funded center that facilities the diabetes-related research of more than 100 Vanderbilt scientists. He conducts diabetes-related research that focuses on pancreatic islet biology, development, function, and transplantation. His research is or has been supported by the NIH, the VA Research Service, the Juvenile Diabetes Research Foundation (JDRF), and the American Diabetes Association (ADA). Dr. Powers is the Director of the Vanderbilt Medical Student Research Training Program in Diabetes, Endocrinology, and Metabolism and the Coordinator for the NIDDK Medical Student Research Program in Diabetes. These two programs enable more than 100 medical students to conduct diabetes-related research each summer at a

NIDDK-supported Diabetes Research Center. Dr. Powers, a physician at Vanderbilt University Medical Center and the VA Tennessee Valley Healthcare System, is listed by Castle Connolly Medical Ltd as one of “America’s Top Doctors.” Dr. Powers received his undergraduate degree from the University of Virginia and his medical degree from the University of Tennessee Center for the Health Sciences. After training in internal medicine at Duke University Medical Center, Dr. Powers trained in Endocrinology and Diabetes at the Joslin Diabetes Center, the Massachusetts General Hospital, and Harvard Medical School.

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Albert Einstein College of Medicine
DK020541

Baylor College of Medicine
DK071289

Boston Area Diabetes Center
DK057521

Columbia University
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Johns Hopkins University and University of Maryland
DK079637

Joslin Diabetes Center
DK036836

University of Alabama at Birmingham
DK079626

University of California at San Diego and University of California at Los Angeles
DK063491

University of California at San Francisco
DK063720

University of Chicago
DK020595

University of Michigan
DK020572

University of Pennsylvania
DK019525

University of Washington
DK017047

Vanderbilt University
DK020593

Washington University
DK020579

Yale University
DK045735

Training Grants Supporting
2013 NIDDK Medical Student Research Program in Diabetes

Grant Number	PI of Training Grant	Institution
T32DK007513-25	Zukin, R. Suzanne	Albert Einstein College of Medicine
T32DK007696-20	Moore, David	Baylor College of Medicine
T32DK007770-11	Melmed, Shlomo	Cedars Sinai Medical Center
T32DK007271-34	Bilezikian, John	Columbia University
T32DK007751-16	Radovick, Sally	Johns Hopkins University
T32DK007260-36	Blackwell, Thomas Keith	Joslin Diabetes Center
T32DK007028-38	Avruch, Joseph	Massachusetts General Hospital
T32DK062710-09	Allison, David	University of Alabama Birmingham
T32DK007571-23	Swerdloff, Ronald	University of California Los Angeles
T32DK007494-29	Olefsky, Jerrold	University of California San Diego
T32DK007418-32	German, Michael	University of California San Francisco
T32DK007011-38	Refetoff, Samuel	University of Chicago
T32DK007245-36	Auchus, Richard	University of Michigan
T32DK007314-32	Birnbaum, Morris	University of Pennsylvania
T32DK007247-35	Schwartz, Michael	University of Washington
T32DK007061-38	May, James	Vanderbilt University
T35DK007383-34	Powers, Alvin	Vanderbilt University
T32DK007058-38	Wysolmerski, John	Yale University
T32DK007120-38	Semenkovich, Clay	Washington University

Some students were supported by the Diabetic Complications Consortium

U24DK076169	McIndoe, Richard	Georgia Regents University

Training Grant Supporting
2013 NIDDK Medical Student Research Program
in Nephrology and Hypertension

Grant Number	PI of Training Grant	Institution
P30DK079341-05	Harris, Raymond	Vanderbilt University

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Abstracts from the Posters of the Students

Participating in the

2013 NIDDK Medical Student Research Program

(For abstract number see Table of Student Participants: pages 5 - 9)

Three-Dimensional Image Analysis of Human Intraepidermal Nerve Fibers

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A. Gordon Smith, M.D., Stephen I. Lentz, Ph.D., and Eva L. Feldman, M.D., Ph.D.
University of Michigan

Diabetic peripheral neuropathy (DPN) is one of the most frequent complications of chronic diabetes mellitus (DM) and is the leading cause of non-traumatic amputations. The current accepted method of diagnosis is the use of skin biopsy to obtain intraepidermal nerve fiber (IENF) density count under bright field microscopy. This project seeks to develop a 3-dimensional fluorescent microscopy technique to quantify morphological characteristics of IENFs that may be affected by the diabetic neuropathy disease process. A 3mm cutaneous punch biopsy was taken from both the distal thigh and distal leg of 20 patients (4 healthy controls, 9 diabetic patients without neuropathy, and 7 diabetic patients with neuropathy), sectioned, stained with fluorescent markers, and fixed onto glass slides. A Leica SP5 confocal fluorescent microscope was used to take Z-series images of the stained tissues and images were processed through Imaris software to render representation of nerve surfaces and filaments. Using this technique, we quantified the morphological characteristics of nerve volume, nerve straightness, and branching behavior. We found that Nerve Volume Ratio (volume of nerve surfaces per volume of epidermis) decreased with disease progression in the distal thigh. Straightness showed a significant increase in both the distal thigh and the distal leg. We also found that small fiber nerves tend to branch more in the distal thigh as diabetes progresses into diabetic peripheral neuropathy. This study demonstrated that 3D image analysis is an objective technique for the characterization and quantification of IENFs. Our measurements offer insight for future research into the effect of these morphological changes and the pathogenesis of DPN.

A Novel, Digital Approach to Quantify Intraepidermal Nerve Fiber Density

Hussein S. Hamid, B.S. (University of Michigan Medical School), Chelsea Lindblad, B.S., John M. Hayes, B.S., Eva L. Feldman, M.D., Ph.D., and Stephen I. Lentz, Ph.D., University of Michigan - Ann Arbor

Diabetic Peripheral Neuropathy (DPN) is the most common complication of diabetes. It is a condition characterized by a distal to proximal loss of cutaneous sensation that begins in the hands and feet. Skin punch biopsies have become a standard method to assess DPN as patients show a degradation of Intraepidermal Nerve Fibers (IENFs). Intraepidermal Nerve Fiber Density (IENFD) is a parameter that measures the number of IENFs that enter the epidermis from the dermis. The most common method to calculate IENFD uses chromagen based immunohistochemistry on skin biopsy sections followed by manual counting of IENFs at a microscope. This technique is limited by the lack of permanent images and counting records for subsequent analysis, and requires a microscope and trained technicians. We developed a novel approach that digitizes brightfield images of skin biopsy sections and uses a protocol for subsequent calculation of IENFDs that can be done at a computer without the need for a microscope. We obtained skin biopsies from the proximal thigh of 20 healthy control patients and analyzed 4 sections per patient (80 sections total). We showed that the IENFDs calculated using our novel digital approach significantly correlates with IENFDs calculated from the standard manual counting method. Our novel method of quantifying IENFD allows for a permanent and transferrable record of skin biopsy sections that expedites training, collaboration, technique standardization, and subsequent analysis without the need to be physically present at a microscope.

Diet-induced obesity impairs Regulatory B lymphocyte proliferation and function

Joseph Hsiao (St. Louis University School of Medicine), Lan Wu, and Luc Van Kaer (Vanderbilt University Medical Center)

Chronic low-level inflammation is a recognized hallmark of obesity and an important player in the development of insulin resistance in Type 2 Diabetes. Conventional B lymphocytes promote this inflammatory response in conjunction with T cells and macrophages. Regulatory B lymphocytes (Bregs) modulate inflammation under a number of chronic inflammatory conditions through secretion of immunoregulatory cytokines, in particular IL-10. Thus, we hypothesized that obese mice would have fewer Bregs and that the Bregs would be less competent in producing IL-10 in their metabolic organs. To test this, we compared the amount of Bregs and the Breg IL-10 levels in mice fed on a 12-week high fat diet against those fed a low fat diet. We used FACS to detect and quantify Breg markers (CD19highB220lowCD5+) in the spleen, peritoneal cavity (PerC), and stromal vascular fraction of white adipose tissue (SVF). While the splenic composition was unchanged, the proportion of Bregs decreased in the PerC (4.2 ± 0.2%) and SVF (4.6 ± 1.1%) of obese mice compared to lean PerC (7.6 ± 2.1%) and SVF (7.8 ± 1.5%). Intracellular staining for IL-10 revealed a similar decrease from 88.1 ± 2.2% in the lean PerC to 71.7 ± 10% in the obese PerC and 36.8 ± 6% in the lean SVF to 22.3 ± 5.7% in the obese SVF. These data suggest a possible therapeutic route for Type 2 Diabetes through modulation of Breg number and/or function in obese mice. Further studies include measurement of glucose tolerance in obese mice supplemented with Bregs isolated from donor mice or in vitro differentiated in culture.

Patients with hereditary cancer predisposition syndromes have defined phenotypes specific to their syndrome.

Celina Jacobi (Washington University School of Medicine), Katie Gettinger, RN, CPNP, Jennifer Henry, BA, Todd Druley, MD, PhD, Beth Kozel, MD, PhD, Marwan Shinawi, MD, Robert Hayashi, MD, Washington University School of Medicine, St. Louis Children's Hospital

Hereditary Cancer Predisposition Syndromes (HCPS) are hereditary conditions that are associated with malignancies that develop in diverse organ systems at any age. There are several well described conditions, such as Beckwith Wiedemann Syndrome, Hemihypertrophy, Pleuropulmonaryblastoma, Familial adenomatous polyposis, and Li-Fraumeni syndrome, that are associated with a restricted scope of malignancies. However, the scope of nonmalignant conditions characteristic of each HCPS is varied and frequently poorly described. We hypothesize that patients with cancer predisposition syndromes have a defined phenotype with a range of specific physical, cognitive, and psychological difficulties that correlate to their diagnosis. In order to characterize each syndrome, we designed a longitudinal database and are currently enrolling pediatric patients with a HCPS diagnosis. Participants' parents will complete a health history questionnaire each year until their child turns 18, assessing their child's physical, cognitive, emotional, and psychosocial health and noting the age of occurrence for each positive symptom. Additionally, the child's medical record will be reviewed to collect information, such as height and weight, limb measurements, and genetic testing. We anticipate that these tools will depict the specific phenotype of each HCPS and demonstrate the evolution of patients' symptoms over time. The current standard of care for most HCPS with poorly defined phenotypes is surveillance for malignancy with frequent screenings until the patient ages out of the high risk population. However, greater knowledge of each syndromes' phenotype will encourage holistic treatment that extends beyond tumor monitoring to improve patients' quality of life and long term wellbeing.

Recurrent Yohimbine (YOH) Administration as a Model for Recurrent Stress

Susan Hill (University of Puerto Rico), Connie West, Jennifer Jay, Aryana Zavosh, Dianne Figlewicz Lattemann, VA Puget Sound Health Care System and University of Washington, Seattle, Washington

We hypothesized that recurrent pharmacological activation of brain noradrenergic pathways sufficiently models recurrent stress such that it can be a model to evaluate food intake/energy homeostasis and motivational behavior. Rats were injected every other day for two weeks with either the adrenergic agent YOH or saline (SAL) in the peritoneal cavity while their behavior was monitored. The rats then participated in a sucrose self-administration protocol for 4 weeks. Using cFos immunocytochemistry quantitation we determined activation of specific areas of the brain. The rats injected with YOH showed increased activity in the central nucleus of the amygdala and the dorsal striatum versus SAL injected rats. Clear distinctions in behavior across all six injections were noted in the YOH- versus SAL-injected rats. The brain activation pattern, difficulties in sleeping, and heightened agitation seen in the YOH-injected rats correlate well with known behaviors in human subjects with PTSD. Preliminary data from the sucrose self-administration tests suggest an increase in food reward amongst the YOH-treated rats, which will be replicated in additional cohorts. All of the data combined imply that dietary education and behavioral modification for food intake may be important factors to include when treating patients with PTSD.

HOX6 GENES ARE CRITICAL MESODERMAL TRANSCRIPTION FACTORS THAT MODULATE PANCREATIC ORGANOGENESIS

Bright H. Kim (University of Michigan Medical School), Brian M. Larsen, Deneen M. Wellik, Division of Molecular Medicine and Genetics, Department of Internal Medicine, University of Michigan Medical Center

Hox genes encode a family of important transcription factors that modulate various developmental processes such as skeletal morphogenesis, neural patterning, and organogenesis. Of the four Hox clusters (A-D) that are organized into thirteen paralogous groups in mammals, the Hox6 paralogous group includes three genes—Hoxa6, b6, c6—that encode critical mesenchymal transcription factors that modulate various aspects of pancreatic organogenesis. We show that loss of Hox6 function produces phenotypic abnormalities of the pancreas that are not seen in wild-type mice embryos. Our lab set out to characterize these phenotypes by comparing epithelial and mesenchyme volume, cellular proliferation, extent of epithelial branching, and endocrine cell mass between Hox6 mutant and wild type embryos. Through immunofluorescence assay and fluorescence microscopy of mouse embryos at different embryonic days, these phenotypic characteristics were measured and quantified. The overall epithelial volume of the pancreas in mutants is significantly reduced compared to littermate controls by embryonic day 11.5 (E11.5). Although there is no significant difference in mesenchyme volume or proliferation, the number of endocrine cells, particularly insulinproducing beta cells, decreases drastically by E18.5. Hox6 mutants also exhibit abnormal epithelial branching and reduced epithelial cell proliferation. These data demonstrate that Hox6 genes are critical mesenchymal transcription factors necessary for the proper development and maintenance of the pancreas. As the function of the mesodermal niche of the pancreas is better understood, regenerative therapies of in vitro endocrine cell development may be improved for diabetic patients.

Mitochondrial translocator (TSPO) Distribution and Localization in the Mouse Brain.

Meredith M. McKenney[1], Misty M. Thompson[2] & Kate L.J. Ellacott[2]
[1]University of Texas Health Science Center Houston
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Mitochondrial translocator protein (TSPO) is an outer-mitochondrial membrane transporter which has many functions including participation in the mitochondrial permeability transition pore, regulation of apoptosis, cholesterol binding and translocation for steroidogenesis, regulation of reactive oxygen species, and production of cellular energy. TSPO has also emerged as a reliable inflammatory marker in disease pathologies due to its up-regulation in activated glia cells (astrocytes and microglia) in the CNS and in macrophages in the periphery. The distribution of TSPO in the "normal" mouse brain has thus largely been characterized by autoradiography and positron emission tomography (PET) with radiolabeled TSPO ligands. A fine mapping study of TSPO expression in the mouse brain has not been performed and was the goal of the current project. Our immunohistochemistry (IHC) data revealed a novel distribution of TSPO throughout the hypothalamus including regions such as the arcuate nucleus and dorsal medial hypothalamus (DMH) (regions important for the regulation of food intake), as well as circumventricular organs (areas lacking a tight blood brain barrier). We next hypothesized that TSPO is localized in glial cells within these hypothalamic regions. As a first step to determine the identity of TSPO-expressing cells, triple-label IHC was performed for TSPO in addition to glial fibrillary acidic protein (GFAP; astrocyte marker) and vimentin (tanycyte/ependymal cell marker). Our preliminary data shows that TSPO co-localizes with vimentin within tanycytes and ependymal cells lining the third ventricle of the hypothalamus, which are involved in nutrient sensing. TSPO did not co-localize with all GFAP-expressing astrocytes and some TSPO-positive cells within the DMH were not GFAP- or vimentin-positive, suggesting that these cells may be microglia or neurons. The distribution and cellular expression of TSPO presented here suggest that TSPO may be involved in the regulation of food intake, energy expenditure and/or nutrient sensing pathways which should be further explored. Future directions include further characterization of the identity of TSPO-expressing hypothalamic cells and evaluating potential regulation of TSPO in the brain by metabolic state.

Inflammation in CKD and CVD: Role of Genetics and IL-1ra (Rilonacept)

Jia Yi (University of Tennessee Health Science Center, College of Medicine)
Adriana M. Hung MD MPH, Vanderbilt University Medical Center

Chronic kidney disease (CKD) is a major public health issue. But even more concerning, patients with CKD are at a high risk for cardiovascular disease (CVD) related morbidity and mortality with the common mechanism being inflammation. The cause of this activated chronic inflammatory response remains unknown; however, recent work has implicated the activation of the inflammasome protein complex as the source of persistent inflammation found in many auto-inflammatory conditions. The inflammasome activates IL-1, a major pro-inflammatory cytokine, as its downstream effector making inflammasome related diseases highly IL-1 dependent. Inhibition of IL-1 with the drug Rilonacept successfully controls the auto-inflammatory response found in these diseases. In a mouse model of CKD, expression of Nlrp3 inflammasome and its resulting cytokines were increased. In addition, Nlrp3 expression in human kidney biopsies showed increased expression of various inflammasome components regardless of disease etiology. In a previous study of end stage renal disease patients, we have shown that IL-1 blockade leads to a quick and drastic 50% drop in Hs-C-Reactive Protein (HsCRP, an inflammatory marker), the largest response described so far in this patient population. Based on these findings, IL-1 inhibition should beneficially minimize the inflammatory response found in CKD.

In this randomized, placebo-controlled, double-blinded pilot and feasibility drug trial for the IL-1 receptor antagonist Rilonacept, we plan to determine if inhibition of the IL-1 inflammatory pathway decreases markers of inflammation, improves markers of vascular health, and slows CKD progression. The study will consist of 55 randomized participants over a 24-week treatment period. We hypothesize that Rilonacept will decrease markers of inflammation and slow CKD progression. Other markers of kidney injury (albuminuria), endothelial function (flow mediated dilation, pulse wave velocity and ADMA), oxidative stress (F2-isoprostane), and insulin resistance (HOMA-IR and the leptin to adiponectin ratio) will also be measured. At this point in time, we have begun screening and recruiting patients. The study will be conducted over the next two years and is anticipated to produce a better mechanistic understanding of the inflammatory consequences of CVD in CKD.

The Effect of Literacy and Numeracy on Self-Care Behaviors in Diabetes Patients

Sneha Venkatraman (University of Tennessee Health Science Center), Laura Chambers, Karen Trochez, Shari Barto and Russell Rothman, Vanderbilt University, Nashville, TN

Diabetes self care behaviors are important in the management of diabetes and may be influenced by patient literacy and numeracy levels. Literacy and numeracy may also have a downstream effect on glycemic control (HbA1C) in diabetic patients and on other outcomes such as perceived self-management, treatment satisfaction, and medication adherence. Limited research has been done on comparing scores on different literacy and numeracy measures and how they correlate with self-care behaviors. We hypothesized that higher levels of literacy and numeracy will be associated with more frequent self-care behaviors, and that subjective measures (SLS-3 and SNS-8) may be more associated with self reported behaviors. Higher levels of literacy and numeracy may also be associated with lower levels of HbA1C and higher levels of treatment satisfaction, perceived self-efficacy, and medication adherence, and objective measures of numeracy (DNT-5) may be more associated with these outcomes. 410 adult participants with Type 2 Diabetes Mellitus were recruited from 10 “safety net” low-income clinics in the middle Tennessee area run by the Tennessee Department of Health. Cross sectional analysis of baseline data from a cluster randomized trial examining the role of a literacy sensitive intervention to improve diabetes care was used. The S-TOFHLA and SLS-3 were used to study literacy, the DNT-5 and the SNS-8 to study numeracy, and the PDQ and SDSCA to study self-care behaviors. The DNT-5 seemed to correlate most with multiple self-care behaviors and was closest to approaching significance with A1C levels, so more research can be done in broadening its use when studying numeracy in diabetes. Subjective and objective measures of literacy and numeracy were more correlated with the PDQ than the SDSCA. More research should be done on the uses of the PDQ in studies of diabetes. By addressing literacy and numeracy issues, this data suggests certain self-care behaviors, especially those involving using data to modify the diet, can be addressed and perhaps improved.

Liver Estrogen Signaling and the Metabolic Response to Different Macronutrient Compositions.
Eric K. Singhi (Medical University of South Carolina), Melissa N. Martinez, John Stafford, MD/PhD, Vanderbilt University Medical Center

Coronary Heart Disease (CHD) is an important health issue in developed countries. In fact, nearly one-third of Americans over the age of 35 die from CHD each year. Although many of the risk factors for CHD are non-modifiable, some risk factors can be controlled for such as an unhealthy diet. The metabolic consequences of overconsumption are especially important to understand as an excess intake of dietary fats and carbohydrates has been shown to have a profound impact on glucose tolerance, insulin sensitivity, and lipid metabolism. Although many of today's popular diet strategies are targeted towards improving the complications of obesity and reducing risks of CHD, identifying individuals who are best served by a specific type of diet remains a challenge. Previous research has demonstrated that women are better protected from CHD risk than men. By contrast, few studies also suggest that high-carbohydrate diets may be more harmful in women than men. With this in mind, we questioned if there is a differential effect of estrogen signaling on glucose *vs.* lipids. Because the liver is a unique organ such that it integrates glucose and lipid metabolism, we decided to explore hepatic estrogen signaling to better understand the mechanistic role that estrogen plays in the metabolic response to different macronutrient compositions. In order to determine the impact of high-fat *vs*. high-sucrose feeding in the presence or absence of hepatic estrogen signaling, we used 12 week old female C57BL/6 mice that were either wild-type or lacking estrogen receptor alpha (ERalpha) specifically in the liver. We fed them diets that were high in fat or high in sucrose for 8 weeks. To assess glucose tolerance, a proven index of insulin sensitivity, we performed intraperitoneal glucose tolerance tests. We also used a high performance liquid chromatography system to examine differences in lipid profiles. We determined that a knockout model of hepatic ERalpha (LKO-ERalpha) worsened glucose tolerance on high-fat diet feeding. Interestingly, female mice with LKO-ERalpha showed improved glucose tolerance on high-sucrose feeding, but their lipid profile was worse than that of the wild-type controls. Thus, we believe that liver estrogen signaling is helpful in the setting of a high-fat diet, but may be harmful in the context of high-carbohydrate feeding. These findings may have important health benefits in helping women choose the most appropriate diet for weight loss, both pre and post menopause.

Stimulatory Effects of Glutamate on Vitamin C Efflux in Synaptosomes

L. Nora Zeidan (Medical College of Georgia), M. Pierce, J. May, Vanderbilt University Medical Center

Vitamin C, VC, is an essential antioxidant that serves to protect the central nervous system from oxidative stress. It has been determined that VC is transported into neurons by Sodium-dependent Vitamin C Transporter (SVCT2), but its efflux mechanism is still undefined. To test the hypothesis that glutamate-induced vitamin C efflux is mediated via a volume-regulated anion channel (VRAC), we introduced both glutamate and a series of pharmacological inhibitors that are known to act on protein channels.

To assess VC efflux at the synaptic bouton, we used a crude synaptosome preparation as a model of the nerve terminal. This preparation allowed for us to introduce pharmacological anion channel inhibitors to the synaptosomes to determine if they alter glutamate-induced VC efflux. Previous studies suggest that the VRAC is sensitive to classical anion channel inhibitor 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), while SVCT2 is sensitive to sulfinpyrazone (SPZ) and phloretin (PHL). We determined that synaptosomes spontaneously efflux endogenous Vitamin C at 37° and the presence of 200μM glutamate increased efflux. DIDS did not inhibit Vitamin C efflux in synaptosomes as expected. On the other hand, sulfinpyrazone and phloretin inhibited transport of Vitamin C, therefore increasing the net efflux out of the synaptosomes. These data suggest that glutamate stimulates increased efflux in synaptosomes, but the specific mechanism remains undefined.

Dietary nitrates and nitrites as a source to reduce inflammation in diabetes and CVD

Joshua Burks (University of Oklahoma), Joan Tupper, Francis Kim; University of Washington Diabetes and Obesity Center of Excellence

Current research shows abnormal cellular inflammation is associated with insulin resistance, obesity, and type II diabetes. Consequently, a reduction in inflammation is believed to restore normal physiologic insulin response. Nitric Oxide (NO) is an important mediator of inflammation that is deficient in these disease states, and recent studies indicate its production from nitrate (NO_3^-) and nitrite (NO_2^-) in the absence of endothelial NO synthase (eNOS). To test whether dietary NO_3^-/ NO_2^- reduced inflammation in adipocytes, 15 mice were fed a diet high in fat and 15 were fed a diet low in fat, and given water containing $NaNO_2$ or vegetable pills rich in NO_3^-. There were also two control groups in which no supplements were given (one for high fat diet and one for low fat diet). Next we used quantitative PCR to measure levels of RNA for inflammatory proteins TNF, CCL-2, CD11b, and others. We observed a 2.5 and 3.0 fold increase in expression of CCL-2 and CD11b, respectively, with the high fat diet compared to the low fat diet. Within the low fat group, those receiving vegetable supplements demonstrated reduced inflammation. These data indicate that in a low fat diet, vegetable supplements reduce inflammation in adipocytes. However, in a high fat diet, there were no trends to indicate that either had an anti-inflammatory effect. Under those circumstances, the NO_3^-/ NO_2^- may not have reached the adipose tissue. Administering the supplements for a greater duration may have changed this outcome. On the other hand, inflammation caused by a high fat diet may be too great to be overcome by dietary nitrate/nitrite supplementation. Further study of the delivery of nitrates and nitrites into tissue presents the opportunity for new therapies to improve insulin sensitivity.

Healthy Food Choices at the Dollar General: Feasible Dietary Advice for Low Income, Rural Populations

Divya Carrigan (University of Alabama School of Medicine), Monika Safford, MD, University of Alabama in Birmingham

Diet plays an important role in preventing and treating chronic diseases and obesity. However, many rural populations lack access to supermarkets selling the fresh produce that is essential for a healthy diet. As a result, many rural residents purchase a substantial proportion of their groceries at more local stores such as the Dollar General, which offer limited options, many unhealthy. Practical advice on how to make healthy food choices at the Dollar General or similar stores is currently not available. It is possible to create a culturally adapted, acceptable educational video on how to make healthy food choices at the Dollar General or similar store. The rural Alabama Black Belt is characterized by steep poverty, among the worst health outcomes in the US and scarce resources, including very few full-service supermarkets. The area is 50-75% African American and educational quality and attainment is generally low. We used the infrastructure of a community-based research program in partnership with Black Belt communities to collaboratively develop an educational video. We used data from focus groups and previous research projects to inform the intervention design. We worked with a research nutritionist and community members to develop and pilot test the video content. Community members were integrated into the video with peer testimonials. In the future, we will show the video to 50 area residents and quantify how useful they find the video and whether it will change their food choices when shopping at the Dollar General. If found to be acceptable, the video will be integrated into a community-based intervention in order to test the hypothesis that the intervention will lead to weight loss and healthier eating patterns in rural Alabama Black Belt residents. The video used a practical framework tailored to the major challenges relating to healthy eating in our targeted community: eating too many calories (large portions and second helpings) and inadequate intake of fresh fruits and vegetables. We developed 3 simple messages: 1) eat fewer second helpings; 2) use the “plate method” (1/4 protein, ¼ starch, ½ fruits/vegetables; and 3) eat fewer fried foods and drink fewer sugar sweetened beverages. The video demonstrated a community member in the Dollar General making choices for snacks, breakfast food, lunch and dinner. We also demonstrated how these purchases can be integrated into healthy snacks and meals. Interspersed into the video were educational segments by the nutritionist and peer testimonials on shopping at the Dollar General store for groceries and the importance of healthy eating for Black Belt residents. The community members who reviewed the script and messages provided enthusiastic endorsement for the direction, strategy and content. This educational video will provide critical missing information about how to make healthier food choices at stores that are frequented by rural residents, but that are largely overlooked in currently available dietary advice. Using just a few key messages about healthy eating specifically targeted at major challenges and barriers to healthy eating in the rural Alabama Black Belt, and using a visual approach to information dissemination is intended to improve comprehension and uptake of the information. Educational materials that are targeted culturally adapted and collaboratively developed may be used in future intervention studies to help individuals improve dietary intake.

Acute Kidney Injury: Role of Amphiregulin in Repair of Proximal Tubular Epithelial Cells

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Acute kidney injury (AKI) is defined as an abrupt decrease in renal function and predominately affects the proximal tubular epithelial cells (PTECs) located in the medullary portion of the kidney. These cells express epidermal growth factor receptor (EGFR) and are able to proliferate by associating with EGFR ligands, including amphiregulin (AREG). It is unknown whether AREG is important for the regeneration of PTECs following AKI resulting from ischemia-reperfusion (I-R) injury. To test this hypothesis, we surgically clamped off the renal artery and vein unilaterally for 30 minutes in equal aged and gendered AREG KO transgenic mice. Blood urea nitrogen (BUN) measurements were taken post-surgery for seven days to assess the level of epithelial repair. Immunoblotting was preformed to assess the level of neutrophil infiltration, phosphorylated EGFR (pEGFR), and AREG following I-R injury. Results revealed that BUN is elevated in AREG KO mice and has a slower return to baseline when compared with wild-type mice. Likewise, AREG KO mice have increased levels of proximal tubular damage following I-R injury. Reverse transcriptase polymerase chain reaction (RT-PCR) showed that macrophages and dendritic cells are the predominant cells producing AREG. These data suggest that this ligand is important in the proliferation and regeneration of the PTECs following hypoxic conditions. Furthermore, AREG may be important in decreasing the rate of AKI reoccurrence seen in many critical patient populations.

Identification of Genes Required for the Response to Metabolic Stress

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Diabetes is associated with an increased risk of cardiovascular disease due, in part, to diabetic dyslipidemia. When excess lipids accumulate in non-adipose tissues, they cause oxidative and ER stress and can lead to cell death. Endothelial cells are particularly sensitive to this lipotoxic stress. We hypothesized that we might identify novel genes involved in metabolic stress responses in endothelial cells through a lossof-function screen using an shRNA library. A genome-wide loss-of-function screen was carried out in immortalized human umbilical vein endothelial cells (iHUVECs) using a lentiviral shRNA screening library and growth under lipotoxic conditions. Following confirmation of resistance to lipotoxicity, shRNA-targeted genes were identified using the BLAST algorithm (NCBI) to identify specific genes. We identified several novel genes that have not previously been implicated in the response to metabolic stress. In future studies, the contributions of these genes to lipotoxicity will be confirmed by directed knockdown in wild type cells and complementation of the palmitate-resistant clones. The function of these genes in the lipotoxic pathway could provide new mechanistic insights into how excess metabolites contribute to diabetes complications and facilitate the development of novel pharmacological therapies.

Using Patient Registries To Recruit Subjects For Clinical Studies

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Background: Subject recruitment remains a significant challenge when conducting clinical studies (includes clinical trials). Identification and availability of patients, time constraints, budget, and entry criteria are factors that affect this problem.
Aim: This study examines patient registries which have been useful for subject recruitment in clinical studies.
Methods: A PubMed search was conducted to identify journal articles on registries used for recruiting subjects for clinical studies, followed by a careful review of all relevant articles containing such information. The search was conducted on 6/26/13, consisted of both MeSH and textword terminology, and limited to English language studies. Information on the ongoing University of Michigan Diabetes Research Registry (UMDRR) was obtained from the team that runs it.
Results: The PubMed search identified 104 papers. Of these, 39 papers appear to be related to registries used for recruiting subjects for clinical studies. These registries cover of a number of diseases/conditions (Parkinson's, atrial fibrillation with risk of stroke, glaucoma, muscular dystrophy, cancer, dementia, epilepsy, cerebral palsy, bipolar disorder, trauma, and other rare diseases and neurological conditions), groups, gender, ages, races (general population, older adults, women, children, twins, and minorities), and many countries (US, UK, Canada, Africa, Australia, New Zealand, and Italy). They operated on multi-national, national, regional, or local levels, and may be based at a single or multi-center. The majority of these registries have shown enhanced patient recruitment for clinical studies. Other studies have compared subject recruiting using registries to electronic medical records (EMR). The EMR provides a larger pool of patients but is less specific. Registries have a smaller pool of patients but are more specific. The UMDRR uses data from EMR and a registry. In 3 years it has recruited close to 3800 patients. The data in the UMDRR includes age, gender, race, BMI, blood pressure, serum lipids, type and duration of diabetes, glucose lowering and other medications, HbA1C, amongst many others. During this time period 12 UM investigators utilized the UMDRR for their clinical research studies. Funding for the investigators has come from private (4), public (7), and non-governmental granting agencies (1).
Summary/Conclusion: A patient registry for a specific disease can enhance and facilitate recruitment of subjects for clinical studies. It allows the identification of potential subjects for clinical studies and is a more efficient way of identifying them.

Effect of γ-MSH on Skin Electrolyte Homeostasis

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The skin interstitium responds to a high salt diet by sequestering excess Na^+, K^+ and Cl^-, creating a hypertonic reservoir to store the excess salt. γ -MSH is a natriuretic melanocortin which regulates blood pressure and sodium metabolism by binding to the melanocortin 3 receptor (MC3R). The $MC3R^{-/-}$ mice are hypertensive on a high salt diet. This study tests the hypothesis that $MC3R^{-/-}$ mice are not able to respond to increased circulating levels of γ-MSH, leading to an increase in sodium accumulation in the skin.

Electrolyte contents in the skin, carcass, and total body were determined through a combination of dry ashing, atomic absorption spectroscopy, and $AgNO_3$ titration. $MC3R^{-/-}$ mice had markedly decreased electrolyte content in skin, carcass, and total body indicating that γ-MSH's effect on electrolyte storage was not specific to the skin. The relationship between water content and electrolyte levels in skin were linear indicating that γ -MSH does not affect the concentration of electrolytes within the skin. Hypertension in these mice is not related to sodium retention.

Effect of ER Stress and Oxidative Stress on b-cell Compensation and b-cell Survival.

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Chronic nutrient excess induces initial compensatory expansion and eventual demise of pancreatic b cells. Nutrient excess are known to cause endoplasmic reticulum (ER) stress and oxidative stress. But the role of these cellular stress in b-cell compensation and survival have not been systemically studied. We have found that overnutrition in zebrafish induces compensatory b-cell genesis. However, with genetic insulin resistance, prolonged overnutrition causes b-cell demise. We hypothesize that ER and oxidative stress response systems may play a key role in influencing compensatory β-cell genesis and their eventual death and will test the hypothesis using both pharmacological and genetic manipulations. Here we report our preliminary findings on several genes pivotal to proper ER stress and oxidative stress including hsf1, nrf2, sirt1, and jnk1. We found that inhibition of jnk1, sirt1, or hsf1 enzymes, essential for resolving various cellular stresses, caused b-cell genesis in the absence of overnutrition. This suggests that cellular stress drives initial compensatory b-cell genesis. We have generated mutations in the aforementioned genes using TALEN mutagenesis. Preliminary analysis of nfe2 mutant that have impaired oxidative stress response showed an increased basal b-cell number, further suggesting a role of cellular stress in compensatory b-cell genesis.

Health literacy is associated with patients' adherence-related knowledge and motivation, but not adherence or clinical outcomes

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Among adults with type 2 diabetes mellitus (T2DM), medication adherence is a strong independent predictor of glycemic control (A1C), but health literacy (i.e., the ability to obtain, process, and apply health information to make health decisions) has been inconsistently linked to medication adherence and A1C. According to the Information-Motivation-Behavioral skills (IMB) model of adherence, medication adherence-related ***information*** and ***motivation*** are determinants of adherence-related ***behavioral skills***, and these factors collectively predict ***medication adherence***, which, in turn, predicts a ***health outcome***. Given mixed evidence on the role of health literacy in predicting medication adherence and A1C, we hypothesized health literacy would be more strongly related to factors more distal to these outcomes (i.e., medication adherence-related information and motivation rather than medication adherence-related behavioral skills, medication adherence, or A1C). We analyzed data from 314 adult patients with T2DM who were recruited from a safety net clinic, had completed a series of interviewer-administered self-report measures, had information collected from their medical record, and had undergone a point-of-care A1C test. We conducted unadjusted and adjusted multivariate regression models (adjusted for demographic and clinic characteristics) to examine the relationships between health literacy and the aforementioned IMB model components. Participants were age 51.8±11.7 years, 65% were female, and 53% were African American/Black. Many participants had low socioeconomic status (32% had <high school degree; 45% had incomes <$10K; and 45% were uninsured), and the average A1C was 8.2%±2.2%. In unadjusted and adjusted models, health literacy was significantly associated with adherence-related information ($\beta=0.15$, $p<0.05$) and social motivation ($\beta=0.24$, $p=0.001$), and there was a trend toward a significant association with personal motivation ($\beta=0.15$, $p=0.055$).

Health literacy was not associated with adherence-related behavioral skills, medication adherence, or A1C in unadjusted analyses. In summary, health literacy was independently associated with adherence-related ***information*** (i.e., having more knowledge) and social ***motivation*** (i.e., having more social support), and may be associated with personal ***motivation*** (i.e., having favorable attitudes/beliefs), but was not associated with adherence-related ***behavioral skills*** (i.e. having more self-efficacy), actual medication adherence, or A1C. Health literacy-appropriate interventions should aim to improve patients' adherence-related knowledge and social support, as studies have consistently shown both factors are strong determinants of self-efficacy, which is amongst the strongest determinants of medication adherence behavior, and medication adherence affects glycemic control.

Glycemic control and anti-diabetic medication usage in older adults with type 2 diabetes mellitus in an inner-city primary care practice

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Approximately 11 million people aged 65 years and older in the United States have diabetes mellitus (DM). Yet, the optimal glycemic target for elders with type 2 DM is uncertain, since most of the clinical trials were conducted largely in the middle-aged population. In early 2013, the American Geriatric Society recommended that the glycemic target for elders with type 2 DM change from a previous recommendation of HbA1c of <7% to a less stringent 7.5-8%. As such, millions of older adults may be able to simplify their drug regimens to reduce risks of hypoglycemia, polypharmacy, and geriatric syndrome. We analyzed data from East Baltimore Medical Center (EBMC) to characterize glycemic control and drug usage by older inner-city adults and quantify the potential implications from a less stringent glycemic target. In 2011, 519 adults aged 65 and older with type 2 DM were seen at EBMC: mean age 73.4 (SD, 6.8); 29% men, 96% African Americans, and mean body mass index 32.3 (7.5) kg/m2. The mean HbA1c was 7.1 (SD: 1.5); 58% of the patients had an A1C value of <7%. 34% of diabetic elders were on medications with an A1C <7%; 9% were taking multiple anti-diabetic medications to achieve this HbA1c level. In addition, 9% of older adults who had a HbA1c level between 7.0% and 7.9% were taking more than one drug. If the glycemic target is raised to <8%, at least 18% of older patients at EBMC may be able to discontinue or reduce their anti-diabetic medication regimens, which could potentially translate to thousands of older adults simplifying their drug regimens in the U.S. and the world.

The role of kynurenine in human Embryonic Stem Cell pluripotency

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Pancreatic β-Cells are damaged in both Type I and Type II diabetes mellitus through different mechanisms. In Type I diabetes, the immune system malfunctions, destroying healthy β-Cells early in life. In Type II diabetes, the overwhelming demand placed on β-Cells to produce insulin leads to β-Cell damage and destruction (Ashcroft et al. 2012). The development of induced pluripotent stem cells (iPSCs) derived from adult cells provides great therapeutic potential for replacing a patient's lost or damaged β-Cells. Since a great deal of research is already in progress optimizing the differentiation of β-Cells (Pagliuca and Melton, 2013), we are focused on examining the stem cell population from which the β-Cells can be differentiated. Human Embryonic Stem Cells (hESCs) exist in two states of pluripotency, an earlier "naïve" state and a later "primed" state. Elf1 cells are a newly derived line of naïve hESCs that are more efficient at differentiating into the endoderm germ layer, which gives rise to pancreatic tissue, than their primed counterparts (Ware et al. in press). We are examining the metabolic pathway of kynurenine – a catabolite of tryptophan – in this naïve Elf1 cell line as well as in the primed H1 cell line to determine whether it plays a role in converting hESCs between the primed and naïve state. Through mass spectrometry, we found that kynurenine accumulates in primed hESCs. In cancer cells, kynurenine binds to the aryl hydrocarbon receptor (AHR) increasing cancer cell motility and proliferation (Opitz et al. 2011). We hypothesize that kynurenine may play a similar role in stem cells, by binding to AHR and increasing the expression of a key developmental target gene of AHR, Embryonic Endoderm Development (EED1). To test this hypothesis, we measured EED1 expression levels in naïve and primed hESCs and found that EED1 expression was increased in primed ESCs. We also tested the effect of adding kynurenine directly to naïve cell media, expecting that it could push the naïve hESCs to a more primed state. After growing naïve hESCs in media with kynurenine we found increased levels of several markers of the primed state (IDO1 and EED1) providing preliminary data indicating that kynurenine can push cells toward the primed state. These results indicate the importance of the kynurenine metabolic pathway in hESC pluripotency and support the value of further research into whether alterations of this metabolite could be useful in creating more naïve iPSCs with greater therapeutic potential.

The food dyes tartrazine and sunset yellow augment the effect of corticosterone on the glucocorticoid receptor.

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In recent decades, the rate of diagnosed diabetes cases in the United States rose in parallel with the production volume of synthetic organic chemicals. With the connection between obesity and type 2 diabetes, recent research has examined the ability of these chemicals to disrupt metabolic signaling in adipose tissue. We looked at lipophilic food dyes with similar chemical properties to glucocorticoid receptor (GR) agonists due to their potential to alter insulin sensitivity and adipocyte differentiation and proliferation. To do so, we transected 3T3-L1 fibroblasts with a GRE-luc reporter and treated the cells with various concentrations of commonly used food dyes alone and in the presence of the endogenous glucocorticoid corticosterone. At treatment concentrations ranging from 1nM to 100 uM, some of the food dyes induced a significant level of luminescence, which is indicative of GR activation. However, none of these levels were comparable to those generated by corticosterone. When treated with 100 uM of the dyes tartrazine or sunset yellow in combination with 10 uM of corticosterone, there were significantly higher levels of luminescence relative to cells treated with corticosterone alone. These higher levels of GR activation suggest that while each dye alone may not be a strong GR agonist, when present with endogenous corticosterone, certain food dyes may be able to interfere with normal adipocyte metabolism and alter insulin sensitivity.

Combined, pioglitazone, a PPARγ agonist, and azilsartan, an ARB, have more effect on the regression of glomerulosclerosis through both the PPARγ and AT receptor pathway.
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Chronic kidney disease (CKD) is characterized by glomerulosclerosis. PPARγ agonists and ARBs activate two different signaling pathways, PPARγ, a transcription factor, and AT_1R/AT_2R. Previous experiments have shown that both classes of drugs slow down the progression of glomerulosclerosis. To test the hypothesis that pioglitazone, a PPARγ agonist, and azilsartan, an ARB, in combination could have a greater benefit to decrease the progression of glomerulosclerosis, we tested this combination in the 5/6 nephrectomy (Nx) model of CKD. We started treatment 8 weeks after 5/6 Nx. We assessed body weight, glucose level, blood pressure, proteinuria, and creatinine clearance at weeks 0,8, and 12 and glomerulosclerosis index, plasminogen activator inhibitor-1 (PAI-1) expression level, podocyte count (WT-1), macrophage count (ED-1), collagen accumulation, AT_1 and AT_2 receptor expression, and PPARγ activity in both biopsy (week 8) and autopsy (week 12) adult male Sprague Dawley rat tissue samples. Four treatment groups, randomized with equal starting sclerosis, were determined as follows: Vehicle – untreated rats (n=7), TAK-497 – Azilsartan, 3mg/kg in DW (n=6), Pio – Pioglitazone, 2.5mg/kg in DW (n=7), and TAK/Pio – Azilsartan and Pioglitazone (n=7). At autopsy, the combined treatment group (TAK/Pio) had the lowest blood pressure and glomerulosclerosis level which links to lower PAI-1 and AT_1 expression. Podocyte density was the highest in the combination therapy group (TAK/Pio) indicating the highest levels of renoprotection. PPARγ activity was also in the combination therapy group (TAK/Pio). Thus, PPARγ agonists and ARBs have more of an effect on the regression of glomerulosclerosis when used in combination than when used individually. Our data supports that these effects are mediated by increased PPARγ activity and decreased AT receptor pathway.

Effects of leptin receptor signaling in AgRP Neurons on Neuronal Activity and AgRP Expression
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Activation of Agouti-related protein (AgRP)-expressing neurons in response to decreased leptin levels is an important adaptive response to starvation, but other leptin-independent signals could also be involved. The goal of this summer research project was to understand how AgRP expression and neuronal activity are regulated by leptin, and the underlying signaling mechanisms. Mice were generated in which leptin receptor is specifically deleted in AgRP neurons. Hypothalamic sections from these mice were then prepared, and immunofluorescence analyses were carried out to examine expressions of cfos, AgRP and several signaling pathways. Data obtained from these experiments has displayed evidence that leptin does not directly affect the activation of AgRP neurons in the hypothalamus, hinting that perhaps other hormones or chemicals in the body are involved in this activation. The experiments performed this summer have provided some level of insight into leptin regulation of AgRP neurons under normal physiologic conditions and helped provide understanding of its dysregulation in obesity.

A Look at Hepatic Gene Expression in a Diet-Induced Obesity Mouse Model

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Untreated prediabetes has a high risk of developing into type 2 diabetes and its associated defective carbohydrate metabolism, decreased insulin secretion, and increased insulin resistance. Type 2 diabetes is most commonly treated with the oral antihyperglycemic drug metformin, which increases insulin sensitivity and suppresses hepatic gluconeogenesis. The hormone betatrophin induces β cell proliferation and increases plasma insulin levels in genetically engineered obese and diabetic mice, suggesting a possible therapy for type 2 diabetes by increasing β cell mass. We hypothesized that liver, white adipose, and brown adipose tissue betatrophin expression would be increased in mice on a high-fat diet and with increased age and obesity. In addition, mice on a high-fat diet treated with metformin were expected to have lower levels of betatrophin because of increased insulin sensitivity. The gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) were expected to be increased in mice on a high-fat diet. A Diet-Induced Obesity (DIO) mouse model was used to study betatrophin expression in 12- and 22-week old mice. Metformin was administered to DIO mice at doses of 12.5 mg/kg body weight (n=6), 25 mg/kg body weight (n=7), and 50 mg/kg body weight (n=6). The levels of hepatic and visceral white adipose tissue betatrophin expression were significantly lower in 22-week old DIO mice. In peripheral white adipose tissue and brown adipose tissue, betatrophin expression did not significantly differ in DIO mice. This may suggest a role in the pathogenesis of T2D; decreased betatrophin and β cell mass could lead to the problems associated with T2D. PEPCK expression in liver tissue was significantly increased in 12-week old DIO mice and significantly decreased in 22-week old DIO mice. G6Pase expression was not significantly different in DIO mice. Administration of metformin is ongoing and gene expression will be analyzed. Future studies will replicate these experiments and confirm betatrophin and gluconeogenic enzyme expression trends.

MicroRNA-495 regulates the expression of the β-cell apoptotic factor, TP53INP1.

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DLK1-MEG3, an imprinted microRNA (miRNA) cluster on human chromosome 14q32 is under expressed in insulin-producing β-cells of Type 2 Diabetes Mellitus (T2DM) pancreatic islets when compared to non-T2DM control islets. In order to elucidate the significance of this misregulation to T2DM pathogenesis, the targets of the 54 miRNAs encoded by the 14q32 locus were identified using high throughput sequencing of cross-linked and immunoprecipitated RNA (HITS-CLIP) of Argonaute (Ago). MiR-495, a member of the 14q32 miRNA cluster, has been shown to regulate important transcription factors in the developing pancreas. A pro-apoptotic factor, p53-Induced Nuclear Protein 1 (TP53INP1), was identified as a miR-495 target. We hypothesize that expression of TP53INP1 increases when the associated miRNA, miR-495, is knocked-down. This increase in expression results in increased β-cell apoptosis and failure, thus contributing to T2DM pathogenesis. To test this hypothesis, we cloned a Tough Decoy (TuD) against miR-495, which sequestered mature miRNA in the cytoplasm through complementary base pairing, thereby allowing for de-repression of target mRNAs. Here we show that transfection of the Hela cells with the anti-miR-495 TuD construct successfully resulted in a two-fold increase in expression of TP53INP1 mRNA, but not protein. Additionally, we cloned this construct into a lentiviral backbone to enable similar studies in human islets. In summary, we have validated that TP53INP1 is a target of the 14q32 miRNA, miR-495, as predicted by HITS-CLIP in human islets. Thus, decreased expression of miR-495 results in increased expression of apoptotic factors like TP53INP1, which can exacerbate β-cell failure and apoptosis. This provides insight into a novel mechanism by which miRNAs may contribute to the pathogenesis of T2DM.

Silk-mediated Delivery of BMP7 to White Fat Leads to Browning and Increased Energy Expenditure

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Obesity is currently a global pandemic, and results from an energy imbalance whereby energy intake exceeds energy expenditure. Excess energy is stored in white adipose tissue (WAT), which is characterized by adipocytes with a single, large lipid droplet. By contrast, brown adipose tissue (BAT) is characterized by multilocular lipid droplets, numerous mitochondria, and dense innervation by the sympathetic nervous system and vasculature. BAT also uniquely expresses uncoupling protein 1 (UCP1), allowing the tissue to undergo thermogenesis. Overall, these qualities make BAT a tissue capable of undergoing high levels of energy expenditure, and make it an appealing target for obesity therapies. Bone morphogenetic protein 7 (BMP7) is part of the transforming growth factor-β (TGF-β) super-family of growth and developmental factors, and our laboratory has implicated BMP7's involvement in 1) brown adipogenesis from committed and uncommitted progenitor cells; 2) increasing mitochondrial activity of mature brown adipocytes *in vitro*; and 3) increasing energy expenditure and decreasing appetite in mice. This makes BMP7 an enticing therapeutic option for the treatment of obesity and related metabolic conditions. However, BMP7 treatment carries the risk of bone-development if delivered to certain anatomical locations and if coupled with cell damage. Thus, we sought a way to deliver BMP7 specifically to WAT in order to induce tissue-resident progenitor cells to differentiate to energy-expending brown adipocytes without off-target effects such as bone-formation. This process of inducing brown adipogenesis in WAT is termed 'browning' and occurs during cold-exposure to provide additional cells for thermogenesis. Therefore, to deliver BMP7 to WAT, we utilized a silk scaffold consisting of a hydrogel carrying BMP7 encapsulated in silk microspheres. Silk scaffolds such as this have previously been used for BMP2-treatments, and silk is non-immunogenic. The silk has been engineered to obtain desirable release kinetics in order to mimic our *in vitro* differentiation studies. We have found that injection of this BMP7-loaded silk to the subcutaneous WAT of mice results in 'browning', including the development of multilocular, UCP1-positive brown adipocytes, and an increase in whole-body energy expenditure. Current studies are aimed at utilizing BMP7-loaded silk in obese mice to reverse body weight.

Assessment of sudomotor function as a predictor of cardiovascular autonomic neuropathy.

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Regulation of cardiovascular and sudomotor function is largely controlled by the autonomic nervous system via the small, unmyelinated C-fibers. In diabetes, damage to these fibers can cause changes in sudomotor function and a serious complication called cardiovascular autonomic neuropathy (CAN). To test the hypothesis that changes in sudomotor function will be an early predictor of CAN, we evaluated its association with standard CAN assessment measures in a cohort of type 1 diabetes (T1DM) patients. A total of 15 TIDM patients and 4 healthy controls were evaluated for CAN using standard cardiovascular autonomic reflex tests and analysis of heart rate variability (HRV). Sudomotor function was assessed as electrochemical sweat conductance (ESC) in the hands and feet, using a SUDOSCAN® (Impeto Medical; Paris, France). Feet ESC, as assessed by the SUDOSCAN®, was not significantly different between the subjects with TIDM and healthy volunteers (79 ±8 μS and 80 ±9 μS; respectively, p=0.74). Similarly, the hand sudomotor function was not significantly different as both study groups had a mean hand ESC of 69 ±10 μS (p= 0.95). This preliminary data showed no significant correlation between the hand or feet ESC and any of the HRV measures. In this cross-sectional pilot study cohort, no associations were found between sudomotor function and measures of CAN. This study is ongoing, evaluating 50 T1DM and healthy controls, who will be longitudinally followed over 3 years. Further evaluation at the conclusion of the study may reveal significant findings.

EPO expression in renal interstitial cells can be characterized via RNA *in situ* hybridization.
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The HIFs control the adult renal response to hypoxia by increasing the expression of EPO by renal interstitial cells, inducing erythropoiesis. In chronic kidney disease, fibrosis results from proliferating interstitial cells, yet EPO production is diminished, causing a renal-based anemia. To investigate this, formalin-fixed, paraffin-embedded tissue sections from adult mouse kidneys were examined for their EPO expression profile using the novel RNA *in situ* hybridization technology RNAScope®. We hypothesized that in the adult kidney, EPO expression will be localized to the interstitium rather than the endothelium and that the population of interstitial cells expressing EPO will increase with hypoxia. Wild-type mice were compared to a genetic model of hypoxia, which was created via Cre-Lox recombination by deleting the HIF inhibitor EGLN1. In wild-type mice, EPO expression was localized to the interstitium and was minimal. In the *Egln1 -/-* model, EPO expression appeared to be elevated selectively in the interstitium, but this could not be validated due to high background signal. Further optimization of RNAScope for FFPE tissues is needed to characterize the mutant model. RNAScope is currently being adapted for use with cell suspension in hopes of combining it with fluorescence-activated cell sorting. In the future, this will allow us to examine the Foxd1 interstitial cell population in isolation and characterize its EPO expression in health and disease.

Type 1 diabetes self-management: Adherence to blood sugar monitoring and record keeping among adolescents with attention problems.

Racquel Wells (University of Massachusetts Medical School), Ellen O'Donnell, Lynne Levitsky, Massachusetts General Hospital/Harvard Medical School

Adherence to Type 1 Diabetes regimens declines in adolescence. Patients with symptoms related to attention deficit hyperactivity disorder are more likely to have poor organizational and attentive skills and therefore may be less adherent to their diabetes regimen. Inattention and ADHD symptoms may be related specifically to poorer blood sugar monitoring and record keeping. Twenty-six adolescents (12 and over) and their parents completed the Diabetes Behavior Rating Scale and Conners' Rating Scale questionnaires. Self-reported responses were analyzed for correlations between adherence to blood glucose monitoring and record keeping and inattention and ADHD-risk behavior of adolescent patients. Mean parent report of adherence was significantly lower for patients ≥ 12 than for patients < 12. Parents and patients responded similarly to questions on frequency of monitoring and record keeping. There was a significant correlation between reporting of monitoring at recommended and expected times and parent reported behavior of inattention and ADHD risk. Rates of record keeping were low among all patients, regardless of inattention/ADHD risk. Physicians should focus on identifying adolescents that are at a higher risk of poorer adherence due to attention problems. Screening should include specific questions related to adherence. Measuring adherence to specific self-management practices in adolescents is a benefit to calculating and maintaining their standard of care. Future studies may utilize more "real time" measures of adherence, as opposed to record keeping, such as electronic reporting and 24-hour recall to compare with self-reporting.

CTRP Metabolic Signaling and Expression in the Brain.

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Adipocytes release “adipokines,” such as Leptin and Adiponectin, through which adipose tissue can act as an endocrine organ, hormonally controlling metabolism and regulating satiety. Adiponectin is closely related to a family of cytokines called C1q/TNF-related proteins (CTRPs), which are all believed to have metabolic effects. We hypothesized that CTRP9, CTRP12, and Myonectin (CTRP15) were all expressed in the hypothalamus and we would be able to observe regulation during different metabolic states. We also hypothesized that these cytokines may have effects on metabolically relevant signaling pathways in hypothalamic neurons. To test our first hypothesis we performed Real-time PCR on hypothalamic tissues from mice that had been exposed to various metabolic states (Ad Libitum Fed, Fasted, or Fasted and Re-Fed). To test our second hypothesis we exposed a hypothalamic neuronal cell line (N38) that had been serum starved to either CTRP9, CTRP12, or one of our control solutions for either 5, 15, 30, or 60 minutes and then performed Western Blot analysis with antibodies targeting metabolically relevant signaling pathways. CTRP12 and Myonectin were both found to be significantly upregulated in the hypothalamus during the fasted state, while CTRP9 showed no regulation under fasted or re-fed conditions. This regulation suggests that CTRP12 and Myonectin have an effect within the hypothalamus during fasting. Also, hypothalamic neuronal cells treated with CTRP9 or CTRP12 for 15 minutes were found to both down-regulate AMPK and MAPK phosphorylation. This data indicates that CTRP9 and CTRP12 could play a similar role to Leptin, which down-regulates AMPK in order to inhibit appetite.

Trust in Physicians and Its Link to Intermediate Diabetic Outcomes

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Trust in your physician as previously been linked to better glycemic control. We examined the relationship between general trust in physicians and diabetic outcomes as measured through blood pressure and glycemic control (A1c). This is a secondary data analysis using cross-sectional data from a community-based trial. Patients were mostly African Americans with diabetes living in southern, rural Alabama. Face-to-face interviews by a trained interviewer assessed general trust in physicians using a previously validated instrument (TMP-11). HbA1c and blood pressure were measured at baseline using a standardized protocol. Categorical variables were compared using chi square tests. Continuous variables were compared using bivariate linear regression. Multivariable analyses included general linear regression models adjusted for gender, income, education, insulin use, medication adherence, number of ambulatory visits, perceived discrimination, and depressive symptoms. 418 patients provided information for the trust scale (6 were missing). Mean age was 59, 75% were female and 87% were African American. High Trust (TMP $\geq$ 30) was associated with older age, less perceived discrimination, and lower medication adherence. In bivariate analyses, hemoglobin A1c (p=0.26) and mean blood pressure (p=0.64) showed no significant association with provider trust. Multivariate adjusted analysis of both A1c and mean blood pressure again showed no association with general trust in physicians. General trust in physicians was not associated with change in glycemic control or blood pressure at follow-up in either unadjusted or adjusted analyses. Though it did not reach statistical significance, higher general trust was associated with higher systolic blood pressure. Future research should further explore these relationships.

High density lipoproteins and serum triglycerides may be associated with colorectal adenomas.
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Colorectal cancer (CRC) is the third leading cause of cancer worldwide and the fourth most common cause of cancer death worldwide. Many factors that have been associated with an increased risk of colorectal adenoma may be associated with the metabolic syndrome, such as abdominal obesity, elevated fasting glucose, and possibly dyslipidemia (elevated triglycerides, lowered high density lipoproteins (HDL)). In this study, we hypothesize that individuals identified with colorectal adenomas will have increased triglyceride levels and decreased HDL levels compared to those with no history of colorectal adenomas. This study included 403 polyp-free controls, 467 non-advanced colorectal adenoma cases and 238 advanced colorectal adenoma cases from the Tennessee Colorectal Polyp Study, a large colonoscopy based case-control study. Non-advanced and advanced adenoma cases had decreased HDL levels (40.2 ± 12.2 mg/dL, 38.8 ± 10.8 mg/dL, respectively) compared to the control group (44.0 ± 13.8 mg/dL, $p<0.001$) and increased triglyceride levels (162.2 ± 81.5 mg/dL, 168.6 ± 95.8 mg/dL, respectively) compared to control groups (145.3 ± 90.2 mg/dL, $p<0.002$). Individuals in the highest quartile for HDL levels compared with the lowest quartiles had an odds ratio of 0.43 (95% CI: 0.26, 0.70) for non-advanced adenomas and 0.21 (95% CI: 0.11, 0.40) for advanced adenomas. Individuals in the highest quartile for triglyceride levels compared with the lowest quartiles had an odds ratio of 1.91 (95% CI: 1.20, 3.03) for non-advanced adenomas and 2.67 (95% CI: 1.49, 4.79) for advanced adenomas. We found that individuals with lower HDL levels and higher triglyceride levels are associated with an increased risk for developing both advanced and non-advanced colorectal adenomas.

Pyridorin – Bringing a Novel Treatment for Diabetic Nephropathy from Bench to Bedside

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Diabetic nephropathy affects 33% of all patients with Diabetes and is the most common cause of End-Stage Renal Disease, with 40% of diabetic patients requiring renal replacement therapy. Pyridorin (pyridoxamine (PM) dihydrochloride) is a crystalline amine ($C_8H_{12}N_2O_2$) that has demonstrated a significant treatment effect in slowing the progression of diabetic nephropathy in Type 2 Diabetes in three Phase II clinical trials. The objectives of this research include understanding in detail the “bench to bedside” process of getting a drug like Pyridorin to the public market while modifying & revising Phase III Pyridorin protocol pertaining to run-in periods for re-submission to the FDA. Research results conclude that bringing a drug like Pyridorin from “bench to bedside” is projected to take ~22 years (1996 to 2018) with a cost of nearly $500M and >2000 patients/animals. In addition, a modified definition of run-in periods should be included in the Pyridorin’s Phase III protocol to increase overall patient recruitment.

Urothelial cytokeratin response to inflammatory bladder injury

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The urothelial response of the murine bladder to injury as it relates to cytokeratin expression has not been well characterized. Chronic bladder injury can lead to a variety of functional problems due to the alteration of the bladder wall. Recently, we identified alterations in urothelial cytokeratins in response to bladder injury using a model of partial bladder outlet obstruction and bladder distension. Proteins have been shown to be upregulated during bladder injury particularly cytokeratin 14 (CK14) and cytokeratin 6 (CK6). We hypothesized that a purely inflammatory injury to the bladder will cause the urothelial cells to upregulate their expression of cytokeratins 14 and 6. In order to test our hypothesis we instilled 50 microliters of E.Coli 05:B55 lipopolysaccharide (LPS) at a concentration of 400micrograms/mL for 60 minutes in the bladders of female ovariecteomized C57Bl/6 mice. Control mice were instilled with 50 microliters of sterile saline. 24 hours after the acute injury, we sacrificed both groups of mice and employed different staining techniques to observe the effects LPS on the urothelial cytokeratin expression and on inflammatory infiltrate. Based on H&E staining, bladders instilled with LPS demonstrated acute inflammation, marked by subepithelial edema and accumulation of neutrophils. Control bladders instilled with saline showed little to no inflammation. Immunofluorescence (IF) staining revealed that CK 14 was upregulated in the bladders that were instilled with LPS and was not upregulated in the bladders that were instilled with saline. IF staining also showed that the expression of CK 6 was unaffected by the instillation of LPS or saline. These results suggest that the instillation of LPS can stimulate an inflammatory response in the urothelium that leads to the increased expression of CK 14. These results were consistent with other forms of bladder injury such as distension and bladder outlet obstruction, but provide evidence that changes associated with cytokeratin expression are not dependent upon physical effects of the injury model.

Insights into the Mechanism of Action of a Potential Drug for the Treatment of Adrenocortical Carcinoma

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Adrenocortical Carcinoma (ACC) is a rare, and highly malignant type of cancer with a poor prognosis and few treatment options. ATR-101 is an orphan drug that was originally intended to lower cholesterol levels in the treatment of atherosclerosis. However, ATR-101 resulted in adrenal toxicity, and thus is now being developed for the treatment of ACC. Currently, the only known target of ATR-101 is Acyl-CoA Cholesterol Acyltransferase (ACAT), an intracellular enzyme that catalyzes the esterification of free cholesterol. Previous studies reported adrenal toxicity in ACAT knockout mice fed a high cholesterol diet, indicating that ACAT activity is essential for adrenal cell viability in the presence of high cholesterol. To test the hypothesis that ATR-101 induces adrenal toxicity by increasing intracellular free cholesterol levels, we examined the effect of a cholesterol-chelating agent, methyl-**β**-cyclodextrin (M**β**CD), on human ACC (H295R) cell viability. H295R cells were incubated with varying concentrations of ATR-101 and M**β**CD for 4 hours and 24 hours. After incubation, we performed SYTOX and ATP assays as separate measures of cell viability. M**β**CD (1-1.5mM) was found to protect H295R cells from ATR-101-mediated cytotoxicity both 4 hours and 24 hours after drug treatment. To further examine the effect of M**β**CD on ATR-101, the expression of cholesterol responsive genes (CHOP, LDLR, IDOL, ABCG1, HMGCR) was analyzed in the presence of ATR-101, M**β**CD, and ATR-101 together with M**β**CD. Consistent with the protective effect of M**β**CD on cell viability in the presence of ATR-101, the induction of the stress response gene, CHOP, by ATR-101 was reversed in the presence of MbCD. M**β**CD induced cholesterol-repressed genes, LDLR and HMGCR, in either the absence or presence of ATR-101, indicating that M**β**CD lowered intracellular cholesterol levels. Furthermore, in either the presence or absence of M**β**CD, ATR-101 inhibited LXR target genes, ABCG1 and IDOL, indicating that M**β**CD does not block entry of ATR-101 into the cell. Together, this is consistent with the hypothesis that M**β**CD counteracts ATR-101 toxicity by lowering intracellular cholesterol levels. Gaining a better understanding of the mechanism(s) of adrenal toxicity by ATR-101 is critical for the development of an ATR-101 therapy for ACC and in the design of alternative ACC drugs.

Accurate Estimation of Total Daily Caloric Needs: A Comparison of Mifflin to Sabounchi Predictive Equations

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The ability to accurately estimate daily caloric needs across a range of body types is critical for research that compares appetite regulation among lean and obese individuals. While the equations proposed by Mifflin et al. are currently considered the "gold standard" for estimating basal metabolic rate (BMR) for the general population, Sabounchi et al. has recently published a set of age- and race-specific equations for estimating BMR. We hypothesized that the Sabounchi equations would provide better estimates such that the sub-populations reporting less fullness after meals would have BMR values that were underestimated by the Mifflin equation, in comparison to the estimation by the applicable Sabounchi equation. To assess hunger and fullness, we collected visual analog scale (VAS) scores from adult subjects enrolled in a twin study of appetite regulation. VAS ratings occurred before and after meals designed to provide a specific percent of the twins' daily caloric needs, as predicted by the Mifflin equation. Hunger and fullness VAS scores were compared to assess standardization of fullness between groups following each meal. The daily caloric needs estimated by both the Mifflin and Sabounchi equations were compared between groups and to VAS data. There were no significant differences in hunger or fullness VAS scores by sex or BMI category. There was a significant effect of race on differences between Mifflin and Sabounchi predictions ($P < 0.0001$). In comparing the Mifflin and Sabounchi predictions for total daily kilocalories to total daily kilocalories consumed during the study, the Sabounchi equations generated a better fit in a linear regression model ($R^2 = 0.33$, $R^2 = 0.48$, respectively). There were no significant differences in the average post-snack VAS fullness score between subjects for whom the Mifflin equation underestimated or overestimated BMR relative to the Sabounchi prediction, though there was a trend in which subjects in the underestimation group reported lower average post-snack VAS fullness scores (38.6±23.1mm) and subjects in the overestimation group reported higher average fullness scores (53.3±21.8 mm). These results suggest that, in research of appetite regulation among a racially diverse population, the BMR prediction equations published by Sabounchi et al. may be more appropriate than the Mifflin equations.

Terminating Type 1 Diabetes with Enhanced CD8 Regulatory T Cells.

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Type 1 diabetes (T1D) results from aberrant interactions between autoreactive T and B cells that lead to the destruction of pancreatic beta cells. While numerous therapies have attempted to quell this autoreactivity by targeting either B cells or T cells individually, none have successfully restored tolerance long term. It is now known that healthy individuals normally restrain T cell-B cell interactions to prevent autoimmunity through the actions of newly described CD8 regulatory T cells (CD8 Tregs). I hypothesized that CD8 Tregs fail to maintain self tolerance in Type 1 diabetes due to deficiency in critical signals for their activation and survival. CD8 Tregs were assessed numerically by flow cytometry in non-obese diabetic (NOD) mice, the primary pre-clinical model of T1D. Their response to the survival factor IL-15 was assessed by ex vivo culture with a superagonist (10ng/mL) and by measurement of IL-15R signaling with phosphoflow cytometry. The biological function of CD8 Tregs was determined in an adoptive transfer experiment. NOD CD8 Tregs were found to decrease in an age-dependent manner and were 25-fold reduced compared to B6 by 16 weeks of age ($p<0.0001$ by two-way ANOVA). These cells failed to expand robustly when stimulated with IL-15 survival signal (20-fold increase in B6 CD8 Tregs; 3-fold increase in NOD CD8 Tregs). We attributed this failure to decreased Lyn signaling following IL-15 stimulation in NOD CD8 Tregs (B6 MFI=1372; NOD MFI= 607.5). This decreased signaling related to enhanced phosphatase activity in NOD mice, perhaps relating to a T1D-associated polymorphism in PTPN22. Importantly, CD8 Tregs were competent to regulate the immune response in NOD mice when provided in a physiologic ratio, suggesting that, if adequately expanded, they may represent an effective cellular therapy to halt the immune response in T1D and permit islet beta cell replenishment.

Diabetes Connect: African American Men's Perceptions of the Community Health Worker Model for Diabetes Care

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Background: African Americans are disproportionately affected by the diabetes epidemic, with higher prevalence and diabetes-related complications compared with non-Hispanic whites. Increasingly, the community health worker (CHW) model has been implemented in an effort to improve health outcomes in underserved populations. While promising, interventions to date are more likely to attract female participants. The purpose of this qualitative study is to explore how African American men living with type 2 diabetes perceive potential benefits and risks of community health worker-delivered diabetes management programs.
Methods: Four ninety-minute focus groups were guided by a trained moderator with a written guide to facilitate discussion on the topic of community health workers (CHWs) and diabetes management. Participants were recruited from the diabetes education database at a local safety-net health system in Birmingham, AL. Two independent reviewers performed content analysis to identify major themes using an iterative, combined deductive and inductive approach.
Results: There were 25 male participants. Mean years living with diabetes was 9.6 (range 1-20). Participants demonstrated knowledge of self-management strategies and identified various hardships including emotional and physical manifestations of diabetes, dietary restrictions and non-adherence, and institutional frustrations with the medical system that contributed to self-management barriers. Participants preferred CHWs be relatable and knowledgeable about diabetes. Preferred CHW duties were to educate, facilitate support groups, provide coaching on daily activities, and link to resources. Potential concerns regarding CHWs included confidentiality and condescension. The participants also discussed the importance of family-focused support dynamics.
Conclusions: Participants identified critical self-management strategies but endure hardships that present barriers to achieving these methods. Many of the strategies and barriers to self-management that participants identified mirrored their preferred CHW duties and traits. Results from this study suggest that African American men in Alabama would participate in and benefit from a community-delivered diabetes management program.

Disruption of normal feeding schedule alters energy metabolism

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The time of day during which food is consumed influences energy homeostasis.[1] The mistiming of food intake which occurs, for example, with shift work may adversely affect energy metabolism and contribute to diseases such as obesity and metabolic syndrome.[2] To model the feeding schedule of a shift worker, C57BL/6 mice were fed a normal chow diet *ad libitum* (LD), or feeding was restricted to the light (L) or dark (D) periods. Since mice are primarily nocturnal, we hypothesized that L-fed mice will develop abnormal energy homeostasis and glucose metabolism. After 4 weeks, the cumulative food intake was lower in L-fed mice (300.59 kcal) compared to D-fed (356.24 kcal, $p < 0.001$) and LD-fed (348.62 kcal, $p < 0.01$), but L-fed mice had similar body weight, fat and lean mass compared to D-fed and LD-fed mice. Oxygen consumption was significantly lower in L-fed mice than D-fed and LD-fed mice. The profiles for blood glucose, liver glycogen, and lipids were also different in L-fed mice as compared to D-fed mice. L-fed mice also had an exaggerated insulin response to feeding. These data suggest that disrupting the normal feeding schedule in mice alters energy metabolism, decreases energy expenditure, and causes insulin resistance, which may predispose to metabolic disorders such as obesity and diabetes. Further research is necessary to determine the long-term effects of restricted feeding on organ-specific energy metabolism.

1. Bray MS et. al. International Journal of Obesity. 2012; 37:843-52.
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A Unique Look at Integrins' Role in Collecting Duct Injury

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Integrins are a family of transmembrane receptors for extracellular matrix components known to play a role in kidney development, function, and disease. To investigate the hypothesis that deletion of the α3 integrin subunit and subsequent loss of integrin α3β1 leads to increased tubulointerstitial fibrosis and collecting duct dilatation, transgenic knockout mice were developed. Immunohistochemistry and western blot analysis were conducted at various time points following unilateral ureteric obstruction (UUO). Preliminary trichrome staining showed an increase in tubulointerstitial fibrosis in α3 knockout kidneys compared to control kidneys after 7 days UUO. Western Blot analysis of papillary and medullary tissue showed increased collagen I expression after 5 and 7 days UUO as compared to control. These results have implications regarding congenital kidney disease and can potentially lead to a better understanding of normal α3β1 integrin function in kidney epithelial cells.

Prior use of the built environment predicts continued use of the built environment for routine family physical activity.

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Families play an integral role in setting healthy lifestyle expectations. For example, children of active parents are up to 6 times more likely to be active themselves. Therefore, any obesity preventive programs need to include the family and start early in childhood. This study examined: 1) physical activity programming preferences of parents and their preschool-aged children; and 2) predictors of actual attendance of physical activity programs in recreation centers. Participants of the study were low-income, minority parent-child dyads (child aged 3-5 years) living in or frequenting areas near designated community recreation centers in Nashville, TN. In this study we developed and administered a survey (n=32) to collect data on participants' process and content preferences for parent-preschool-aged child physical activity programming. Also, we performed a step-wise analysis to assess what factors predicted parent and child attendance at ongoing monthly community events (n=33). We found that parents and their preschool-aged children prefer 60-minute long fitness classes, dance/movement classes, and family swim times on Tuesdays and Saturdays from 4:00-8:00pm, and they are willing to spend up to $5 to participate. Predictors of actual attendance of physical activity programming in the community recreation center included attending prior sessions that took place in that setting. In fact, every one additional face-to-face session attended resulted in participants being 1.61 times more likely to attend a monthly community event. However, fifteen of the nineteen demographic, previous attendance, physical activity, and built environment perception factors studied were not significant in predicting monthly community event attendance. These results suggest that familiarizing families with active use of their existing built environment can lead to increased utilization of their built environment for routine physical activity.

Altered brain activity in type II diabetes – implications for cognition.

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An increasing volume of literature indicates that type II diabetes is a risk factor for a range of neurological complications, including deficits in cognition and dementia. Research conducted in mild cognitive impairment (MCI) and Alzheimer's disease (AD) patients has indicated that aberrant deactivation of regions within the default mode network (DMN) may serve as one of the earliest signs of AD onset. To test the hypothesis that type II diabetics would exhibit similar patterns of neurological activity within the DMN, we used fMRI to analyze activation and deactivation patterns during a recognition task. The recognition task performed in the MRI scanner was split into two stages; during the encoding stage patients were presented with colored images, while during the recognition stage patients were presented with the same image series and asked to indicate by the press of a button whether the images had previously presented in red or green. We used the FSL software package to perform general linear model multiple regressions comparing activation and deactivation patterns between subject cohorts during the recognition task relative to rest. We controlled for age during the final fMRI regression analysis; cohorts were well matched with respect to other potentially confounding variables such as IQ, task performance, and presence of the ApoE4 allele. Relative to T2DM subjects, controls exhibited significantly greater deactivation in the anterior cingulate cortex (ACC) and the caudate nucleus during the recognition task; T2DM patients exhibited greater activation than controls within the same regions. In conclusion, the T2DM subjects in our cohort exhibited altered patterns of brain activity similar to that seen in AD patients, demonstrating persistent activation of the ACC, a key region of the DMN, during a cognitive task. The relative deactivation observed in the caudate nucleus of control subjects is likely a reflection of hyperactivation of the caudate among T2DM subjects, which may be a compensatory mechanism used to preserve cognitive function in the absence of DMN deactivation. These observations have implications for the identification of pre-clinical biomarkers of cognitive decline in patients with T2DM.

Rescuing the blockade of wild-type proinsulin misfolding by mutant proinsulin via compounds similar to ERO1α holds promise in the treatment of diabetes mellitus.

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Misfolded mutant proinsulin causes a blockade in wild-type insulin release via intermolecular disulfide bonds, resulting in the dominant-negative behavior of diabetes pathologies as seen in the Akita mouse strain and certain types of human diabetes mellitus. Overexpression of the *ERO1α* gene has been shown to rescue this blockade of wild-type proinsulin misfolding. It is our hypothesis that compounds with similar properties to the sites of *EROα1* protein crucial to rescuing this blockade hold potentially therapeutic effects. To test this hypothesis that these compounds may increase wild-type insulin production in the presence of mutant proinsulin, we cotransfected 293T cells with both mouse wild-type (mWT) and mouse Akita (mAK) insulin genes and chased samples in the presence of potential “rescue” compounds. Chased samples were labeled with the isotope S35 and separated by gel electrophoresis to analyze respective amounts of preproinsulin and proinsulin in samples with and without the “rescue” compounds. Compared to mWT transfected 293T cells, the cotransfected mWT + mAK 293Tcells show reduced proinsulin and elevated preproinsulin levels on gel electrophoresis. This illustrates that wild-type (pre)proinsulin folding is blockaded in the presence of a mutant proinsulin relative to wild-type proinsulin alone. Although experiments are still in progress, we expect that cotransfected mWT+mAK cells treated with *ERO1α* similar compounds will show varying elevations in proinsulin compared to the untreated cotransfected cells, but these levels remain lower than mWT proinsulin levels. We hope to show that the compounds that rescue the folding blockade in this mutant model, relatively rare among the causes of human diabetes pathology, will extend their ability to increase wild-type proinsulin folding to other causes of diabetes mellitus that also result from higher ratios of protein misfolding. Once we select the *ERO1α*-like compound with the greatest potential to protect wild-type proinsulin folding in the presence of mutants, we will test this compounds effects on β cells of diabetic mice with higher misfolding occurrence not caused by genetic mutation.

B-Catenin and K-ras synergize to form Wilm's tumor with concurrent p53 pathway modulation.
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Dr. Peter Clark, Dr. David DeGraff, Vanderbilt University.

Humans can develop pediatric kidney tumors called Wilm's tumors. If one identifies the specific genes that cause Wilm's tumor, or that concomitantly change expression levels in the tumor tissue, then diagnosis and eventually drug targets for therapy are expedited. Characterizing genetic determinants in the mouse model can help actualize these future therapies. When thegenes K-r as and β-Catenin are overexpressed in a mouse, it develops a renal tumorhistologically identical to a h uman Wilm's tumor. Microarray analysis on mouse tumor tissueshowed modulated expression levels of gene targets in the p53 tumor suppressor pathway.Immunohistochemistry stained mice tissue specifica lly for p53. In tissue with K-ras andβ-catenin overexpression, p53 staining is positive surrounding the tu mor. RT qPCR measuredlevels of gene expression of p53 pathway associated genes. Combination mut ants β-Cateninand Kras were compared with controls. This PCR array analysis identified genes, such a sc-Jun, Traf1, and Dapk1, that had significant expression changes in the combination mutantwhen com pared to either mutant individually. The expression is modulated in a non-additivefashion in K-ras + β-c atenin mutant tissues, which can explain the phenotype of Wilm's tumor inonly double mutant mice. Th ese genes individually represent targets for therapy in the future,and together represent an identifying fi ngerprint for diagnosis and prediction.

Finding a Needle in a Haystack: Insights into Electronic Record System.

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In 2011, the University of Michigan introduced MiChart, an electronic health record(EHR) as a tool to store patients' medical information. In a pilot study, we evaluated the usefulness of MiChart using an electronic research alert (ERAs) as a tool for locating lean hypertensive patients to measure their plasma adrenal steroid levels. We wanted to assess whether the ERA's was a useful tool for identifying this rare phenotype with the hope that the results would help us in modify the system for further use in Cardiovascular, Metabolic, and Diabetes Research. The criteria for a lean hypertensive participant was a male between the ages of 18-70 with a systolic blood pressure of over 140, or a diastolic blood pressure of over 90, or were taking anithyptertensive medications. Once filtered through the initial pre-screen, our screening staff would then identify potential candidates for the clinical study. Of 660 patients identified by the ERA, 556 were excluded on the grounds of potentially-confounding comorbidities. Some patients diagnosed with HTN were taking a drug known or suspected to raise blood pressure or were found to have other characteristics suggestive of secondary hypertension. 44 patients were deemed eligible for participation of a blood draw. Thus far, 20 of the 44 eligible candidates have been contacted by mail, and 5 of these candidates have agreed to participate. This pilot study experience suggested additional exclusion criteria such as alcohol abuse, sympathetic overactivity, and adrenal tumors to be used in a future, larger study. Looking ahead, we are now working on a modified ERA that would trigger when a lean hypertensive patient schedules an appointment and hope that this modification would make it easier to recruit patients straight from the clinic.

Zebrafish betatrophin Expression is Induced by Insulin Resistance and Over Nutrition

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Reduced insulin-producing pancreatic β-cell mass is a central feature of both type 1 and type 2 diabetes. Current pharmacological treatments and insulin injections cannot fully substitute for endogenous β-cell function to prevent hyperglycemia. Replacement or regeneration of β-cell mass is a potentially better therapeutic treatment. Research by Yi et al. identified betatrophin, a hormone that specifically increases β-cell mass in insulinresistant mouse models, raising hopes for regenerative β-cell therapy in humans. The uncertainty of the mechanism by which betatrophin induces pancreatic β-cell proliferation is the biggest obstacle for ongoing research. Based on sequence similarity, conserved synteny, and the detection of transcripts in adult liver-tissue we identified a zebrafish betatrophin ortholog (z-betatrophin). This research study aims to determine whether z-betatrophin is a functionally conserved zebrafish ortholog. The spatial and temporal dynamics of z-betatrophin expression during development was studied. Our results suggest that z-betatrophin is liver specific and its transcription begins 6 days post fertilization (dpf). In order to determine whether nutrition state or insulin resistance regulates z-betatrophin expression we studied 6 dpf embryos from wild type models and zebrafish muscle insulin resistance models (zMIR). Our results suggest z-betatrophin expression is stimulated by lipid rich nutrient and insulin resistance. In addition, we utilized a heat shock (hsp70l) regulated and a liver specific (fab10) transgenic constructs to study the effect of ectopic expression of z-betatrophin and h-betatrophin (human betatrophin) on pancreatic β-cell number of wild type models. Although, z-betatrophin did not have a significant effect, we observed a significant increase of pancreatic β-cell number in 6 dpf embryos with ectopic expression of h-Betatrophin. These results positively identify a functional betatrophin system in zebrafish, providing an in vivo platform to identification and functional characterization of Betatrophin signalling pathways.

Pancreatic islet cell osmotic fluctuations activate L-type Ca^{2+} channels, stimulating hormone secretion

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Alpha and beta cells of the pancreatic islet secrete the hormones glucagon and insulin, respectively, which play key roles in maintaining glucose homeostasis. Despite their opposite responses to glucose, both cell types depend on Ca^{2+} influx for hormone secretion. Because it has been shown that islet cell swelling accompanies elevations in concentration of extracellular glucose, we hypothesized that glucose-induced changes in islet cell shape affect Ca^{2+} entry and hormone secretion. Cell swelling and shrinkage were provoked by changing extracellular osmolarity and by ionic amphipathic molecules, chlorpromazine (CPZ) and picric acid (TNP), which preferentially insert into either the inner or outer monolayer of the plasma membrane and cause membrane curvature. Hormone secretions in the presence CPZ and TNP were quantified using an ELISA-based assay. A number of different channel inhibitors were used to elucidate specific channels involved in regulating Ca^{2+} activity. Based on our experiments, we found that both islet cell swelling and shrinkage activate mechanosensitive L-type Ca^{2+} channels in a depolarization-independent manner. Glucagon and insulin secretion are enhanced in both low and high glucose conditions in the presence of either CPZ or TNP. Experiments performed in the absence of extracellular Ca^{2+} showed that intracellular Ca^{2+} stores are released when cells undergo shrinkage. In conclusion, changes in islet cell volume and the resultant stress on the plasma membrane activate mechanosensitive L-type Ca^{2+} channels, stimulating hormone secretion.

The Effect of Nonsynonymous Mutations in *Mtgr1*and *Mtg8* on Repression Capability

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Myeloid Translocation Gene, Related Protein-1 (MTGR1) and Myeloid translocation Gene 8 (MTG8) are two members of a family of genes originally identified as the target of chromosomal translocation in acute myeloid leukemia (AML). *Mtgr1* and *Mtg8* function as transcriptional corepressors and serve as scaffolding proteins upon which other transcriptional repressors, histone deacetylases and transcription factors assemble. Nonsynonymous mutations in *Mtg8* (P167T) and *Mtgr1*(P187T) have been identified in Human colon carcinoma by The Cancer Genome Project These mutations map to the NHR1 domain, a region important for repressor function. Specifically, this region is known to bind E-Box proteins and be involved in recruiting other components of the repression complex. We hypothesize that these mutations will reduce repression efficiency and result in regulatory deficits of target genes. To test this hypothesis we used site-directed mutagenesis to model the mutations in Mtgr1 and Mtg8 as GAL4 fusion proteins. We performed reporter assays using a GAL4-TK-Luciferase reporter to test for the global ability of the mutants to repress transcription. In order to assess whether the mutants affected more specific MTG-regulated pathways we used the SuperTopFlash assay to evaluate their impact on WNT signaling. Sanger Sequencing confirmed that plasmids contained the desired mutations and successful protein expression was confirmed via immunoblotting. The Gal4 luciferase assay demonstrated the global repression ability of the *Mtg8* and *MtgR-1* proteins but did not show a statistically significant difference in repression between the mutant and wild type proteins. The SuperTopFlash assay is still ongoing but will offer more specific data on the effect of the mutation. This data overall suggests that the mutation sites do not interfere with the translation or the global repression ability of the protein. We hope to continue to work with these mutants and identify what functional deficits' may lead to their role in human colon cancer.

Type I Diabetes Diagnosed in the Parents of Children with Type I Diabetes

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The majority of adult new onset cases of diabetes are largely classified as Type II diabetes (T2D), prompting the presumptive misdiagnosis of many patients that have Type I diabetes (T1D). Little is known about why some patients develop T1D at older ages compared to those who develop the disease in childhood and not much information is available about the diagnosis or course of T1D in a parent when one of their children is already diagnosed with T1D. Onset of T1D in adults seems to be associated with lower HbA_{1C} and C-peptide levels and milder signs of metabolic decompensation compared to children. It is hypothesized that adults that have a child with T1D will present with less severe symptoms compared to their children and may be misdiagnosed with T2D. We performed a retrospective chart review of 26 patients, consisting of 13 parent-child pairs, in order to analyze age at diagnosis, ethnicity, gender, initial HbA_{1C}, C-peptide level, personal and family history of autoimmunity, BMI, lipid profile, and presence of β-cell autoantibodies (GAD65, ICA). The average age of adults diagnosed with T1D was 44.6 ± 6 years (range 33-60). Children were diagnosed at a mean age of 9.0 ± 4.7 years (range 1-18), with 23% presenting in diabetic ketoacidosis. Children had an initial HbA_{1C} of 9.6 ± 2.9% with an average C-peptide level of 0.7 ± 0.5 ng/mL, while adults had an initial HbA_{1C} of 8.3 ± 2.0% and significantly higher average C-peptide level of 1.3 ± 0.6 ng/mL. 92% of patients were Caucasian and all patients tested positive for antibodies. 46% of adults presented with a BMI $\leq$25 and a favorable lipid profile of high HDLs (59.3 ± 15.7 mg/dL) and low triglycerides (72.7 ± 34.8 mg/dL). 58% of adults were misdiagnosed with T2D. These data suggest that adults with T1D are often incorrectly diagnosed with T2D despite having a first degree relative with T1D, indicating that parents with diabetes who also have a child diagnosed with T1D should be evaluated for T1D. The following characteristics of Caucasian ethnicity, presence of autoantibodies, lean BMI, history of autoimmunity, and a favorable lipid profile should generate suspicion of a T1D diagnosis rather than a T2D diagnosis in adults.

IDOL controls peripheral lipid composition.

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Cellular cholesterol levels are regulated by two independent transcriptional pathways governed by the Liver X Receptors (LXRs) and Sterol Regulatory Element-Binding Pathway (SREBPs). The sterol sensitive LXRs directly regulate the Inducible Degrader of the LDL Receptor (IDOL), an E3 ubiquitin ligase that inhibits lipid uptake through degradation of its targets: the LDL receptor, the VLDL receptor and ApoER2. A previous short-term study (11 weeks) of IDOL knockout mice on high-fat Western diet discovered perturbations in the metabolic profile of the peripheral tissues. To further understand the physiological effect, a long-term study (11 months) challenging WT and IDOL knockout mice with western diet was undertaken. We believe that a prolonged exposure will further uncover the metabolic contribution of the LXR-IDOL axis. Serum and tissue samples were harvested and characterized. Western blot analysis revealed a marked increase in the VLDL receptor expression in the white adipose (WAT) of the *Idol*-null animals. An associated increase in triglyceride and cholesterol levels within the WAT was detected indicating an enhanced lipid storage capacity in the IDOL knockout animals. We conclude chronic western diet exacerbated the metabolic dysregulation in the *IDOL*-null mice. These observations demonstrate that IDOL directly affects lipid uptake in the adipose tissue. Understanding the biological impact of the LXR-IDOL pathway may lead to the development of novel therapeutics for the treatment of metabolic diseases.

Assessing energy expenditure of sedentary behavior in youth using whole-room indirect calorimetry

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In epidemiological and clinical studies, energy expenditure (EE) is usually assessed using self-reported activity and corresponding metabolic equivalents termed MET. MET values are defined as the ratio of the metabolic rate for a particular activity to a resting metabolic rate (RMR) value of 1.0 $kcal \cdot kg^{-1} \cdot h^{-1}$. The Compendium of Physical Activities (Compendium) assigns MET intensity values to common activities in adults; we examined the validity and applicability of these MET estimates for sedentary behaviors, which utilize 1-1.5 METs, to children. We enlisted eighteen healthy youth to spend approximately 24 hours under in-person surveillance in a whole-room indirect calorimeter that generated high-precision EE measurements. Participants engaged in two task sessions: self-paced ambulatory exercises comprised the morning activities, and sedentary activities comprised the afternoon session. This study demonstrates cautionary use of adult compendium values to estimate the energy cost of individual sedentary behaviors in children. It also remains unclear if 1.5 METs is an appropriate cutoff value to define sedentary behaviors as a whole in younger populations. Further characterizations of activity patterns in youth are necessary to improve public health initiatives that look to reduce health-related outcomes like obesity, diabetes and other disease states.

The Effects of Insulin Detemir on Behavior in Obese and Insulin Resistant Individuals

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Insulin exerts opposite modulatory influences on dopamine transporter (DAT) and norepinephrine transporter (NET) activity via PI3K/Akt signal transduction pathway; reduced insulin signaling decreases DAT and increases NET surface expression. This suggests that impaired insulin signaling in CNS (e.g. in diabetes, obesity) may blunt DA clearance in striatum, while enhancing DA/NE clearance in prefrontal cortex (PFC), having direct and measureable effects on stop signal task (SST) performance. We predict that short-term insulin detemir treatment rescues SST performance in obese, diabetic volunteers by increasing striatal DA clearance and/or decreasing PFC DA/NE clearance. To test this hypothesis, volunteers performed the SST in fMRI scanners without and then with amphetamine (AMPH), before and after a four-week long insulin detemir or control treatment. Effect of AMPH, which increases PFC and striatal extracellular [DA] and [NE], will inform whether impaired SST in diabetes reflects low PFC [DA] and/or [NE] or high striatal [DA]. Insulin detemir arm showed significant AMPH-induced improvement in stop signal response time (SSRT) at both measurement weeks ($p<0.05$ at week 2, $p<0.005$ at week 6) whereas control arm had significant improvement only at week 2 ($p<0.05$). The two arms did not differ statistically in SSRT except at week 6 after AMPH administration. No other significant differences were identified for any other measures of SST performance, such as critical stop signal delay (cSSD) or median go response time (mGRT). All participants had improved SSRT in week 2 suggesting that they had lower than optimal [DA] in areas associated with response inhibition, such as PFC. Subcortical regions, such as striatum, may also play a role in the observed responses due to the magnitude of AMPH-induced improvements in both measurement weeks. These areas are good targets for future region of interest (ROI) fMRI studies to identify neural correlates of the observed differences in SST performance.

Peroxisome Proliferator-Activated Receptor β/δ Regulates Angiogenic Activity in Mouse Müller Cells

Jack Li (Texas A&M Health Science Center College of Medicine), Vanderbilt University

Neovascularization is the dysregulated growth of blood vessels, and in the eye it is a critical pathologic feature of diabetic retinopathy, retinopathy of prematurity, and age-related macular degeneration. Peroxisome proliferator-activated receptor β/δ (PPAR-β/δ) is a ligand-activated transcription factor that regulates angiogenic cell behaviors. In this study, we employed retinal angiogenesis models to explore the hypothesis that PPAR-β/δ regulates angiogenesis through regulation of vascular endothelial growth factor (VEGF) and angiopoietin-like-4 (*angptl4*) gene expression. We propose that hypoxia induction of VEGF and angptl4 expression in mouse Müller cells (MMC) is PPAR-β/δ-dependent. Retinal MMC cultures were established from wild type and PPAR-β/δ null mice. Culture lysates were collected and assayed for *angptl4* and *VEGF* using qRT-PCR. MMC were treated with the PPAR-β/δ antagonist GSK0660 in 2% FBS growth medium at 0.01uM, 0.1uM, or 1.0uM. Both *VEGF* and *Angptl4* expression increased with duration of hypoxia and was highest after 12 hours of hypoxia. GSK0660 treatment reduced expression of *VEGF* and *angptl4* at 1uM concentration. Paradoxically, PPAR-β/δ knockout MMC revealed increased expression of *angptl4* and *VEGF* under hypoxia compared to wild type. These results may explain the counter-intuitive finding that genetic and pharmacologic manipulation of PPAR-β/δ yield opposite effects on retinal angiogenesis in the mouse oxygen-induced retinopathy model. Our findings suggest that PPAR-β/δ may regulate *VEGF* and *angptl4* not through direct induction, but rather potentially through trans-repression.

Loss of nuclear receptor *nhr-25* leads to abnormal fat accumulation following endoplasmic reticulum stress

Jane Shin (Texas A&M Health Science Center), Jennifer Mamrosh, David Moore, Baylor College of Medicine

Endoplasmic reticulum (ER) stress is highly implicated in metabolic diseases such as diabetes, atherosclerosis, and non-alcoholic fatty liver disease. A conserved set of signal-transduction pathways, collectively termed the unfolded protein response (UPR), provides chaperones, expands the ER, and decreases protein load in order to resolve ER stress occurring from the accumulation of misfolded proteins. In a previous study, a loss of liver receptor homolog-1 (*Lrh-1*) resulted in liver lipid accumulation in mice following ER stress from tunicamycin (TM) treatment. We predict that loss of the nuclear hormone receptor- 25 (*nhr-25),* a homolog of LRH-1, will also result in abnormal fat accumulation in *C. elegans* when treated with TM. In order to quantify lipid accumulation, we utilized a triglyceride assay kit and a protein assay kit for normalization of lipids to total protein to compare wildtype worms (N2) and *nhr-25* mutants when treated with vehicle or TM. A higher lipid concentration in the *nhr-25* worms was observed when treated with TM. Moreover, in order to determine factors dependent on *nhr-25* that were also required for ER stress resolution, a siRNA screen was performed. N2 worms treated with TM were fed *E. coli* containing siRNA plasmids in order to systematically inactivate individual genes post-transcriptionally. The screen covered 95% of 232 genes that we selected from a previous microarray experiment performed in our lab, which identified genes that *nhr-25* mutants could not induce in response to ER stress. From the screen, 35 unique siRNAs resulted in significantly decreased survival or development following TM treatment. In the future, we would like to determine if any of the new components identified as required for ER stress resolution also confer a fat phenotype following ER stress. Ultimately, we would like to understand novel mechanisms of ER stress resolution in order to develop potential drug targets for metabolic diseases.

Developing a time efficient and cost effective model of diabetes mellitus using ins:NTR-mCherry zebrafish and metronidazole.

Andrew Brown (Michigan State University College of Human Medicine), Weibin Zhou, Justin Blaty, University of Michigan

Diabetes mellitus is often studied using time consuming animal models and expensive chemicals. However, recent advances in the genetic modification of zebrafish may allow development of a cheaper and more efficient model of the disease. To test the hypothesis that a diabetic state can be induced in zebrafish with low costs and rapid development, we applied metronidazole (MTZ) to ablate transgenic fish ins:NTR-mCherry, in which a bacterial nitroreductase (NTR) is expressed specifically in pancreatic beta cells under control of the insulin promoter. MTZ is converted to a cytotoxic compound by NTR only in the beta cells and thus results in beta cell ablation, mimicking type one diabetes. Various parameters including drug dosage, feeding schedule, volume of fish tank, and type of food were adjusted while measuring effectiveness of cell ablation via mCherry florescence of beta cells and toxicity via death rate. We have found that 2.5mM and 5mM MTZ solutions are most effective in ablating beta cells of the pancreas while minimizing toxicity. Studies regarding other parameters are ongoing, but will be refined in order to maximize beta cell ablation, growth rate, survival rate, and efficiency in creating a diabetic model. Once the beta cell ablation protocol has been optimized, future tests will include fasting blood glucose levels, limb regeneration, and oil red assay for detecting concentration of circulating lipids. Using MTZ to create low insulin conditions in the genetically modified fish will potentially be an effective way of making a more economical animal model of diabetes. Such a model has substantial implications for future studies, and may help to facilitate a more rapid understanding of the disease and future pharmaceutical interventions.

Peroxisome Proliferator-Activator Receptor-β Regulates Angiogenic Cell Behaviors and its Effects on Choroidal Neovascularization

Nicole Prabhu (University of Oklahoma College of Medicine) Sara Savage and John Penn, Vanderbilt University

Age related macular degeneration (AMD) is leading cause of blindness in people over 60. The most severe form, known as “wet” AMD, is believed to result from atrophy of the choriocapillaris, causing the retinal pigment epithelial cells (RPE) to become hypoxic and secrete vascular endothelial growth factor (VEGF). VEGF stimulates choroidal neovascularization (CNV) and can cause scarring in the macula and severe central vision loss. Peroxisome proliferator-activated receptor-β (PPAR-β) is a transcription factor that stimulates the release of VEGF. We hypothesize that PPAR-β plays a dual role in CNV. First, we suggest that PPAR-β may promote production of VEGF by retinal pigment epithelial cells (RPE). Secondly, we suggest that PPAR-β may stimulate angiogenic behaviors of choroidal endothelial cells (CEC). To test our hypothesis, we tested the effect of PPAR-β agonism and antagonism on *in vitro* human RPE and CEC (HCEC) and in the mouse laser-induced CNV (LCNV) model. The PPAR-β antagonist, GSK0660, lowered VEGF secretion by RPE cells ($p<0.002$) and reduced cell proliferation in HCEC ($p<0.0001$). However, the antagonist showed no effect in the *in vivo* studies. Interestingly, the PPAR-β agonist, GW0742, did not show any significant changes in our RPE and HCEC *in vitro* studies, but showed both an increase in lesion size and lesion leakiness in our LCNV studies. While these data are preliminary and further experiments must be conducted to complement our results, it appears likely that PPAR-β is involved in choroidal neovascularization. Therefore, targeted treatments may constitute a novel therapy for the wet form of age-related macular degeneration.

The origin of reprogrammed, insulin-producing intestinal cells

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Diabetes mellitus results in the gradual destruction of pancreatic β-cells and/or, eventually, their insulin-secreting abilities. Since islet transplants currently involve low supply/viability and serious immunocomprise, focus is directed towards finding a new, autologous source of β-cells for these patients. Previously, we demonstrated that intestinal crypt cells in an immunocompetent mouse can be reprogrammed into insulin+ cells that functionally and phenotypically resemble endogenous pancreatic β-cells. Gut development studies have also shown that Ngn3+ progenitors give rise to enteroendocrine cells. Thus, we hypothesize that Ngn3+ progenitor cells could be the source of our reprogrammed, insulin-producing cells. We combined our *in vivo* reprogramming system with an Ngn3+ genetic lineage tracing system, generating "NCVB" transgenic mice carrying four alleles: **N**gn3CreER, R26**C**herry, **V**illinrtTA and R26Tet**β**. The cells were first labeled by giving the NCVB mice tamoxifen, followed by doxycycline to induce the β-cell reprogramming. The reprogrammed tissues were then genetically traced to their hypothesized origins, the enteroendocrine progenitors, by observing for co-localization of lineage (Cherry) and reprogramming (GFP) signals. We observed that ~35% of the reprogrammed (GFP+) intestinal cells co-stained with insulin, with <10% of GFP+ cells positive for the Ngn3+ lineage tracing marker (Cherry) and ~3% of GFP+ cells positive for both the Ngn3 marker and insulin. We also noticed that among all Ngn3+ labeled cells, ~30% contain the reprogramming marker (GFP), but only a subset has both GFP and insulin. Altogether, our results show that the reprogrammed insulin+ intestinal cells can originate from both Ngn3 and non-Ngn3 lineages. This gives insight that although enteroendocrine progenitors are a possible source of insulin-producing cells, there are other lineages within the crypts that can be reprogrammed similarly, with implications for a larger and more versatile reservoir of new β-cells for the diabetes patient.

SLC1A5 expression in Colon Cancer as Function of Grade: Justifying Imaging with [^{18}F]-4F-GLN.
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It has been shown that intestinal cells in the colon use primarily Glutamine (Gln) as a source of carbon and energy. Specific to Gln is a transporter, SLC1A5, which is expressed in cells to acquire increasing amounts of Glutamine, may be overexpressed to cancer cells because of an addictive quality that cancer cells exhibit towards Gln. It is our hypothesis that the pathology grade of the cancer cells are directly related to the amount of SLC1A5 expressed on the cells. Surgical core samples have been analyzed from 140 patients (97 with colo-rectal cancer and 43 with resulting Liver Metastases) stained for SLC1A5. Using a subjective scoring method based on the staining intensity, the measure of tumor pathology grade is correlated with the degree of staining for colon/liver samples. Just over 50% of Grade 2 cancers exhibited elevated SLC1A5 levels in the Medium to High ranges. An IHC index calculated with this new method showed a similar proportion of SLC1A5 levels in primary tumor and liver metastases as seen in Figure 5. These results justify the interpretation of evaluating SLC1A5 levels with [^{18}F]-4F-Gutamine using *in vivo* xenografted athymic nude mice models were generated by implanting human colon cancer cells.

Recruitment in Multi-Center Type II Diabetes RCT: Challenges & Solutions Jeremy Miles, Albert Einstein College of Medicine, Einstein Diabetes Center

In June 2013, the Albert Einstein College of Medicine began recruitment for the GRADE randomized clinical trial, a study comparing the effectiveness of various diabetes medications. Novel methods of recruitment have been implemented at Einstein. However, many challenges have emerged during the initial phases of recruitment. The goal of this research is to analyze the initial recruitment data, and by doing so, we will be able to identify the successes and drawbacks that we've encountered thus far. Three main approaches have been taken to enroll patients into GRADE. First, there is a sifting of potentially eligible patients using an electronic query of the Montefiore Medical System EMR. These patients then receive letters and phone calls about GRADE. The second tactic is an academic detailing of Montefiore clinic sites. This academic detailing includes formal meetings with site directors and PCPs and distributing fliers and brochures around the Montefiore clinics. Lastly, if a patient wants to discuss the study with his/her PCP or is scheduled for any of the GRADE visits, a note in the EMR is sent to the PCP. So far, our EMR query has identified 740 eligible patients from four Montefiore clinics. We have spoken to 153 of these patients and, to date, 36 are scheduled for a screening visit. There were many patients who were ineligible because they had diabetes for more than five years. The EMR query picked up these patients even though they were ineligible because the EMR at Montefiore is only 2-3 years old and does not have exact onset dates of diabetes for all patients. 7 % of the patients we spoke to wanted to discuss the study with their PCP before entering the study. Of the patients who were eligible, 34% declined to participate in the study. While we have been moderately successful up until now it is clear that we can improve our recruitment approach. We need to refine our EMR query software so that ineligible patients do not appear on our patient list. Strategic phone calling is important for future recruitment. Moreover, there seems to be a disconnect between the PCPs at Montefiore and the research focus at Einstein. We need to continue efforts to keep the GRADE study on the minds of the PCPs, whether it is through academic detailing, notes through the EMR or publicizing GRADE throughout the Einstein-Montefiore community. Pinpointing these barriers will allow us to adjust our recruitment strategies and enhance patient involvement in the GRADE study for the future.

The role of extracellular matrix deposition and capillary density in insulin resistant *Cyp2c44(-/-)* mouse skeletal muscle.

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Cyp2c44, a mouse cytochrome P450 (Cyp) epoxygenase, converts arachidonic acid to epoxyeicosatrienoic acids (EETs). *Cyp2c44(-/-)* mice lack the major vascular Cyp enzyme responsible for production of vasodilatory and pro-angiogenic EETs. When assessed by hyperinsulinemic-euglycemic clamps, *Cyp2c44(-/-)* mice have decreased insulin-dependent glucose uptake response in skeletal muscle and adipose tissue. However, *ex vivo* skeletal muscle insulin-dependent glucose uptake appears to be similar in *Cyp2c44(-/-)* compared to wild-type. One possibility is that impaired tissue perfusion *in vivo* contributes to insulin resistance in *Cyp2c44(-/-)* skeletal muscle. We hypothesize that *Cyp2c44(-/-)* mice have an increase in extracellular matrix (ECM) abundance and/or a decrease in capillary density impairing tissue perfusion which causes insulin resistance in skeletal muscle. An accumulation of ECM could prevent insulin from readily diffusing from the blood to the muscle tissue, and fewer capillaries could limit the blood flow and insulin delivery to muscle tissue. Using immunofluorescent staining we quantified ECM abundance and capillary density in frozen sections of *Cyp2c44(-/-)* and wild type skeletal muscle tissue. We used collagen IV as a marker of ECM and CD31 as a marker of endothelial cells in capillaries and analyzed the immunofluorescent signal per area to determine the percent area covered by either collagen IV or CD31. The collagen IV abundance per area was similar between *Cyp2c44(-/-)* and wild type skeletal muscle tissue (14.8 ± 1.09% vs. 14.5 ± 0.11% in *Cyp2c44(-/-)* vs. wild type; $p = 0.81$), indicating that excess ECM accumulation does not explain the skeletal muscle insulin resistance in *Cyp2c44(-/-)* mice. Quantification of CD31 staining will be similarly applied to analyze capillary density in *Cyp2c44(-/-)* mice. Investigation of the mechanism of insulin resistance in *Cyp2c44(-/-)* mouse muscle tissue will further elucidate how the Cyp2c44-derived EETs contribute to insulin sensitivity. This work may lead to further investigation of the connection between EETs and vascular changes associated with insulin resistant states and possible pharmacological manipulation of EETs metabolism to improve insulin sensitivity.

3,5- Diiodo-L-Thyronine (T2) treatment improves hepatic insulin sensitivity but not hepatic steatosis in rats fed an unsaturated fat diet.

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Selective thyroid hormone mimetics that specifically target the nuclear thyroid hormone β-receptor have been shown to potently decrease plasma lipids without the deleterious cardiac and bone effects associated with thyroid hormone itself. However, while these agents effectively decrease hepatic lipid content in fat fed rats, they also induce insulin resistance. In contrast, the thyroid hormone metabolite, 3,5- Diiodo-L-Thyronine (T2) has been reported to decrease hepatic lipid content and improve glucose tolerance. This compound does not activate the classical nuclear thyroid hormone receptors and thus may avoid the toxicity seen with TRβ specific agonists. We hypothesized that T2 treatment would improve hepatic insulin sensitivity by decreasing hepatic triglyceride content and improving hepatic insulin signaling. To test this hypothesis, rats were placed on a high fat diet and treated with T2 or vehicle at a dose of 0.025 mg/kg i.p. for 10 days. Tissue specific insulin sensitivity was evaluated by hyperinsulinemic-euglycemic clamp studies, and biochemical and molecular analyses were performed in both fasting and hyperinsulinemic conditions. There were no significant differences in body weight, adipose mass, liver triglyceride content, plasma glucose, and non-esterified fatty acids between T2 and vehicle treated rats. Hepatic insulin sensitivity was slightly improved in the T2 group. Specifically, insulin suppressed endogenous glucose output by 74% ± 10 in T2 treated rats, as compared with 46% ± 5 in vehicle treated rats ($p=0.04$). There was no difference in basal endogenous glucose production or insulin stimulated peripheral glucose disposal. Insulin signaling as assessed by Akt phosphorylation was improved; insulin stimulated Akt phosphorylation was increased by 260% in T2 treated animals ($p=0.003$). T2 did not to act through classical nuclear thyroid hormone receptors, for gene expression of DIO1 and *hairless* were not increased in tissues of treated animals. There were no significant differences in expression of genes of hepatic lipogenesis (FAS, DGAT 2), hepatic gluconeogenesis (G6Pase), fatty acid oxidation (PPARα, PGC1-α, FGF21) or adipokines (adiponectin and leptin). Activation of ERK1/2 has been proposed as a non-genomic pathway for thyroid hormone action. Thus, we quantified hepatic ERK1/2 phosphorylation and found a small (15%), but significant increase ($p=0.04$) with T2 treatment. In summary, while 10 days of T2 treatment did not ameliorate NAFLD in fat-fed rodents it did lead to subtle improvements in hepatic insulin sensitivity, which may be due to the activation of non-classical signaling pathways. Further exploration of these pathways could hold promise for developing new agents for treating insulin resistance.

Glutamate Uptake in Rodent Primary Cortical Astrocytes

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Gamma-aminobutyric acid (GABA) is the main inhibitor neurotransmitter in the mammalian central nervous system. Previously, we showed that GABA acts within the ventromedial hypothalamus (VMH) to suppress the counterregulatory hormone responses to hypoglycemia. More importantly, recurrent exposure to hypoglycemia (RH) increases VMH GABA levels which in turn, suppress the counterregulatory responses to subsequent bouts of hypoglycemia. Our current research suggests that following RH, VMH GABAergic neurons may adapt to using alternate fuel substrates such as lactate to meet their metabolic needs. Hence, during hypoglycemia when glucose levels are low, lactate may prevent VMH GABAergic neurons from sensing a fall in glucose and thus, GABAergic inhibition of the counterregulatory responses is maintained. The purpose of this study was to identify the mechanisms that increase lactate production in the VMH during RH. Lactate is primarily derived from astrocytic metabolism. As opposed to GABA, glutamate in the VMH stimulates the counterregulatory responses during hypoglycemia and following its release, this excitatory neurotransmitter is removed from the extracellular space by glutamate transporter-1 (GLT-1), which is located on astrocytes. Because the uptake of glutamate is coupled to an energy dependent Na^+-K^+-ATPase, an increase in glutamate uptake leads to greater glycogenolysis and an associated increase in lactate production by the astrocytes. In prior studies, we showed that extracellular lactate levels can be reduced in RH animals by reducing VMH GLT-1 expression. Thus, in the current study, we tested the hypothesis that recurrent glucose deprivation can increase glutamate uptake in astrocytes using primary rat astrocyte cultures. Astrocytes were repeatedly incubated in low glucose medium for 90 minutes for three consecutive days and subsequently, the uptake of ^{14}C-labelled glutamate was assessed. Astrocytes exposed to recurrent episodes of glucose deprivation had a greater tendency to take up glutamate compared to astrocytes maintained in normal glucose medium. Together, our data suggests that the observed increase in astrocytic glutamate uptake may enhance lactate production by astrocytes and contribute to counterregulatory failure.

Patient skin fibroblasts may serve as a robust cell-based model in screening for potential drug therapies for Wolfram Syndrome

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Wolfram Syndrome (WFS) is a rare autosomal recessive disorder characterized by insulin-dependent diabetes mellitus, optic nerve atrophy, diabetes insipidus, deafness, and neurological dysfunction leading to death in mid-adulthood. WFS is caused by mutations in the WFS1 gene, located on the short arm of chromosome 4, which results in endoplasmic reticulum (ER) stress-mediated apoptosis. There are no known direct treatments for WFS, and current management efforts primarily focus on addressing the complications of WFS. For our studies, we devised a reporter system for the expression of CHOP, an integral gene involved in the activation of ER stress-mediated cell death. With this system, we identified two pharmacologically active compounds, Sunitinib and PD-407824, particularly effective in reducing CHOP expression in human embryonic kidney cell lines under ER stress. In light of these results, we are now considering skin fibroblasts from Wolfram patients as viable cell-based vehicles to elucidate the efficacy of these candidate compounds in mitigating ER stress-mediated apoptosis. Preliminary investigations have already revealed a number of patients expressing higher levels of CHOP as compared to control lines. We plan to treat these lines with Sunitinib and PD-407824 and follow CHOP expressivity. Future studies will extend drug treatment to neural progenitor and β cell lines.

Resveratrol Enhances Insulin Sensitivity and Promotes "Browning" of Subcutaneous Fat in Insulin Resistant Humans

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Insulin resistance is a hallmark of type 2 diabetes mellitus (T2DM) and thus an important therapeutic target. Detrimental metabolic effects of white adipose tissue in obesity include increased macrophage infiltration, oxidative stress, and cytokine production. Resveratrol (RSV), a plant-derived polyphenol that is abundant in the skin of grapes and red wine, is believed to have anti-inflammatory and anti-aging effects. In animal models, RSV improves insulin sensitivity and mitochondrial function via activation of NAD-dependent deacetylase sirtuin-1 (Sirt1), a regulatory enzyme, and AMP-kinase. We hypothesize that resveratrol will have beneficial metabolic effects on subcutaneous adipose tissue in insulin resistant humans, reducing inflammatory cytokines and oxidative stress. RSV may also promote "browning" of white adipose tissue, a potentially advantageous shift in metabolic characteristics and mitochondrial function. Therefore, to determine resveratrol's effect on insulin sensitivity in humans, we performed 6-hour euglycemic-hyperinsulinemic clamp studies to quantify insulin sensitivity and endogenous glucose production in overweight human subjects (n=17, age 55±3 yr; BMI =28±1 kg/m2; HOMA-IR= 5.3±0.9) before and after 28 days of treatment with RSV 2000 mg daily in randomized, double-blinded, placebo controlled fashion. Subcutaneous fat biopsies were performed at baseline and post- RSV. RSV treatment increased glucose uptake by 40% in these insulin resistant subjects. "Browning" gene expression in whole fat increased or trended upwards with RSV, consistent with a transitional "beige" adipose phenotype. UCP1 expression increased after treatment (48% increase; $p<.05$), while ZIC1 and PGC1α expression trended upward (128% and 32% increase, respectively). "Beige fat" gene expression in whole fat also increased or trended upwards after RSV including CD137 (61% increase; $p<.05$), SHOX2, TMEM 26, and LHX8 expression (115%, 58%, 99% increase, respectively). Gene expression of the insulin-sensitizing hormone adiponectin also increased substantially (76%, $p<.05$). Together, these findings suggest that resveratrol may be an important therapeutic agent to enhance insulin sensitivity in insulin resistant humans, at least in part through anti-inflammatory effects and promotion of "browning" or "beiging" of white adipose tissue.

Muscle specific deletion of integrin-linked kinase improves insulin resistance in high fat-fed mice.

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During the progression of skeletal muscle insulin resistance, changes occur in the composition of the surrounding extracellular matrix (ECM). High fat diet feeding in mice causes inflamation, which leads to increased levels of collagen and hyaluronan in the ECM. Increased collagen deposition activates integrin receptors in the ECM. Integrins are a family of cell surface receptors that interact with the ECM and mediate a variety of bidirectional signaling. Previous studies have shown that activation of the collagen-integrin α2β1 interaction contributes to the development of diet-induced muscle insulin resistance. Integrin-linked kinase (ILK), a downstream component of the integrin signaling may be key in linking ECM remodeling to insulin resistance. To investigate this, a muscle specific ILK knock out mouse line was generated and studied. We hypothesize that muscle-specific deletion of ILK would improve muscle insulin action in high fat-fed mice. The ILK knockout mice and their wild type (WT) littermate controls were fed either a low fat chow diet or a high fat diet for 15 weeks. The high fat diet derived 60% of its calories from fat. At 19 weeks, a hyperinsulinemic-euglycemic clamp experiment was performed to assess insulin sensitivity of the mice. Western blot analysis was done to determine insulin signaling in gastrocnemius and vastus lateralis muscles. Plasma samples taken during the clamp experiment were also analyzed to determine circulating insulin levels before and during the clamp. ILK deletion did not affect the body weight or composition of the mice regardless of diet. Muscle insulin sensitivity was not affected by ILK deletion in the chow-fed mice as glucose infusion rate (GIR), endogenous glucose production (endoRa), and glucose disappearance rate (Rd) were the same between WT and the KO mice. In contrast, in the HF-fed mice ILK deletion markedly improved muscle insulin resistance as shown by increased GIR and Rd. Using P-AKT as a downstream insulin signaling marker, we showed insulin signaling to be greatly increased in the ILK KO mice during HF feeding. The insulin ELISA data shows this increase in insulin action and insulin signaling is not due to disproportional insulin levels in these KO mice compared to their WT counterparts. While the male WT and KO mice had comparable insulin levels at both baseline and during the insulin clamp, the female KO mice instead showed lower insulin levels at both basal and insulin-stimulated states. In conclusion, our results suggest that muscle ILK is a pivotal mediator in linking ECM remodeling and high fat diet-induced muscle insulin resistance. This work adds to the growing pool of support for the ECM modeling of skeletal muscle insulin resistance. As the exact role of the ECM is more reveled it has possibility to open many new areas of discover and potential drug design for patients with diabetes.

Effects of Nicotine use on expression of VEGF, ETS-1, and Nephrin in a diabetes model. Bobby Sistani (University of Alabama in Birmingham School of Medicine) Ping Hua, Phillip Chumley, Edgar Jaimes, UAB

Tobacco smoking has been proved to be an independent risk factor accelerating the progression of diabetic nephropathy, the most common cause of Chronic Kidney Disease in the United States. Among the numerous harmful substances found in tobacco, nicotine is one of the highly active compounds that maybe acquired through active and passive smoking. The db/db mouse strain has been used as a model of type 2 diabetes. In this study, we investigated the effects of nicotine on the levels and presence of nephrin, VEGF, and ETS-1 in the kidney. Eight weeks old db/db mice (C57BLKS/Jlepr) and control non-diabetic mice (C57BLKSJ) were divided in 2 groups each: control mice on tap water, control mice on nicotine (100 ug/ml) in the drinking water, diabetic mice on tap water, diabetic mice on nicotine. The mice were maintained in their respective treatment groups for 10 weeks. Kidneys were then harvested and saved for histology and molecular biology. Western blot densitometry studies indicate a significant decrease in nephrin levels with nicotine use. Further data and results will be reported later. These observations will provide insight for the pathogenesis of diabetic nephropathy with nicotine use and future treatment options.

Mechanisms Underlying Diabetes-Accelerated Osteochondrogenic Differentiation and Ectopic Calcification of Blood Vessels

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Vascular calcification, the “hardening” of blood vessels, is prevalent in patients with Type 2 diabetes mellitus. Although often asymptomatic at early stages, diabetic patients with vascular calcification showed a heightened risk for cardiovascular morbidity and mortality, stroke, and lower-limb amputation compared to those without. Vascular calcification is an actively regulated, cell-mediated process, similar to bone development and remodeling. Using a genetic fate mapping strategy, we recently identified vascular smooth muscle cells (SMCs) as the major cell source that contributes to the enhanced osteochondrogenic differentiation and calcification of diabetic blood vessels. In addition, receptor for advanced glycation-end products (RAGE), Runx2, a critical transcription factor for bone and cartilage formation, and active form of extracellular signal-related kinase (p-Erk) were associated with SMCs undergoing osteochondrogenic differentiation. Furthermore, SMCs isolated from healthy arteries cultured in high glucose procalcific medium at levels of hyperglycemia showed increased Runx2 expression and matrix calcification compared to normal glucose procalcific cultures. There was also an increase in RAGE and p-Erk in SMCs cultured in high glucose procalcific medium. Since the cytoplasmic domain of RAGE (ctRAGE) has an Erk docking site and phosphorylation of Runx2 through p-Erk is critical for osteoblastic differentiation, it is possible that ctRAGE stabilizes p-Erk via its binding, leading to prolonged activation of Runx2 through formation of RAGE-p-Erk-Runx2 complexes, thereby enhancing osteochondrogenic differentiation and calcification of SMCs. To test this possibility, we induced osteochondrogenic differentiation of vascular SMC with high glucose, procalcific medium. Cell lysates were collected at days 2, 6, and 14 of the treatment. Monoclonal antibody specific for p-Erk was used to precipitate the RAGE-p-Erk-Runx2 complexes followed by a dissociation of the complexes and Western blotting for Runx2, RAGE, and total Erk. We found that there was indeed a formation of the RAGE-p-Erk-Runx2 complex in high glucose cultures as evidence by a ~60 kDa Runx2 band and a ~45 kDa RAGE band found in the p-Erk-precipitated cell lysate. Our results are the first to demonstrate the presence of RAGE-p-Erk-Runx2 complexes, a likely cause for the enhanced osteochondrogenic differentiation and calcification of vascular SMCs under hyperglycemia.

HIV-1 accessory viral protein R (Vpr) and hepatic de novo lipogenesis: Implications for the pathogenesis of fatty liver disease.

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Fatty liver disease is defined as abnormal lipid accumulation in hepatocytes, associated with liver function abnormalities with a high rate of progression to cirrhosis and liver cancer. Fatty liver, often associated with insulin resistance and dyslipidemia, occurs frequently in HIV patients. While some of these metabolic abnormalities may result from adverse effects of antiretroviral drugs used to treat HIV, the intriguing possibility that the virus per se may be responsible for some of the defects has not been fully explored. The goal of this project is to determine if the HIV virus can cause fatty liver disease.

The specific hypothesis is that the HIV-1 accessory protein viral protein R (Vpr) can induce fatty liver disease. Vpr acts *in vitro* as a corepressor of PPARγ, a critical regulator of lipid metabolism and adipose insulin sensitivity (2), and recent data from our lab suggests that it also corepresses PPARα. Vpr's effects on PPARγ lead to hyperlipolysis and increased flux of fatty acids into the liver; its effects on PPARα block fatty acid oxidation in the liver. Both of these effects would promote fatty liver. The focus of my project is to determine whether there is a third Vpr-mediated mechanism that contributes to fatty liver, namely, whether and how it can promote hepatic de novo lipogenesis. I explored this mechanism using tissues from two mouse models: a transgenic model, expressing Vpr under control of the PEPCK promoter in liver and adipose tissues, and a pharmacologic model, wherein synthetic Vpr is administered subcutaneously through an Alzet pump. Fatty liver developed in both mouse models. Notably, Vpr is present in the plasma of both mouse models, suggesting that it can act in a hormone-like manner.

Our data show that (in both mouse models) Vpr enhances the mRNA and protein expression of both SREBP 1c and ChREBP, master regulators of hepatic lipogenesis. Vpr concomitantly increases the mRNA and protein levels of key lipogenic gene targets of SREBP1c - FASN and SCD-1 – and decreases Ser79 phosphorylation of ACC. These effects demonstrate that Vpr in the circulation is sufficient to upregulate hepatic lipogenesis and contribute to the development of fatty liver.

These novel findings raise the following critical question that is currently being addressed in the lab - how does Vpr cause all these effects? We hypothesize that Vpr interacts with and coactivates LXRα, the master regulator of both SREBP1c and ChREBP.

Understanding and Overcoming Barriers to Kidney Transplantation

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BACKGROUND: Socioeconomic and demographic disparities in kidney transplantation persist despite interventions designed to improve accessibility. A proportion of referred patients never present for transplant evaluation, and this group is a vulnerable population requiring additional assistance. The primary aim of this study was to compare educational, psychological and social factors between patients referred for kidney transplantation who do not initiate and those who successfully complete the evaluation.
METHODS: Recruited patients were referred for kidney transplant evaluation at Vanderbilt Transplant Center (VTC) November 2012-June 2013. Participants who did not complete the evaluation visit were matched on race and month of visit with patients who did complete transplant evaluation visit. Data was collected via a standardized phone-based survey determining demographic factors, socioeconomic status (SES), health literacy and numeracy, pre-evaluation visit concerns about transplantation, quality of life, Internet use and systems of social support. Analysis included Chi-square, Fisher's exact test, and t-tests.
RESULTS: Sixty-four patients were matched for a total of thirty-two matches. No demographic data, including age, SES, martial status, and distance to VTC, affected evaluation no show or completion. Quality of life and health literacy and numeracy also were comparable. Prior evaluation at a different transplant center was significantly correlated with no show ($p=0.023$). Not being on dialysis ($p=0.026$) and using the Internet for healthcare information ($p=0.043$) were significantly associated with evaluation completion. No significant differences existed between pre-evaluation patient concerns of no shows versus those of completed participants. Affording medications after transplant was most identified among all participants when asked their foremost concern about the evaluation visit (27.1%). However, affording medications after transplant was the most cited pre-evaluation concern among no shows (71.9%), while the length of time to wait for a transplant was the most cited concern among completed participants (62.5%).
CONCLUSIONS: Patients' decisions to present to the initial kidney transplant evaluation visit are complex and require further examination than can be ascertained via a simple phone survey. Patient attitudes and motivation may have a significant impact on evaluation completion. A future study using focus groups will further explore the nuances of motivations and attitudes towards transplantation of patients who do and do not complete their evaluation visits.

Efficacy and comparative effectiveness of commercial weight loss programs: Preliminary results from Weight Watchers

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INTRODUCTION: Nearly two-thirds of U.S. adults are overweight or obese, which places them at risk for diabetes and hypertension. Patients often turn to commercial weight loss programs, like Weight Watchers (WW), yet the efficacy of programs like these is unclear.

OBJECTIVES: We aimed to determine the weight loss and blood pressure lowering benefits among WW participants, as well as compare the effectiveness of WW to medically supervised counseling on weight loss.

METHODS: We searched MEDLINE from 1 January 2002 to 13 June 2013, as well as included articles from a prior comparative effectiveness review. Paired investigators independently screened results to assess eligibility, and then abstracted data. We synthesized results qualitatively by outcome (mean %weight change, mean systolic blood pressure (SBP) change, mean diastolic blood pressure (DBP) change).

RESULTS: We included 10 trials. WW participants achieved up to a 7.5% weight loss and improved their blood pressure by lowering their SBP up to 6.5 mmHg and DBP up to 4.4 mmHg. As compared to medically supervised counseling, a majority of trials (n=8) favored WW for achieving up to 3.6% greater weight loss.

SUMMARY: We found that WW participants lose weight and improve their blood pressure, which suggests that WW may be a reasonable option for patients. Given our findings, health insurers who provide benefits coverage for programs such as WW may help more patients access this program.

Hepatocyte specific knockout of SRSF3 in mice shows similar activation of insulin signaling in response to insulin treatment as wild-type

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Serine/arginine-rich splicing factor 3 (SRSF3) is a member of the serine/arginine rich (SR) family of RNA binding proteins that play a crucial role in constitutive and alternative splicing, as well as other RNA metabolism. It has been demonstrated to regulate splicing of the insulin receptor (*INSR*) gene *in vitro* in hepatoma and HEK cell lines and *in vivo* in mouse liver. Hepatocyte specific deletion of SRSF3 (SRSF3HKO) causes increased expression of insulin receptor IR-A, which lacks exon 11. The 12 amino acids encoded by exon 11 broaden the receptor binding specificity such that IR-A binds IGF2 with high affinity. As SRSF3HKO mice show high expression of insulin-like growth factor 2 (IGF2) and increased insulin sensitivity when compared to control littermates we checked insulin receptor signaling in the SRSF3HKO mouse model. The mice were treated with insulin, injected intraperitoneally (IP), at 3 months of age. Their livers were harvested and protein was extracted. Western blots were used to detect activation of the insulin-signaling pathway in SRSF3HKO and control livers. Insulin receptor (INSR), glycogen synthase kinase 3 beta (GSK3-β), phosphoinositide-dependent kinase 1 (PDK1), protein kinase B (AKT), and extracellular signal-regulated kinase 1 and 2 (ERK1/2) activation were checked. We found that SRSF3HKO mice show similar insulin signaling in response to insulin treatment when compared to wild-type. Ongoing studies will explore the effects of IGF2 treatment in SRSF3HKO liver.

Understanding the Function of the Discoidin Domain Receptor 1

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Discoidin Domain Receptors (DDRs) are members of the receptor tyrosine kinase (RTK) superfamily of receptors and are shown to be upregulated in kidney fibrosis, atherosclerosis, arthritis, and cancer. Currently two different DDRs, namely DDR1 and DDR2, have been described, and five subtypes of DDR1 (a-e) have been identified. Of these, the DDR1b has been shown to contain an NPXY motif, which is important for binding scaffolding proteins that control receptor activation and function. The scaffolding protein talin has been shown to bind the NPXY motifs of the integrin matrix receptors. However, whether talin binds and controls DDR1 function is unknown. The goal of this study was to determine whether talin binds DDR1b and whether this binding controls DDR1b function. We hypothesize that the presence of the NPXY motif on the DDR1b cytoplasmic tail is a talin-binding site and this binding is important for regulating DDR1 function. To test our hypothesis, the talin cDNA was inserted into the pGEX vector and this vector was then subcloned into BL21 DH3 Gold bacterial cells to produce the desired GST-tagged talin protein. The GST-tagged talin protein was successfully produced upon induction of bacteria with IPTG, and the purified protein is functional and able to bind DDR1.The next step in our research is to determine whether talin binds only the NPXY motif. To do this, we will determine the ability of talin to bind DDR1b vs. DDR1a, which does not contain an NPXY motif. We will also determine whether binding of talin to DDR1 controls receptor function. Understanding how talin modulates DDR function may enhance development of new treatment methods to block unwanted receptor activation in fibrotic disease.

Insulin secretion in postpubertal adolescents and adults with cystic fibrosis and pancreatic insufficiency is reduced despite "normal" glucose tolerance.

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With improved survival among individuals with cystic fibrosis (CF), cystic fibrosis related diabetes (CFRD) has emerged as a significant comorbidity that is associated with worse pulmonary function, worse nutritional status and increased mortality. To improve the understanding of the pathophysiology underlying development of CFRD, we are conducting a study to characterize insulin secretion and secretory capacity in subjects with CF across the spectrum of glucose tolerance. To test the hypothesis that individuals with CF and pancreatic insufficiency demonstrate impaired insulin secretion, patients $\geq$ 16years of age were characterized according to their annual Oral Glucose Tolerance Test (oGTT); pancreatic insufficient with normal glucose tolerance (PI-NGT) were defined as individuals with 2 hr plasma glucose < 140 mg/dl. Subjects underwent Glucose Potentiated Arginine (GPA) testing during which the acute insulin responses to arginine were determined under fasting (AIR_{arg}), 230 mg/dl (AIR_{pot}) and 340 mg/dl (AIR_{max}) hyperglycemic clamp conditions. To date, 10 PI-NGT subjects completed the study and when compared to healthy controls (n=14). PI-NGT subjects were significantly younger, had lower BMI, and trended towards higher one-hour plasma glucose levels than controls. There was a trend toward lower AIR_{arg} in PI-NGT than control (18.9 ± 3.1 vs. 31.0 ± 4.5 μU/ml, p=0.07); furthermore, the glucose-potentiated acute insulin responses to arginine were significantly lower in PI-NGT subjects for both AIR_{pot} (61 ± 6 vs. 130 ± 14 μU/ml, $p < 0.01$) and AIR_{max} (70 ± 15 vs. 169 ± 22 μU/ml, $p < 0.01$). Similarly, the acute C-peptide response to arginine was lower under fasting, 230 mg/dl and 340 mg/dl hyperglycemic clamp conditions (0.8 ± 0.1 vs. 1.7 ± 0.2, 2.1 ± 0.5 vs. 5.9 ± 0.6, and 2.9 ± 0.4 vs. 5.8 ± 0.6 ng/ml; $p \leq 0.01$ for all). Insulin sensitivity (M/I) was not different between the groups (0.38 ± 0.14 vs. 0.41 ± 0.06 (mg x min)/kg, $p = 0.643$). These results indicate that patients with pancreatic insufficient CF manifest significantly reduced β-cell secretory capacity despite "normal" glucose tolerance. The extent to which this decrease in insulin secretion reflects pancreatic exocrine damage extending to endocrine tissue vs. an inherent β-cell or insulin secretion pathway defect is not known but is the subject of ongoing investigation.

Spautin-1 inhibits TUG cleavage and GLUT4 trafficking

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To increase glucose uptake in fat and muscle, insulin stimulates the translocation of GLUT4 glucose transporters from intracellular storage vesicles to the cell surface. Insulin signals through PIST proteins to stimulate endoproteolytic cleavage of Tether containing UBX domain for GLUT4 (TUG). TUG cleavage mobilizes intracellular GLUT4 and produces the novel ubiquitin-like modifier TUGUL. Cleavage is likely mediated by a protein that is a member of the ubiquitin-specific peptidase (USP) family, but the identity of this protein is not known. Here, we tested the hypothesis that USP10 or 13 is required for TUG cleavage in 3T3-L1 adipocytes. These USP isoforms act on Beclin-1, a PIST-binding partner implicated in autophagy, and are specifically inhibited by the pharmacologic agent, spautin-1. We observed decreased proteolytic processing of TUG in spautin-1 treated cells, compared to controls, as assessed by immunoblots of both the amino- and carboxyl- terminal TUG cleavage products. Ongoing experiments are examining the effect of spautin-1 on GLUT4 translocation, and are studying the potential roles of USP10 and USP13 using RNAi and overexpression. These experiments may identify the TUG protease and will potentially link autophagic processes to GLUT4 translocation.

Understanding the Role of Nicotine in the Progression of Kidney Disease in Diabetic Mice

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Kidney damage is a major complication of both type I and type II diabetes. Tobacco and/or nicotine use among diabetics is a risk factor for the progression of kidney disease. With more than 285 million diabetics and 1 billion tobacco users worldwide, discovering the mechanisms by which this occurs is critical to improving our understanding of the disease process and to develop strategies to minimize harm to the diabetic population. We hypothesized that tobacco related kidney damage is mediated in large part by specific nicotinic acetylcholine receptors (nACHr), in particular, the α7 present in the kidney. To test this hypothesis, we administered nicotine to commercially available C57 mice lacking the α7 nACHr and to wild type C57 mice, both diabetic and non-diabetic. Preliminary data suggests that nicotine induced kidney damage is mitigated by the lack of the α7 nACHr, with both proteinuria and albuminuria relatively decreased in the α7 nACHr -/- animals on nicotine versus wild type. After sacrifice, further analysis will be conducted to characterize and determine the extent of kidney damage, and the degree to which lack of the α7 nACHr protects the kidney from nicotine.

Effects of lifestyle modification and metformin on "beige" fat hormones, FGF21 and irisin, in HIV patients with metabolic syndrome

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Brown adipose tissue (BAT) is a unique fat depot that can regulate metabolism through increased energy expenditure. Browning of white adipose tissue (WAT), a process known as "beiging," can be stimulated through hormones fibroblast growth factor 21 (FGF21) and irisin. These novel hormones may have a potential role in improving metabolic parameters through the upregulation of mitochondrial uncoupling protein 1, a key regulator of thermogenesis in BAT. Data from non HIV-infected patients suggests FGF21 may be increased in patients with obesity and metabolic dysfunction, suggesting a potential resistant state or compensatory increase in the context of metabolic dysregulation. Few data are available for irisin in obese or metabolically challenged patients. HIV-infected patients are at increased risk for cardiometabolic complications, including metabolic syndrome, and we have previously demonstrated that intervention with lifestyle modification (LSM) and/or metformin in HIV-infected patients significantly improves metabolic indices, such as maximal oxygen consumption and prevention of coronary plaque progression. The potential for "beiging" in HIV-infected patients may be a mechanism by which these metabolic complications improve. Few studies have measured FGF21 in HIV-infected patients, and to our knowledge, irisin has never previously been measured in HIV-infected patients. We took advantage of a recently completed experimental paradigm and investigated for the first time the long-term effects of LSM and/or metformin on the levels of FGF21 and irisin in HIV-infected patients with metabolic syndrome. We hypothesized that one year of LSM and metformin would increase serum levels of FGF21 and irisin and be associated with improvements in metabolic parameters in this cohort if there was a direct effect of these interventions to "beige" WAT. 50 participants with HIV infection and metabolic syndrome were previously enrolled in a randomized, placebo-controlled trial with a 4 group 2x2 factorial study design: No LSM-placebo, LSM-placebo, No LSM-metformin, and LSM-metformin. Participants in the LSM groups were provided with three supervised exercise sessions per week of aerobic and resistance training and weekly dietary counseling. Participants were asked to take metformin 500mg or identical placebo twice a day with a dose increase to 850mg twice a day after 3 months. Although not statistically significant, a trend was observed among the four groups at 12 months such that the change in baseline FGF21 levels decreased with LSM. When the cohort was stratified by LSM vs. no LSM, participants in the LSM group had a significant decrease in FGF21 from baseline compared to the no LSM (-12.3 [-51.9, 24.6] vs. 42.1 [-0.3, 150.3], P=0.01). In univariate analyses, change in FGF21 was inversely associated with change in resting energy expenditure (ρ=-0.34, P=0.046) and change in VO_{2max} (ρ=-0.38, P=0.02) after 12 months of intervention. Although change in irisin levels was associated with change in systolic blood pressure (ρ=0.47, P=0.004), no other significant associations were demonstrated. The observed decrease in FGF21 levels with LSM and inverse associations with change in exercise parameters may reflect a response mechanism whereby FGF21 resistance is attenuated, or compensatory increases are reduced with long-term diet and exercise modification, leading to decreased levels of circulating FGF21. These data suggest that exercise does not directly affect FGF21, but rather promotes an improved metabolic milieu, which results in a decrease in FGF21 in direct proportion to the improvement in VO_{2max}. The small sample size may have limited the ability to observe direct changes in cardiometabolic parameters in relationship to changes in FGF21 and irisin after intervention with LSM and/or metformin. Further studies are needed to assess whether changes in FGF21 levels and improvements in energy expenditure in HIV-infected patients are associated with "beiging" of WAT.

Effects of a high fat diet on melanocortin gene expression and peptide levels in the mouse hypothalamus.

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The hypothalamic melanocortin system consisting of POMC and AgRP neurons is crucial in regulating food intake and body weight. POMC is synthesized in the arcuate nucleus of the hypothalamus and cleaved to various peptides, including α-MSH and β-EP (Figure 1). α-MSH binds to melanocortin receptors (MC-R) 3/4 and is associated with reduced food intake and decreased body weight. AgRP is an MC-R4 antagonist and produces the opposite physiologic effects. In order to examine the response of the melanocortin system to a high fat diet, we have measured of POMC and AgRP mRNA and peptide levels after 3 days, 1 week, and 8 weeks exposure to a 60% Kcal high fat diet (HFD) that causes diet-induced obesity (DIO). We have also measured levels of the POMC processing enzymes (PC1, PC2, CPE) and PrCP, which degrades α-MSH. We are testing the hypothesis that diet-induced obesity resistant mice (DIO-R) will exhibit changes that lead to a more robust activation of the melanocortin system. Mice fed a HFD for 3 days exhibited higher plasma levels of leptin and insulin, and increased POMC peptide and mRNA in the arcuate nucleus ($p<0.05$). AgRP levels were unchanged. After 1 week on a HFD, POMC mRNA was no longer elevated but AgRP mRNA was significantly suppressed ($p<0.001$). Although there were no significant changes in peptide levels of POMC or AgRP, the α-MSH/POMC and β-EP/POMC ratios were decreased, consistent with reduced POMC processing. These findings correlate with lower levels of α-MSH ($p<0.01$), β-EP ($p<0.05$) and AgRP ($p<0.05$) peptide content in the remaining hypothalamus surrounding the arcuate nucleus. Mice on the 8-week HFD gained more weight than control mice (13.54g vs 4.22g, $p<0.01$), however the weight gain varied among the mice. The DIO-resistant mice gained an average of 7.3g while the heaviest mice gained an average of 13.66g ($p<0.001$). Peptide and mRNA levels of POMC, AgRP, and the processing enzymes will be measured as described in above experiments. These data suggest that the melanocortin system has different responses to acute and chronic high fat feeding and that the response may differ in DIO and DIO-R mice.

Role of colonic epithelial cell protein talin-1 in *C. rodentium* infection in vitro

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Background: L-arginine uptake in the cell is mediated by cationic amino acid transporter (CAT) proteins, and CAT2 is the inducible form. We have shown that $CAT2^{-/-}$ mice exhibit exacerbated colitis in the dextran sulfate sodium model of epithelial injury and repair, but less colitis in the *Citrobacter rodentium* infection model. *C. rodentium* is a rodent pathogen that is closely related to enteropathogenic *Escherichia coli* (EPEC), an important cause of human diarrhea; both pathogens act by forming attaching and effacing lesions on intestinal cells. The translocated intimin receptor from EPEC interacts directly with the host cell protein talin-1, and a resulting complex of bacterial and cellular proteins is involved in downstream signaling. Recent data from our laboratory has shown that in mice infected with *C. rodentium* there is less talin-1 present in $CAT2^{-/-}$ vs. wild-type colon tissues. Our aim was to investigate the role of talin-1 in a *C. rodentium* infection model in vitro. Methods: Conditionally-immortalized young adult mouse colon (YAMC) epithelial cells were stably transduced with control and talin-1 shRNA. Talin-1 mRNA levels were assessed to confirm knockdown of talin-1 in order to select clones for experiments. Cells were activated with *C. rodentium* at a multiplicity of infection of 200. We assessed levels of talin-1 and the NF-κB subunit p65 by immunofluorescence at 30 min, bacterial adherence at 4 h, and mRNA levels for the pro-inflammatory chemokines KC and MIP-2 by real-time PCR at 4 h. Results: Adherence of *C. rodentium* to YAMC cells decreased from 13.7 ± 5.5 to 1.93 ± 0.6 CFU/10e3 cells ($p < 0.05$) in cells with talin-1 knockdown. In cells transduced with talin-1 shRNA there was less talin-1 protein expression and nuclear p65 levels were decreased when compared to cells transduced with control shRNA. With *C. rodentium* activation, KC levels were increased by 1082 ± 402-fold in control cells and decreased by 66.9 ± 9.9% ($p < 0.05$) with talin-1 knockdown; similarly, MIP-2 levels were increased by 221 ± 82.9-fold in control cells and decreased by 71.3 ± 14.2% ($p < 0.05$) with talin-1 knockdown. Conclusions: These findings suggest that talin-1 is essential for *C. rodentium* adherence to colonic epithelial cells and induction of host inflammatory responses. Thus, additional studies of the role of L-arginine in the regulation of talin-1 are warranted, and talin-1 could represent a therapeutic target in enteric infections and/or inflammatory bowel disease.

Impact of a Mind-Body Medicine Cardiac Wellness Program on Patients with Diabetes

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The Cardiac Wellness Program (CWP) at the Benson-Henry Institute for Mind-Body Medicine is a 13-week lifestyle intervention program designed to improve cardiovascular risk factors and exercise endurance, improve stress management, and promote mindfulness and the physiologic relaxation response. Diabetes is a well-known co-morbid risk factor for patients with cardiovascular conditions. To test the hypothesis that patients with diabetes would demonstrate improvement in diabetes outcomes after completing the CWP, we performed a retrospective chart review of patients completing the CWP. Using electronic medical record data, we compared Massachusetts General Hospital (MGH) CWP patients with diabetes (CWP-DM) to two cohorts, CWP patients without diabetes and MGH patients with diabetes (MGH-DM) who received usual care diabetes services matched to CWP diabetes patients on age, sex, race, hemoglobin A1c (HbA1c), Charlson comorbidity score, and period of observation. Of 457 CWP participants from 2008 to 2013 who had records in the MGH practice-based research network, 85 (19%) had diabetes and received their care at MGH affiliated practices. Compared to CWP participants without diabetes (n=372), CWP-DM had worse baseline high-density lipoprotein (57 vs. 49 mg/dl, $p<0.01$), Charlson comorbidity Score (3.1 vs. 5.5, $p<0.01$) and a higher burden of congestive heart failure (6 vs. 16%, $p<.01$), hypertension (78 vs. 96%, $p<.01$), EMR-recorded coronary artery disease (CAD) (16 vs. 40%, $p<.01$), and depression (22 vs. 34%, $p<.05$). There were no significant changes in lipids in any group, but both groups appeared to have similarly decreased rates of blood pressure and statin medication use after completing the CWP, although the reduction in CWP-DM was not statistically significant. CWP-DM participants did not have improved glycemic control after completing the program, with a -0.08 (SD 0.88) change in HbA1c. Consistent with severity of disease, CWP-DM patients experienced more hospitalized inpatient days compared to CWP participants without diabetes (2.05 vs. 1.31, $p<0.01$). Compared to MGH-DM patients (n=153), CWP-DM patients experienced a higher burden of CAD and depression, but had no difference in clinical outcomes after participation in the CWP. Logistic regression models predicting HbA1c reduction of greater than 0.5% showed that CWP participation did not increase the odds of glycemic improvement even after adjusting for baseline differences in comorbidity. Although cardiac wellness programs and relaxation response training have been shown to be beneficial for many medical conditions, our study results suggest that diabetes-specific components of such programs may be needed to improve health status and diabetes clinical outcomes in patients with diabetes. Furthermore, it is possible that the higher burden of disease in diabetes patients may hinder their ability to succeed in intervention programs that do not account for their severity of illness.

Expression and purification of type 1 diabetes autoantigen recombinant proteins.
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Cellular and humoral responses to islet autoantigens can be used as markers of the disease process that leads to Type 1 Diabetes (T1D). The development of these immune responses precedes the onset of symptomatic T1D and provides a manner to reliably screen for risk of developing T1D. A reliable source of autoantigens is of extreme importance for the development of such assays. A major advance in the field has been the transition for testing for nonspecific islet cell antigens using frozen sections to recombinant protein based assays to measure autoantibodies. The Gateway® cloning system offers an alternative to traditional cloning techniques involving restriction enzymes and ligation by employing unique recombination enzymes. By cloning islet autoantigens into the Gateway® system, expression of islet autoantigen recombinant proteins in the appropriate expression system for a given assay is facilitated. DNA fragments encoding 3 previously identified islet autoantigens in T1D, SLC30A8 (ZnT8), PTPRN (IA-2), and G6PC2 (IGRP), were cloned into pDONR221 and pET160-DEST vectors, propagated in One Shot® TOP10 Chemically Competent E. coli cells and confirmed by DNA sequencing. Future studies will allow for the efficient expression and purification of these recombinant proteins based on the Lumio tag recognition sequence and polyhistidine tag sequence contained within the pET160-DEST vector. The production of T1D autoantigen recombinant proteins can help facilitate the development of novel, reproducible cellular and humoral *in vitro* assays that screen for immune responses in T1D.

Analysis of Kidney Resident T Cell Receptors in Angiotensin II-Induced Hypertension

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T cells in the kidney and vasculature have been shown to play a role in hypertension. Our lab has discovered that CD8+ T cells exhibit dominant transcript lengths in three subtypes of the Vβ subunit of the T cell receptor (TCR) in kidneys of hypertensive mice, Vβ3, Vβ8.1, and Vβ17. The CDR3 region of TCRs is the region of the receptor that binds the antigen. In order to characterize these three subtypes of TCRs, we sequenced the DNA of the CDR3 region. We hypothesize 1) that the kidney resident CD44+ memory T cells will have a greater proportion of Vβ3, Vβ8.1, and Vβ17 T cell receptors in hypertensive mice and 2) that there are specific T cell receptor CDR3 region sequences on Vβ3, Vβ8.1, and Vβ17 positive T cells that predominate in the kidneys of hypertensive mice. In order to test our hypothesis, we infused Angiotensin II into C57BL6/J mice for 14 days (490ng/kg/min). Vehicle infused mice were used as controls. We performed flow cytometry on kidney cells to look at the Vβ subtypes of CD44+ cells. Results showed a modest increase in CD44+ memory T cells that were Vβ8.1+ in the hypertensive mice (n=5), but this increase was not statistically significant (13.2% ± 1.9 vs. 10.7% ± 0.8, mean ± S.E.M.). No increase was seen in the Vβ3 and Vβ17 positive T cells. In order to sequence the CDR3 region of the TCRs, kidney CD8+ T cells were sorted, and mRNA was used for cDNA synthesis. We performed nested PCR with cDNA using primers specific for Vβ3, Vβ8.1, and Vβ17. PCR products were cloned using TA cloning and sequenced to obtain the CDR3 region sequence. Vβ3 and Vβ17 cells expressed dominant CDR3 sequences in the angiotensin II-infused mice that were not observed in the control mice. The presence of dominant Vβ3 and Vβ17 TCR sequences suggest that these cells are responding to a neo-antigen present in hypertension. These observations, if further studied, may provide a better understanding of the formation of the suggested neo-antigen in hypertension.

Homeostatic Response to Hepatic Lipid Metabolism in the Absence of ApoB.

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Apolipoprotein B (apoB) is an essential component in the assembly and secretion of VLDL; the abnormal increase in plasma VLDL is directly associated with the progression of atherosclerosis. The knockdown of apoB using antisense oligonucleotides (ASO) results in decreased VLDL secretion after six weeks of treatment in mice maintained on a high fat diet. Despite this decrease in triglyceride secretion with apoB ASO-treatment, there is no hepatic steatosis as compared to control ASO-treated mice. An increase in macroautophagy of the ER was observed as well as an increase in fatty acid oxidation of lipids that become trapped in the ER and are delivered to the lysosome by autophagy, thus preventing the accumulation of lipid. Using HuH-7 cells, a human hepatoma cell line, in which apoB had been genetically knocked out (gift from Kiran Musunuru, Harvard University), we attempted to create an in vitro model in which to further study the effects on hepatic lipid metabolism that were observed in the mice after 6 weeks of apoB knockdown. The HuH-7 cell lines studied included a control (B10) and two apoB knockout (KO) (D9- and C1-) lines. To first confirm that apoB is not being synthesized or secreted, an apoB label experiment was conducted using 35S-methionine to label newly synthesized apoB, where cells were treated with 0.4 mM oleic acid (OA) or BSA only. A fatty acid oxidation study in these cell lines using a 16-hour label (14C-OA) sought to measure the quantity of 14C CO2 and acid soluble metabolites produced at the end of the label. As expected, there was apoB secretion observed in the control cells that had increased with the addition of OA, while the apoB KO lines produced no apoB in the presence or absence of OA. Measurements of triglyceride cell mass showed no significant difference among the three cell lines in either growth media or in the presence of 1 mM OA, similar to what was observed in the apoB ASO model. Fatty acid oxidation after the 16-hour label yielded a significant increase in 14C CO2 and acid soluble metabolites in both apoB KO lines as compared to the control. These data suggest that the increased oxidation of lipid in the apoB KO lines contributes to the lack of accumulation of triglyceride cell mass. Further studies seek to investigate whether the observed results can be explained by an upregulation of autophagy in the ER, thus providing an in vitro model that examines the path of hepatic lipid metabolism in the absence of apoB.

MAPK pathways regulate miR-222 expression in cardiomyocytes

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Exercise is an important lifestyle modification that can ameliorate conditions such as diabetes and heart disease. Increasing evidence suggest that microRNAs play important roles in the regulation of normal and pathologic changes in the heart. One such microRNA, miR-222, was previously found by our lab members to be upregulated in hearts of mice in swimming and running models. Since miR-222 expression was also previously found to be induced *in vitro* with 10% FBS, which activates multiple signaling pathways, including the MAPK signaling pathways, we hypothesize that miR-222 expression can be induced via MAPK pathways. Selective inhibitors of MAPK pathways were added to neonatal rat ventricular myocytes (NRVMs), which were pretreated for 1 hour before 10% FBS was added to each of the samples. After half and 24 hours of incubation in 37˚C, protein and RNA was isolated from cells, respectively. A Western blot assessed the effectiveness of the MAPK inhibitors. MicroRNA qualitative reverse transcription polymerase chain reaction (qRT-PCR) was used to measure the expression of miR-222. In order to find where these pathways act in the transcription and post-transcriptional modifications that result in increased mature miR222 expression, primary miR-222 levels of the same RNA samples were assessed by qRT-PCR. Cardiomyocytes, in which ERK1/2, JNK, and p38 pathways were inhibited, expressed lower levels of miR-222 relative to the stimulated controls without inhibitors, while PI3K inhibition showed no significant difference. The qRT-PCR results shows decreased pri-miR-222 levels in the presence of the MAPK inhibitors. FBS stimulates miR-222 expression through the ERK1/2, JNK, and p38 pathways, which regulate the transcription of miR-222 from gene to primary miRNA.

Diabetes and incident Parkinson's Disease: Results from the Atherosclerosis Risk in Communities (ARIC) Study.

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Diabetes has been associated with multiple neurologic complications including neuropathies and dementia. Prior cohort studies in Taiwanese and Finnish populations demonstrated an increased risk of Parkinson's Disease(PD) in patients with type 2 diabetes(T2DM); however no studies have been conducted in a U.S. population. Therefore, we conducted a prospective analysis to examine the association between pre-existing T2DM and PD in the Atherosclerosis Risk in Communities(ARIC) study, a population based cohort study of approximately 16,000 middle-aged Caucasian and African American adults from four geographically different U.S. communities. T2DM was defined by 1) self-report of physician's diagnosis, 2) use of anti-diabetic medications, 3) fasting glucose ≥ 126mg/dL or, 4) non-fasting glucose ≥ 200 mg/dL. PD was defined by a combination of medical records and physician confirmation if available. The Cox proportional hazards model was used to determine the independent risk of T2DM and PD. All data analysis was performed using STATA statistical software. For the present analysis, 15,291 total individuals were included. At baseline,1,823(11.9%) individuals had T2DM. Over a mean follow-up of 19.8 years, 91 (30.02 cases/100,000 person-years) individuals developed PD. Incidence rates were similar between individuals with and without diabetes, 29.95 and 30.57 cases per 100,000 person-years, respectively(p=0.95). The unadjusted hazard ratio (HR) of PD associated with T2DM was 1.02(95% confidence interval: 0.54-1.91,p=0.96). After adjustment for age, race, sex, and other potential confounders the HR was 0.83(0.29-1.81, p= 0.49). In conclusion, in a large U.S. population-based cohort, we found no evidence for an association between prevalent T2DM and the risk of PD. The inconsistencies observed in studies of T2DM and PD to date may be due to a lack of statistical power within any one individual study or the definitions used for PD themselves.

Characterization of the Metabolic Effects of Growth Hormone in the Somatotroph Insulin-Like Growth Factor-I Receptor Knockout (SIGFRKO) Mouse

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Growth hormone (GH) is an important regulator of fuel metabolism and body composition throughout life. GH is also well known for its lipolytic properties and in an era of increasing obesity, several studies have investigated the utility of GH in decreasing obesity. In order to further investigate the role of GH in body metabolism, I performed metabolic studies on the SIGFRKO mice, which have been shown to produce modestly elevated GH and IGF-I serum levels. On a regular diet, these mice exhibit decreased weight gain, decreased fat mass and percent body fat, suggesting GH is an important regulator of lipid metabolism. When challenged with a high fat diet, I hypothesized that the SIGFRKO mice would demonstrate a similar protective metabolic phenotype as observed in SIGFRKO mice on regular diet. To test this hypothesis, 4 week old SIGFRKO and control mice were placed on a high fat diet (60% kcal from fat) for approximately 7 weeks and then indirect calorimetry was performed (Comprehensive Lab Animal Monitoring System). SIGFRKO mice had an overall decrease in VO_2 measurements when compared to the controls (2499 ± 12.20 vs. 2613 ± 13.05 ml/kg/hr, $P<0.0001$). A decrease in VCO_2 measurements was also observed in the SIGFRKOs (1932 ± 10.53 vs. 2046 ± 11.21 ml/kg/hr, $P<0.0001$). The decrease in VO2 and VCO2 suggests a decrease in both basal and resting metabolic rate, which differs from the previous results from mice on a normal diet. Energy expenditure calculated using the Weir formula, which is dependent on VO_2 and VCO_2, was significantly lower in SIGFRKO vs. control mice during both dark and light cycles throughout the experiment (dark: 12.01 ± 0.07391 vs.13.76 ± 0.08643 kcal/hr $p<0.0001$). This was expected given the lower measurements of both VO_2 and VCO_2. Locomotion was increased in the SIGFRKO mice in both open field activity (228.7 ± 7.336 vs. 181.0 ± 7.580 ± counts $P<0.0001$) and rearing motions (50.34 ± 3.375 vs. 36.91 ± 3.279 counts $P<0.005$), indicating they were more physically active when compared to the control mice. The Respiratory Exchange Ratio (RER) was lower in the SIGFRKO mice when compared to the control mice (0.7723 ± 0.0008239 vs. 0.7879 ± 0.001229 $P<0.0001$), suggesting a relative increase in lipid oxidation in SIGFRKO mice compared to controls. SIGFRKO mice on HFD consumed significantly less chow when compared to the control mice throughout the experiment. This suggests an ability to more efficiently utilize fat as a fuel source. Overall, these studies suggest that modestly elevated levels of GH lead to improved metabolic parameters and more efficient utilization of fat based on indirect calorimetry. These studies also suggest GH alone cannot protect an animal from diet induced weight gain as shown in older SIGFRKO mice on regular chow. In part, weight gain seen in these mice may be due to an increase in lean muscle mass, which will be studied with Echo-MRI. In addition, given the differences of food consumption or energy intake, the role of elevated GH on the satiety center in the ventromedial hypothalamus would also be of interest. Clinically, further investigation into the mechanism by which GH targets adipocyte function in SIGFROK will help delineate the benefits of GH in order to address the obesity epidemic.

Thyroid Hormone as a Regulator of *Cdkn2a (p16^{INK4a})* in Pancreatic β-cells

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Type 2 Diabetes mellitus (T2D) is characterized by a reduced mass of pancreatic β-cells and dysfunctional insulin secretion that cannot meet the demands of the body. The decrease in β-cell mass has been attributed to an age-related decline in β-cell proliferation and has been linked to increased expression of the cell cycle inhibitor p16/Ink4a. High thyroid hormone (T3) levels have been linked to decreased lifespan in mammals, including humans; strategies that lengthen lifespan, such as caloric restriction, in turn decrease circulating thyroid hormone levels. We have shown that T3 is an effective *in vitro* strategy to enhance β-cell functional maturation during the postnatal period. However, a possible simultaneous effect of thyroid hormone as an aging accelerator through its increase in cell metabolic rate has yet to be determined. Considering the replicative fate of a β-cell may be determined by interactions between maturation inducers and cell cycle inhibitors, we hypothesized that thyroid hormone may regulate the expression of aging marker and mediator: *Cdkn2a (p16^{INK4a})*.

This potential relationship was interrogated in a series of experiments. First, freshly-isolated C57Bl/6 mouse islets were cultured in the presence or absence of 100pM T3 for 6, 14, and 24 hours in RPMI 1640. Following RNA isolation and cDNA synthesis, qPCR-measured *Cdkn2a (p16^{INK4a})* mRNA expression was significantly increased in T3-treated islets at 14 hours, suggesting *Cdkn2a* is a direct target of thyroid hormone and its receptor (T3R). Using Transfac/Match databases (BIOBASE), we searched for homodimeric T3R or heterodimeric T3R/Retinoic acid receptor (RXR) putative binding sites (thyroid response elements or TREs) in the mouse *Cdkn2a* region. Reported TRE matrix sequences were validated and curated into a matrix bundle, then matched against the 20kb mouse *Cdkn2a* coding sequence plus 1.5kb up- and downstream flanking regions. Candidate TREs in *Cdkn2a* were evaluated first by their matrix similarity score (fidelity of core 5bp sequence), followed by proximity to a second T3R or RXR binding site, relationship to gene landmarks, sequence conservation (rat and human homology), and appearance in other database queries (Ali Baba). Ultimately, primers were designed for five putative TRE sequences distributed along the 23kb region. DNA fragments immunoprecipitated by T3R antibodies (CHIP assay) tested our database-identified TREs by evaluating enrichment of p16 expression at these sites. Finally, p16 expression was enhanced in MIN6 cells treated with T3 or T3R agonists, further supporting a conserved, and likely direct, homeostatic relationship between these regulators of β-cell function. Future experiments will be needed to determine how the thyroid hormone-p16 dynamic controls various β-cell functional states.

The acute effects of EGF and heregulin on transcription factors associated with the regulation of cholesterol levels in wild type and *Dsk*5 mutant mice.

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The *Dsk5* mutant mice have a Leu863Gln mutation within the tyrosine kinase domain of their epidermal growth factor receptor (EGFR) that leads to an increase in kinase activity in all cells with EGFR expression. Male but not female +/*Dsk*5 mice have high hepatic and circulating cholesterol levels. The mechanisms responsible for this are not yet known. Several clinical studies have shown a positive correlation between circulating EGF levels and hyperlipidemia. The research team hypothesized that injection with EGF or heregulin, the ligands to which the tyrosine kinase receptors EGFR and ErbB3 bind respectively, would lead to an increase in the expression of transcription factors associated with cholesterol synthesis and that this increase would be more pronounced in the *Dsk5* (+/*Dsk*5) compared to the wild type (+/+) mice. Four month old male +/+ and +/*Dsk*5 mice were injected intraperitoneally with PBS(control), EGF or heregulin (0.4µg/gm body weight; n=3 per group with six groups total). Ninety minutes after injection, the mice were sacrificed, their liver specimens homogenized, liver protein concentrations were measured and then analyzed for transcription factor expression via immunoblot. The +/*Dsk*5 mouse showed elevated basal expression of transcription factors associated with the positive regulation of cholesterol synthesis. Mice injected with EGF or heregulin demonstrated an increase in the transcription factors: Egr-1, SREBP-1 and SREBP-2 associated with cholesterol synthesis. Heregulin was shown to increase the processing and expression of SREBP-1 and SREBP-2 in +/+ mice. This pilot study demonstrates that the *Dsk*5 mouse exhibits increased basal levels of transcription factors associated with cholesterol synthesis and that EGF and heregulin positively affect the expression and activation of these transcription factors. These findings have implications in the understanding of the mechanism by which dyslipidemia occurs. Identifying the pathways that lead to dyslipidemia in humans may lead to the use of ErbB kinase inhibitors as a potential form of therapy in managing this disease.

Using DEXA-derived Visceral Adipose Tissue to Predict Cardiometabolic Risk

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Abdominal obesity, especially the visceral component of adipose tissue (VAT), is strongly associated with metabolic and cardiovascular risk in humans. The differences in gender and race with regard to body composition and metabolic risk have also been demonstrated with VAT associated risk. Dual energy X-ray absorptiometry (DEXA) can accurately measure body composition with high-precision, low X-ray exposure, and short-scanning time. We previously showed strong correlations between DEXA and MRI whole body composition, with coefficients of variation of ≤2% for DEXA-derived adiposity measures. With a new fully automated analysis software to measure VAT, we determined the strength of associations between DEXA-derived VAT and other known indicators for diabetes and cardiovascular disease risk in subjects by gender, age, race, and metabolic disease state. We collected anthropometrics, vital signs, lipid profile, and DEXA whole body composition scan for 326 subjects with BMI 21.1 – 49.9 kg/m^2 & age 18 to 69 y. We then performed the non-parametric Spearman correlation analysis and found that in subjects overall, DEXA-derived VAT is associated with BMI (rho=0.556, p-value<0.0001), waist circumference (rho=0.648, p-value<0.0001), serum TG (rho=0.487, p-value<0.0001), % body fat (rho=0.442, p-value<0.0001), % trunk fat (rho=0.605, p-value<0.0001) and % android fat (rho=0.612, p-value<0.0001). We also found that with regard to gender, DEXA-derived VAT is associated with HOMA-IR in females only, and with total cholesterol, LDL and % gynoid fat in males only. With regard to race, DEXA-derived VAT is associated with SBP in Caucasians only, with total cholesterol and LDL in African Americans only, and with serum TG and HsCRP in Caucasians and African Americans only. These observations have implications for VAT associated risk in diabetes and cardiovascular disease.

Expression profiling in the hypothalamus of *Rpgrip1l* deficient mice.

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RPGRIP1L, a gene located in proximity to *FTO* (Fat Mass and Obesity-associated gene), encodes a protein that localizes to the transition zone of the primary cilium. Hypothalamic *Rpgrip1l* expression decreases in fasted mice, suggesting that RPGRIP1L hypomorphism may lead to increased food intake. Mice heterozygous for *Rpgrip1l* (*Rpgrip1l*$^{+/-}$) are hyperphagic leading to increased adiposity. In an effort to identify a pathway through which *Rpgrip1l* controls food intake, we performed expression profiling in the hypothalamus of *Rpgrip1l*$^{+/-}$ mice by RNA deep sequencing (RNAseq). From a total of 90 genes that demonstrated statistically significant expression changes, only 34 were of known function. Clustering revealed their involvement in three major biological processes: neuron plasticity, protein trafficking to the membrane/cilium, and neuron development. Further experiments are warranted for the elucidation of the mechanism by which *Rpgrip1l* controls energy homeostasis.

Facilitating Anemia Treatment Risk Communication for Patients with Kidney Disease: Decision Aid Trial Feedback.

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BACKGROUND
Anemia is quite common in kidney disease patients: around 90% of dialysis patients and 15% of chronic kidney disease (CKD) patients receive treatment. Although there are alternate therapies for treating anemia in kidney disease patients, including the administration of iron or the use of blood transfusions, ESAs continue to be the primary form of treatment. To further patient education of their care, condition, and treatment options, in this study we developed a shared decision aid, to be used in collaboration by both physicians and patients. The objective of this study was to obtain feedback from healthcare providers and patients who are the target audience for this tool.
HYPOTHESES
We hypothesize that through this feedback process, physicians will address barriers to patient care, and patients will address improvements in knowledge and satisfaction with the use of this tool.
METHODS
A convenience sample of providers and patients in the department of nephrology provided feedback on the shared decision making aid. This did not require them to answer any questions about themselves. Questions about the quality and comprehensibility of the graphs and diagrams, as well as the clarity of the explanations provided in the aid were on the feedback form. Other features such as font size and readability were also addressed.
RESULTS
18 providers and 39 patients in the department of nephrology were asked for their feedback on the survey. Comments were made on the first two graphs and the risk chart (question 4) of the shared decision making aid by patients and providers alike. The presence of a normal value bar on the bar graph in question 1 was deemed to be confusing, and some patients were confused about what they were supposed to do to answer those first two questions. 8 of the 18 providers and 18 of the 39 patients made remarks that led to revisions in an updated decision aid. With regard to the risk chart, there was confusion about what the smiley faces represented. Comments suggesting revision would be necessary were made by 9 of the 18 providers and 25 of the 39 patients. Based on these comments, a revised tool was developed. The Suitability Assessment of Materials was used to evaluate the second version of the aid, and after 5 reviews, the revised decision aid received an average score of 37.2 out of 42, which is considered to be a superior rating.
CONCLUSIONS
The feedback collected suggests that this tool could be beneficial in lessening provider-patient barriers and improving risk communication, making patients more aware and more active in their role, and ultimately, leading to improved outcomes in quality of life and other measures. Ultimately, the comments and suggestions provided by responders allowed us to create a revised decision making tool to be used in a future study, making compliance and comprehension more likely. Future research directions include a pilot study, in which patients will be divided into a control group that does not use the decision aid and a treatment group that receives the decision aid. Patient understanding of anemia and their overall satisfaction will be measured in order to measure the efficacy of the decision aid.

Chronic kidney disease results in altered HDL-microRNA content and delivery to endothelial cells

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Chronic kidney disease (CKD) is a major health and economic burden for >20 million individuals in the United States. Reports have demonstrated that subjects undergoing hemodialysis for CKD have dysfunctional high-density lipoproteins (HDL) related cholesterol efflux. A function of HDL is transportation and delivery of microRNAs (miRNAs) and untranslated regions (5' and 3' untranslated regions) of messenger RNA (mRNA)—small, non-coding RNAs that mediate post-transcriptional gene expression—to recipient cells, including human coronary endothelial cells (HCAEC). In this study, it has already been shown by Dr. Vickers' group that macrophages transport miR-92a to HCAEC where it is loaded onto Argonaute-2 RNA-induced silencing complexes. Additionally, the group has found that CKD results in significant down-regulation of miR-16, miR-26b, miR-222and 6 miRNAs in the miR-92a genomic cluster. Another consequence of CKD is that it results in the differential distribution of miRNAs across HDL sub-fractions, which are associated with HDL's capacity to transport miRNAs to HCAEC. This is also important because it has been demonstrated that small, dense, protein-rich HDLs display atheroprotective properties . However, it has been determined that CKD alters miR-16 and miR-26b sub-fraction profile—these miRNAs are present in large, light, lipid-rich HDL, likely compromising the atheroprotective properties of HDL.

***Helicobacter pylori* upregulates Snail via p38 MAPK- and TGFβ-mediated signaling to induce epithelial-mesenchymal transition and disruption of apical-junctional complexes.**

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Introduction and background: *Helicobacter pylori* infection is the strongest known risk factor for gastric adenocarcinoma, one of the most common causes of cancer-related death worldwide. Increased motility and invasiveness are hallmarks of cancer cells and define the process of epithelial to mesenchymal transition (EMT). Snail is a cytoplasmic protein that localizes to the nucleus upon activation, induces EMT, and represses transcription of components of the apical-junctional complex, all of which subsequently increase cancer risk. Preliminary data suggests *H. pylori* suppresses expression of claudin-7, a tight junction component associated with carcinogenesis. Furthermore, in bronchial epithelial cells, claudin-7 is negatively regulated by p38 MAPK- and TGFβ-dependent activation of Snail (1).
Hypothesis: *H. pylori* upregulates Snail in gastric epithelial cells via p38 MAPK- and TGFβ-mediated signaling.
Methods: MKN28 gastric epithelial cells were co-cultured with the *cag*$^+$ carcinogenic *H. pylori* strain 7.13, and expression of Snail was determined by Western blot and Real-time PCR. Prior to co-culture with *H. pylori*, MKN28 cells were pre-treated with inhibitors of p38 MAPK (SB203580, 10μM) or TGFβ signaling (SB431542, 10μM). Isogenic *cagA, slt,* and *cagE* null mutants of strain 7.13 were generated to examine the effects of specific *H. pylori* virulence factors.
Results: MKN28 cells infected with *cag*$^+$ carcinogenic *H. pylori* strain 7.13 expressed significantly higher levels of *snail* transcript (1.9-fold induction, $p<0.001$) and Snail protein (2.5-fold induction) compared to uninfected cells. Levels of Snail protein expression in *H. pylori*-infected cells were significantly attenuated following inhibition of p38 MAPK or TGFβ signaling ($p<0.05$). No significant increase in the level of *snail* transcript was observed in MKN28 cells infected with isogenic *cagE* or *slt* null mutant strains, whereas cells infected with the *cagA* isogenic mutant strain expressed *snail* transcript levels similar to *cag*$^+$ *H. pylori* strain 7.13.
Conclusions: *In vitro, H. pylori* increases Snail expression in gastric epithelial cells, which requires p38 MAPK and TGFβ intracellular signaling pathways. *H-pylori*-induced upregulation of Snail occurs via a *cag* PAI-dependent mechanism. These findings identify Snail as a key mediator of epithelial responses with carcinogenic potential that develop after infection with *H. pylori.*

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Effect of salt intake and aldosterone exposure on aldosterone clearance

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Aldosterone plays a key role in the renin-angiotensin system. Sodium restriction and low blood pressure increase aldosterone to stimulate renal sodium reabsorption. Aldosterone also induces the inflammatory cytokine IL-6. Increased inflammation in the body can lead to negative effects on the cardiovascular system such as atherosclerosis, cardiac remodeling, and cardiac failure. Inappropriately elevated aldosterone can also worsen insulin resistance, impair insulin secretion, and contribute to resistant hypertension. Normally, aldosterone is metabolized in the liver where it is enzymatically degraded by various enzymes such as 5α-reductase, 3α-hydroxysteroid dehydrogenase, and β-glucuronidase. Past research has focused on the detrimental effects of excess aldosterone; however, there has been less focus on the differences in aldosterone clearance rates between racial groups, gender, and salt intake, which may result in increased concentrations of the circulating hormone. Here we tested the hypothesis that salt intake as well as characteristics such as race, gender, and exposure time to the hormone alter aldosterone clearance. Patient data from a clinical study performed by Drs. Brown and Luther at Vanderbilt University was used to calculate aldosterone clearance. During this study aldosterone was infused into 40 participants (10 with metabolic syndrome, 18 females, 33.28 ± 10.49 years old, 10 black Americans) at a rate of 0.7μg/kg/hour for 12 hours starting at 10pm and ending at 10am. Aldosterone concentrations were measured 5 and 12 hours after infusion. We analyzed the effects of race, gender, salt intake, and exposure on clearance of the hormone. Race did not significantly alter the aldosterone clearance rate in black Americans versus white Americans at both 5 hours ($p=0.28$) and 12 hours ($p=0.40$). In a similar fashion, gender did not significantly alter aldosterone clearance rate at 5 hours ($p=0.72$) and 12 hours ($p=0.36$) after initial infusion. Conversely, duration of exposure to exogenous aldosterone significantly increased the aldosterone clearance rate from 68.34 ± 9.58 L/hr at 5 hours to 176.14 ± 45.18 L/hr at 12 hours ($p=0.03$) after infusion. Interestingly, the plasma aldosterone concentration markedly decreased from 147.67 ± 84.81 ng/dL to 79.33 ± 22.35 ng/dL after infusion. Dietary sodium did not significantly alter aldosterone clearance rates at 5 hour ($p=0.21$) and 12 hour ($p=0.43$) time points. Taken together, our findings suggest that continuous exposure to increased aldosterone concentrations may lead to an adaptive increase in the rate of clearance of the hormone.

The Effect of High Glucose on Proximal Tubule Cells

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The proximal tubule is the key tubular segment responsible for glucose reabsorption in the kidney. Among patients with diabetes, proximal tubule epithelial cells are exposed to high levels of both protein and glucose, leading to the production of growth factors and cytokines that may promote tubulointerstitial fibrosis. One such pro-fibrotic growth factor produced by injured proximal tubule cells (PTC) is TGF-beta. In order to determine how the TGF-beta Type II receptor (TBR-II) affects PTC production of Collagen IV, we used TBR-II $^{-/-}$ PTC and TBR-II ^{floxed} PTC in vitro. Using cell culture techniques, we exposed the cells to media of varying glucose concentrations. Western blot analysis demonstrated that the presence of the TGF-beta Type II receptor increased PTC production of Collagen IV regardless of glucose concentration and osmolarity. Additional western blot data showed an increase in Collagen IV production by PTC exposed to mannitol, suggesting further research may be necessary to study the use of mannitol as an osmotic control. In order to determine the paracrine effect of high glucose-exposed PTC on neighboring cells, fibroblasts were exposed to conditioned media from TBR-II ^{floxed} PTC cultured on transwells. Protein quantification data suggests that high glucose may induce fibroblast proliferation. These data suggest that TGF-beta signaling may mediate PTC production of Collagen IV in response to high glucose. Further, PTC exposed to hyperglycemia may produce factors that influence neighboring fibroblasts to proliferate. These results highlight the necessity for further research in the proximal tubule response to hyperglycemia.

“Understanding cell-type specific gene expression in alpha cells regulated by DNA enhancer elements”

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Normoglycemia is dependent upon the homeostatic balance between insulin and glucagon action. These hormones are secreted from specialized cells in the pancreatic islets of Langerhans; insulin being secreted by β-cells and glucagon by α-cells. To understand the actions of these hormones, normoglycemia, and the diabetic disease state it is necessary to understand the biology of these cell types. The pancreatic α cells are poorly understood and the aim of this project is to gain a better understanding of the DNA elements that define the gene expression profile unique to this cell type. This project takes data from a ChIP-seq screen that identified ~200 putative elements near genes that are highly expressed in α cells, and were also associated with conserved regions of the genome. These regions of the genome were taken to test the hypothesis that these elements will drive gene expression specific to alpha-cells. Of the 200 DNA sequences, 30 were chosen for further screening in selective enhancer activity specific for alpha cells. From theses 30 elements, I chose 10 to validate in luciferase enhancer screens. DNA primers were made for each of the potential enhancer elements and used in a PCR reaction in order to amplify each DNA element. Each DNA fragment was then cloned into a vector and transformed into competent *E. Coli* cells. Bacterial colonies were selected to grow in liquid cultures and mini-prepped in order to isolate the plasmid DNA containing our inserted target DNA. Isolated plasmid DNA were submitted for DNA sequencing to ensure proper insertion of the elements into the respective plasmid. In vitro recombination between our entry clones (containing our DNA enhancer element of interest) and a pGL4.23 destination vector (containing the *luc2P* reporter gene) was performed such that the enhancer will drive Luciferase expression through a minimal promoter. Plasmids that were successfully recombined were then transfected into alpha and beta cell lines and tested for expression of luciferase. Of the elements that underwent the luciferase assay, 2 showed a significant increase in luciferase signaling when compared to the controls. Based on the outcome, it appears that these 2 elements show potential for being considered DNA enhancer elements unique for controlling gene expression in α cells. Successful identification of these DNA enhancer elements will be important in understanding how α cells maintain their identity and how these cells play a physiological role in controlling an individual’s blood glucose levels through its expression of proglucagon.

Metabolomic Analysis with Varying Glucose Concentrations in Renal Proximal Tubular Epithelial Cells

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How elevated glucose levels initiate diabetes-related complications over time remains unclear. Recent studies identified regulation of pyruvate dehydrogenase complex and AMPK in diabetic kidney disease; however the regulation of these pathways and metabolic regulation by high glucose in proximal tubular cells is largely unknown. We undertook a comprehensive metabolomic analysis of cultured human proximal tubular epithelial cells (PTEpiC) treated with normal and high glucose conditions in order to identify key metabolites that are changing in these cells during exposure to high glucose conditions and determine which metabolic enzymes of interest are affected by high glucose conditions. A primary culture of human renal proximal tubular epithelial cells (ScienCell) (P3-P6) were made quiescent in serum-free media with EpiCGS, Pen/Strep, 0.5% FBS for 2.5 hours, and then treated with 5.5mM glucose (normal) and 25mM glucose (high) media with 2% FBS, EpiCGS, Pen/Strep. Over 50 metabolite levels were measured by GC-MS in cell culture media of cells treated with high glucose (HG) or normal glucose (NG) conditions for 72 hours. Protein from PTEpiC was analyzed by SDS-page gel electrophoresis, followed by Western blot probed with antibodies against pAMPK, AMPK-α (Cell Signaling), pPDH, and PDH (Invitrogen). Diabetic mice with kidney disease have reduced pAMPK, and increased pPDH in kidney cortex. In cultured PTEpiC, pAMPK is reduced and pPDH is increased in HG conditions vs. NG conditions at 72 hours. In cell culture media from PTEpiC treated with HG for 72 hours, Lactic Acid, Pyruvic Acid, Malic Acid, Succinic Acid, 5-oxo Proline, and 3-Methyl Glutaconic Acid are increased vs. NG conditions. Decreased pAMPK levels and increased phospho-PDH in cells in high glucose conditions could be indicative of a cataplerosis state, in which there is a reduction of carbon flow into mitochondria under conditions of caloric excess. This follows previous in vivo experiments showing decreased pAMPK and increased phospho-PDH levels in diabetic mice and humans. Increased lactic acid, and to a smaller extent increased pyruvic acid, are shunted away from the mitochondria via deactivation of PDH (increased p-PDH) in HG conditions. Increased 5-oxo Proline, and slight increase in 3-Methyl Glutaconic Acid, Succinic Acid, and Malic Acid metabolites suggest the possible rise in leucine breakdown activity in order to shunt more carbon skeletons and hence energy into the TCA cycle other than pyruvate. Additional metabolic flux experiments are underway to follow a stable isotope tracer molecule within the cells' metabolic pathway and identify metabolic changes taking place and specific pathways affected.

Metformin reduces oxidative stress and mitochondrial dysfunction induced by hyperglycemic conditions in models of Barrett's esophagus and esophageal adenocarcinoma

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Esophageal adenocarcinoma (EAC) develops in a premalignant columnar metaplasia known as Barrett's esophagus (BE) in the setting of gastroesophageal reflux disease (GERD). Central adiposity predisposes individuals to GERD and has been linked to insulin resistance, metabolic syndrome, diabetes as well as emergence of a pro-inflammatory state; however, the role of hyperglycemia in the pathogenesis of BE and EAC has yet to be explored. In these patients, reflux of gastric acid and bile leads to inflammation, production of excessive levels of reactive oxygen species (ROS) and oxidative stress in cells of the esophageal mucosa. These changes damage mitochondria, further increasing ROS levels, and other cellular components including DNA. Such damage can result in altered gene expression patterns, induction of metaplasia, transformation of the epithelium, emergence of BE and progression to EAC. We hypothesize that metformin, via its effects on AMPK and mitochondria homeostasis, reduces mitochondria dysfunction and limits oxidative stress and DNA damage in cellular and mouse models of BE and EAC. Normal human esophageal cells, human BE cells, and human EAC cells were cultured under conditions that mimic diabetic conditions and control normal glycemic conditions. A mouse model overexpressing the pro-inflammatory cytokine IL-1β targeted to the oral cavity, esophagus, and squamous forestomach via control of the L2 promoter was also used to investigate the transition from normal squamous epithelium to BE and EAC. Our results demonstrate that hyperglycemic conditions increase oxidative stress and mitochondrial dysfunction, which is ameliorated with metformin treatment. Human BE and EAC cells also exhibit substantial decreases in mitochondrial genes *MT-ND6* and *MT-CO1*. Consistent with our *in vitro* studies, L2-IL-1β mice showed higher levels of protein oxidation and decreased expression of mitochondrial proteins. Taken together, our results demonstrate the role of hyperglycemic conditions and chronic inflammation to promote the disruption of typical esophageal epithelial architecture and transition to BE and EAC, while metformin may serve as a useful pharmacological agent to decrease oxidative stress and hinder this progression.

The Involvement of STAT Signaling Downstream from the Neurokinin 3 Receptor in GnRH Neurons

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Gonadotropin-releasing hormone (GnRH) neurons are at the apex of the hypothalamic-pituitary-gonadal (HPG) axis and the main regulators of fertility and reproduction. GnRH neurons are regulated by neuronal pathways involved in energy homeostasis and metabolism. Interestingly, infertility is often associated with diabetes and metabolic syndrome. Thus, the investigation of the pathways and neuropeptides that regulate GnRH neuron secretion can offer deeper insight into the mode in which metabolism affects reproduction.
Neurokinin B is a neuropeptide that has been shown to regulate GnRH secretion. Mutations in the genes that encode neurokinin B and its receptor, NK3R, were identified in humans with absent puberty and infertility. Recently, neurokinin B was shown to directly regulate GnRH neuron secretion; however, the cellular pathways NK3R signals are currently unknown. Previously, treatment of GT1-7 cells, an immortalized GnRH neuronal cell line, with senktide, a NK3R agonist, was shown to induce c-fos gene expression by activation of protein kinase C. Truncation analysis and cis-mutations identified the SIE within the -400 to -200 bp upstream of the transcription start site in the c-fos promoter as required for this induction. STAT1 and STAT3 transcription factors have been shown to bind to the SIE site in other systems, suggesting NK3R signaling may activate of the JAK-STAT pathway. Here, we employ transient transfections and electrophoretic mobility shift assays (EMSA) to test the hypothesis that STAT1 and STAT3 are activated downstream from NK3R. We find that suppression of STAT signaling through the overexpression of suppressor of cytokine signaling 3 (SOCS3) results in a repression of c-fos promoter activity. Preliminary data from EMSA suggest that STAT3 binding to the SIE may also change in response to the activation of PKC. These results suggest STAT signaling may be a downstream target of NK3R.

Antisense oligonucleotides to 24-dehydrocholesterol reductase (DHCR24) potently reduce target gene and protein expression in atherosclerosis-prone mice but exhibit LXR-dependent modulatory effects in a tissue-specific manner.

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Macrophage foam cells play an integral role in the initiation and progression of atherosclerosis by participating in a maladaptive inflammatory response. Recent lipidomic analysis suggested that the accumulation of desmosterol, a cholesterol biosynthesis pathway intermediate, within macrophage was responsible for suppression of proinflammatory responses through liver X receptor (LXR)-dependent pathways. We hypothesize that increases in cellular desmosterol using antisense oligonucleotides (ASO) targeting 24-dehydroxycholesterol reductase (DHCR24), the enzyme catalyzing the conversion of desmosterol to cholesterol, would effectively potentiate its anti-inflammatory and anti-atherogenic effects. To this end we performed twice weekly injections of control (Scr) or DHCR24 ASO for three weeks in C57bl/6 and $LDLR^{-/-}$ mice fed either normal chow (NC) or high fat, high cholesterol (HFHC) diet. Serum, liver, skin, spleen and macrophages were harvested for analysis. The treatment regimens were well tolerated in all groups. Treatment with two separate DHCR24 ASOs significantly reduced liver DHCR24 mRNA levels (14% and 25% of Scr ASO) in NC fed mice. This was mirrored by a concomitant decrease in DHCR24 protein levels. Previous studies indicate a comparable decrease in DHCR24 corresponded to >7-fold increases in circulating and hepatic desmosterol concentrations. However, treatment with DHCR24 ASO failed to induce classic LXR-responsive genes (ABCA1, ABCG1) in the liver. Cholesterol levels in wild-type mice fed a HFHC diet for 3 weeks showed a modest increase compared to NC fed mice (34.8 ± 3.3 mg/dL NC; 47.8 ± 5.9 mg/dL HFHC). Although DHCR24 mRNA levels showed similar reduction following ASO treatment as did NC-fed mice, expression of LXR-responsive genes remained unchanged. It has been suggested that a hypercholesterolemic state may be necessary for desmosterol to act as a potent LXR ligand. $LDLR^{-/-}$ mice on HFHC diet for 3 weeks demonstrated profound hypercholesterolemia, serum cholesterol 528.8 ± 55.1 mg/dL. However, despite reduced liver DHCR24 mRNA (9% of Scr ASO), LXR-responsive genes again showed no modulation. Remarkably, thioglycollate-elicited macrophages in $LDLR^{-/-}$ animals treated with DHCR24 ASO (5% of Scr ASO in macrophage) demonstrated a corresponding induction in LXR-responsive genes (ABCA1: 1.6-fold, ABCG1: 2.2-fold of Scr ASO) with concomitant suppression of SREBP-dependent pathways (FAS: 50% of Scr ASO). Our results suggest desmosterol acts in a tissue-specific manner, primarily exerting its anti-inflammatory and anti-atherogenic effects in lipid-laden macrophages. Furthermore, these observations establish mechanistic insights for future investigation into DHCR24 inhibition as a potential anti-atherosclerotic therapy.

Diabetes and mental health traits

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'Diabesity', obesity-dependent type 2 diabetes, is not merely a metabolic disease; it may be comorbid with some mental health disorders. Thus, metabolic and mental stress may mechanistically impact each other. To test which biological markers of diabesity best predict mental health traits, we performed several correlation analyses in a cohort of 62 subjects with diabetes and obesity, who have been involved in the Insulin Detemir in Obesity Management (IDIOM) study. We used visceral adipose tissue (VAT) and VAT normalized by soft tissue (VAT/ST) to quantify abdominal adiposity; and Homeostasis Model Assessment (HOMA), Quantitative Insulin-sensitivity Check Index (QUICKI), and C-peptide to assess insulin resistance. Several psychological tests were also administered including Barratt Impulsiveness Scale (BIS-11); Binge Eating Scale (BES); Eating Attitudes Test (EAT-26); and Three Factor Eating Questionnaire (TFEQ). Our results show a positive correlation between: VAT/ST percentage and BIS-11 Total Score (R^2=0.123; p≤0.039); and C-peptide and BES Total Score (R^2=0.117; ρ≤0.021). The data also showed a negative correlation between: VAT/ST percentage and EAT-26 'Dieting' (R^2=0.151; ρ≤0.023); VAT and TFEQ Cognitive Restraint of Eating (R^2=0.232; ρ≤0.004); and QUICKI and TFEQ Disinhibition (R^2=0.167; ρ≤0.006). In our cohort the data suggest that as visceral adiposity increases, behavioral control decreases and as insulin sensitivity decreases, self-awareness decreases. If common mechanisms of impairment for metabolic and neuropsychiatric diseases are identified, we can develop a more holistic approach toward these patients, and identify predictive biomarkers and pharmacological targets effective in the clinical management of both conditions.

Visuomotor performance in *KCNJ11*-related monogenic diabetes varies by mutation type and age of sulfonylurea treatment

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The *KCNJ11* gene encodes the Kir6.2 subunit of the ATP-sensitive potassium (K_{ATP}) channel found on the surface of beta cells and neurons. Mutations of *KCNJ11* may result in neonatal diabetes mellitus (NDM) in isolation or NDM with associated neurodevelopmental delay. Individuals with *KCNJ11* mutations may be safely transitioned from insulin injections to oral sulfonylurea (SU) therapy but the effect of this treatment on neurodevelopment is poorly understood.

We aimed to identify any visuomotor differences between individuals based on *KCNJ11* mutation type, age of sulfonylurea transition, and age of diabetes diagnosis. 34 individuals were assessed using Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI) and the supplemental Visual Perception and Motor Coordination tests. We divided patients into 3 groups based on mutation type. Group A, 9 patients with Val59Met/Ala mutations with a distinct phenotype with clear evidence of speech, motor, and cognitive dysfunction; Group B, 13 patients with any *KCNJ11* mutation other than Val59Met/Ala; Group C, 12 unaffected sibling controls. Subjects ranged from 2.5 to 21 years and were evaluated at the 2010 and 2013 Monogenic Diabetes Family forums in Chicago.

A correlation was noted between age of SU initiation and VMI scores in both groups A and B ($r = -0.8625$ and -0.5785, $p<0.05$ for both). VMI scores were lowest in group A ($p<0.05$) but no difference was seen between group B and the control group C. The age of diabetes diagnosis did not correlate with VMI scores in either group A or B.

In preliminary analysis, neurodevelopmental dysfunction in *KCNJ11*-related diabetes varies according to mutation type and age of sulfonylurea initiation. Our data suggests that early transition to SU therapy may have significant impact on neurodevelopmental outcomes in children with *KCNJ11* mutations.

Characterizing bone marrow-derived macrophages of *Ctnnb1*$^{fl/fl}$ *LysM cre* mice

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Macrophages play a significant role in maintaining tissue homeostasis as well as producing robust immunity against pathogens. However, intracellular signaling pathways mediating these responses are not fully elucidated. Recent studies have shown β-catenin to limit an inflammatory response in intestinal dendritic cells by signaling a tolerogenic state. To test the hypothesis that Wnt signaling is critical for macrophage function, we inactivated the expression of β-catenin by breeding *LysM-Cre* with *Ctnnb1-loxP* mice. PCR genotyping confirmed the presence of the Cre gene in the mutant. Bone marrow monocytes were isolated and cultured in M-CSF to induce differentiation to macrophages. Cells were stimulated in varying concentrations of LPS to induce cytokine production. The results of an IL-6 ELISA assay suggested that β-catenin$^{m_\phi-/-}$ expressed less inflammatory cytokines than β-catenin$^{fl/fl}$. On the other hand, qPCR results suggested a higher expression of both anti-inflammatory cytokines and inflammatory cytokines in β-catenin$^{m_\phi-/-}$. These conflicting data suggests that Wnt signaling may not have a critical role in macrophage function. However, further investigation is necessary to obtain conclusive results.

Glucose tolerance in adults with post-traumatic stress disorder.

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In patients with post-traumatic stress disorder, the odds of developing Type 2 diabetes mellitus is 2-3-fold higher than in patients with other psychiatric disorders. However, the mechanisms leading to this increased risk are unclear. Possible factors include increased insulin resistance, disrupted cortisol levels due to hyperarousal, disturbed sleep, and abnormal levels of adipocytokines (such as adiponectin). We analyzed data from a case-control study to test the hypothesis that individuals with PTSD (who are otherwise healthy) have increased insulin resistance, compared to healthy controls. Secondarily, we assessed whether differences in cortisol levels, adiponectin levels, nightly sleep duration, or slow wave sleep might mediate these effects. A total of 93 subjects matched for gender and age were enrolled and underwent the following assessments: 75-gram oral glucose tolerance test to determine glucose tolerance and insulin resistance, in-home actigraphy to quantify nightly sleep duration, overnight polysomnography to assess slow wave sleep, and overnight blood sampling in a clinical research center to assess cortisol secretion and adiponectin levels. Subjects with PTSD (n=44), compared to controls (n=49) had a significantly higher HOMA-IR (homeostasis model assessment of insulin resistance) score (median [IQR]; 2.5 [1.6-3.6] in PTSD vs. 2.0 [1.6-2.5] in control, p=0.03), 0 minute insulin level (9.8 [7.1-14.1] vs 8.9 [7.4-10.5], p=0.05), 120 min insulin level (27.2 [18.6-56.1] vs 24.1 [15.7-36.3], p=0.02), and insulin AUC (88.2 [60.9-140] vs 68.2 [44.6-87.8], p=0.006). After adjusting for potential confounders, including age, BMI, gender, and smoking, subjects with PTSD had higher levels of insulin 120 min (by 44% compared to controls, 95% CI= 3.3%-102%, p=0.03) and insulin AUC (by 29% compared to controls, 95% CI= 2.7%-63%, p=0.03). Due to significant interaction between gender and group (p=0.005), fasting glucose level was further stratified by gender. Fasting glucose levels in men with PTSD were 9.7% higher (95% CI=0.3%-20%, p=0.04) than in male controls. There was no significant difference in overnight cortisol AUC, high molecular weight adiponectin levels, or nightly sleep duration between the two groups. Although slow wave sleep time was significantly different between the 2 groups (mean +/- SE; 41 minutes +/- 5 in PTSD vs 58 +/- 4 in control, p=0.01) adjustment of the multivariable model for SWS did not alter our findings. In summary, we found that subjects with PTSD, compared to healthy controls, have an abnormal insulin response to a glucose challenge. Furthermore, individuals with PTSD, especially males, have higher fasting glucose levels than healthy controls, after adjusting for potential confounders. These differences in glucose and insulin metabolism are not mediated by adiponectin, overnight cortisol secretion, nightly sleep duration, or slow wave sleep.

Survey Design for "Local Patients, Local Stories:" Using Local Patients' Stories and Community Resources for Healthcare Provider Cultural Competency Training

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Cultural competency training has been suggested by the Institute of Medicine as a way to help eliminate health disparities. Although they have been shown to increase healthcare providers' overall knowledge and confidence regarding cultural beliefs of minority groups, cultural competency training programs have not had success in improving patient health outcomes. We believe that by providing healthcare providers with a cultural competency training program that focuses on the providers' local patient populations and their patients' unique barriers to care, highlights success patients have in overcoming barriers to care, informs them on local resources and community assets and increases self-awareness of personal racial biases, then it will consequently increase providers' awareness of the Latino culture and improve the care they provide their Latino patients with diabetes. There are no well studied, validated scales to assess provider cultural competency for the measures we desired. We conducted a literature review and found five scales that were studied, reviewed and had adequate internal consistency. We then developed a survey tool to measure provider's knowledge of the community, attitudes, behaviors and skills, Latino cultural beliefs and effectiveness in caring for Latino patients. The survey was pilot tested with 18 members of the Biological Sciences Division to assess if each question evaluates one of the desired measures and for clarity and readability. Feedback was used to create a 30-item questionnaire. It will be administered to 40 local healthcare providers prior to, immediately after and 3-months after the hour-long pilot training program. We hope that the "Local Patients, Local Stories" training program will lead to changes in health outcomes of patients and serve as a model for future cultural competency trainings.

AN INVESTIGATION OF ARTERIAL STIFFNESS AS A CARDIOVASCULAR RISK FACTOR IN KIDNEY TRANSPLANT RECIPIENTS

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Kidney transplant recipients have a higher mortality rate than population controls predominantly due to increased risk of cardiovascular disease (CVD). This increased risk stems mainly from exacerbated traditional and non-traditional risk factors. Immunosuppressive drugs, especially calcineurin inhibitors, may worsen non-traditional atherosclerotic risk factors and contribute to arterial stiffness. We hypothesized that new kidney transplant recipients would have increased arterial stiffness 12 months post-transplant compared to baseline and that phase angle (PA), a marker of generalized morbidity, would increase and inversely correlate with the degree of arterial stiffness. We prospectively studied arterial stiffness in 64 kidney transplant patients using pulse wave velocity (PWV) and assessed PA using bioelectrical impedance analysis (BIA) at baseline and 12 months post-transplant. Change in PWV score and change in PA over 12 months were assessed using the Wilcoxon signed-rank test. Correlation between PWV and PA was estimated with the Spearman's rank correlation test. Multivariable linear regression was used to assess the effect of change in creatinine (kidney function) on PWV at month 12 with adjustment of covariates using a propensity score.
No change in PWV score was detected between baseline and 12 months (9.5 ± 3.1 and 9.5 ± 2.9, respectively), however there was a significant increase in PA detected between baseline and 12 months (6.4 ± 1.7 and 8 ± 2.1 respectively, $P= 7.51^{-6}$). On univariable analysis, change in creatinine was not significantly associated with PWV at month 12. PWV score at month 1 (Rho= 0.9, $P= 7.74 \times 10^{-19}$), age (Rho= 0.6, $P= 3.19 \times 10^{-7}$), CVD history pre-transplant, diabetes pre-transplant, and hyperlipidemia were significantly associated with a higher PWV score at month 12. Pre-transplant diabetics (vs. non diabetics) had a significant increase in the ΔPWV score between month 1 and 12 (0.36 ± 1.93 and -0.22 ± 1.03 respectively, P=0.03). On multivariable analysis using a propensity score, the only significant predictor of PWV score at month 12 was PWV score at month 1 ($p < 0.0001$). PA at month 1 negatively correlated with PWV score at month 1 (Rho= -0.3, P= 0.02), but PA at month 12 did not correlate with PWV at month 12. PWV score did not worsen over a 12 month period possibly due to the short follow-up period, however this cohort will be followed over 36 months, and we believe our predicted changes will be seen at that time. PA did significantly increase, indicating improved health among our transplanted cohort. PA did negatively correlate with PWV at month 1 but not 12, suggesting that PA does not reliably reflect arterial cardiovascular health in kidney transplant recipients.

STARS Overexpression in Cultured Myocytes Induces Alterations in Downstream Insulin Receptor Signaling that Correspond to Insulin Resistance.

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Insulin resistance precedes the onset of Type 2 Diabetes (T2D) by many years, supporting the idea that early detection of insulin resistance may be an important method for improving diabetes prevention and management that has yet to be utilized efficiently and routinely in clinical care. **St**riated muscle **A**ctivator of **R**ho **S**ignaling (STARS), an activator of MKL1-dependent SRF pathway genes, has been shown to be upregulated not only in those with T2D, but also in insulin-resistant patients with a family history of T2D. In addition, inhibition of SRF induces insulin sensitivity in mice. Therefore, better understanding of the STARS-SRF pathway and its implications in insulin signaling may support it as a means of early resistance detection and may bring to light potential therapeutic targets. We proposed that overexpressing STARS in myocytes would induce characteristics of insulin resistance in regards to protein and gene expression downstream of the insulin receptor. Myocytes of the rat L6 line were treated with insulin (100 nM) for multiple incubation periods and stages of myocyte differentiation. RNA and protein were extracted after treatment and analyzed via qRT-PCR and western blot analysis. STARS gene expression was increased both with insulin treatment and with cell differentiation. Notably, Egr1, an MKL1-independent downstream SRF immediate expression gene, was also more highly induced with insulin treatment in STARS-overexpressing cells, which may imply a compensatory mechanism outside of the STARS-SRF-MKL1 pathway that responds to the proposed STARS-induced insulin resistance. Although genes involved in fatty acid and mitochondrial oxidative metabolism often have implications in metabolic disorders, a significant change in MLYCD or PGC1 gene expression was not observed in STARS-overexpressing cells. However, phosphorylation patterns of key regulatory proteins in insulin receptor signaling were significantly altered in these cells. In particular, data suggest an overall decrease in induction of proteins involved in active insulin signaling (P-Akt and P-ERK1/2) and decreased insulin induction, yet increased basal activity, of mTor signaling (measured by P-S6) in STARS-overexpressing myocytes. Indeed, decreased phosphorylation of Akt and ERK1/2 in response to insulin treatment suggests that STARS-overexpressing cells exhibit a degree of insulin resistance. Furthermore, increased basal levels and decreased insulin induction of mTor signaling in STARS-overexpressing cells is consistent with observations in patients with T2D and metabolic dysfunction, and thus reveals important connections with STARS function and pathogenesis.

Retinoic Acid Signaling in Ischemia-Reperfusion Acute Kidney Injury (I/R-AKI)

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Acute kidney injury (AKI) is a rapid decrease in kidney functions and a risk factor for end stage renal disease (ESRD). Injured cells can de-differentiate, re-differentiate, and re-populate damaged regions in the kidney post-injury. Retinoic acid (RA) signaling plays an essential role in cellular de-differentiation and proliferation of appendages and organs after injury in zebrafish and amphibians. Previous research from our lab has shown that targets of RA signaling are up-regulated post-AKI and mice treated with RA inhibitor BMS493 have decreased proximal tubular epithelial cell (PTEC) proliferation and increased fibrosis. We also showed that retinoic acid response element positive (RARE+) cells are localized in the outer medulla. We hypothesize that RARE+ cells are proliferating and de-differentiating PTECs. We used RAREhsp68lacZ transgenic mice (which produce the gene product β-galactosidase) to detect the expression of RA signal activation via immunohistochemistry by using β-galactosidase antibodies. The injury model used for the reporter mice was unilateral I/R-AKI with 26 minutes of ischemia time. Paraffin-embedded tissue sections from uninjured and day 3 I/R-AKI mice were co-stained with β-galactosidase antibodies and various markers (DBA, PCNA, LTA, and Kim1) to determine the localization and proliferation of RARE+ cells after I/R-AKI. From epifluorescence microscopy, we found that RARE+ cells are DBA+ collecting duct epithelia in the uninjured and injured kidney. Some RARE+ cells are LTA+ or Kim1+ PTECs after injury and some RARE+ cells express the S phase proliferation marker PCNA, but the majority of PCNA+ cells are RARE-. The data suggest that there is induction of RARE+ cells in a subset of PTECs after I/R-AKI. However, the relationship of RARE+ cells with cell proliferation and de-differentiation remains to be established.

Exercised Rat Plasma Improves the Survivability of Mesenchymal Stem Cells Even Under Metabolic Stress

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Heart disease and subsequent myocardial infarction are major problems affecting the health of Americans today, particularly Americans with diabetes. A possible cardiac therapy being pursued now is using stem cells to regenerate cardiomyocytes where there is necrosis. Before the stem cells can cause cardiomyocyte differentiation and proliferation, they must first be able to survive in the given environment. We hypothesized that if mesenchymal stem cells (MSC's) are grown under conditions mimicking different metabolic states it will affect their proliferation and survivability, which would be relevant for treating diabetic patients who suffered heart attacks. In order to test this hypothesis, MSCs were cultured with different combinations of heat-inactivated plasma from exercised rats, heat-inactivated plasma from sedentary rats, various concentrations of metformin, and various concentrations of insulin. The proliferation of the MSCs was then measured by doing a BrdU Assay, and the viability and cytotoxicity were measured by doing a MultiTox Assay. The MSCs had significantly greater survivability (viability/cytotoxicity ratio) when cultured in exercised plasma (3.8 $\pm$ 0.2) versus sedentary plasma (2.3 $\pm$ 0.2). When cells initially treated with exercised plasma were switched to sedentary plasma, the sedentary plasma knocked down the survivability (2.1 $\pm$ 0.2) mitigating any protective effects from the exercised plasma. In addition, when cells initially treated with sedentary plasma were switched to exercised plasma, the exercised plasma was unable to rescue the viability of the MSCs (2.3 $\pm$ 0.3). When metformin, an AMP kinase activator, was added to the cultures, cells treated with exercised plasma had significantly greater survivability and proliferation than cells treated with sedentary plasma at all concentrations of metformin. Although exercised plasma was protective, this protective effect decreased with increasing metformin concentrations. When insulin was added to the cultures, there was no clear trend or difference between cells treated with exercised or sedentary plasma. These results suggest that exercised plasma is more conducive to a greater MSC survivability than sedentary plasma. Also, although metformin kills MSCs, exercised plasma decreases the amount to which the MSCs are killed by metformin. This information could be beneficial for using MSCs in the future to better treat patients with different metabolic conditions.

ApoE variants act via different mechanisms to regulate ER stress in macrophages.

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The development of atherosclerotic plaques is largely determined by the inability of macrophages to efflux excess cholesterol resulting from unregulated uptake of oxidized LDL, causing the formation of foam cells that have increased susceptibility to apoptosis, as well as immune system activation. Apolipoprotein E (apoE) and low-density lipoprotein receptor-related protein 1 (LRP1) are critical for preventing atherosclerosis, acting independently and cooperatively. ApoE serves to prevent atherosclerosis by promoting cellular cholesterol efflux, and in the atheroma apoE is primarily produced by macrophages due to cholesterol loading. There are three common polymorphisms of apoE in the population: apoE2, apoE3, and apoE4. They are associated with different risks for coronary heart disease. Dysfunctional mutants of apoE, such as $apoE_{cys142}$, are known to cause accelerated atherosclerosis and severe hyperlipidemia. The goal of this research was to focus on the apoE variants in macrophages to determine the differences in susceptibility to ER stress death. To do this, peritoneal macrophages were isolated from $apoE^{-/-}$ mice as well as $apoE^{-/-}LRP1^{-/-}$ DKO mice and incubated for 40 hours with concentrated virus at a MOI of 30 to express the different apoE variants. The macrophages were then treated for 24 hours with 10 μg/ml tunicamycin and TUNEL stained to determine the levels of apoptosis. The findings showed significant decreases in apoptosis of macrophages expressing apoE2 and apoE3 compared to the $apoE^{-/-}$ macrophages, but macrophages expressing apoE4 and $apoE_{cys142}$ showed a significant increase in apoptosis. When compared to the $apoE^{-/-}LRP1^{-/-}$ DKO macrophages, $ApoE_{cys142}$ showed significantly increased apoptosis in the absence of LRP1. The remaining apoE variants with $LRP1^{-/-}$ background did not show significant changes compared to the double knockout macrophages, but apoE3 showed slightly less apoptosis. These results suggest that apoE2 protects against macrophage apoptosis via interaction with LRP1, and apoE3 exerts partial protective effects against macrophage apoptosis independent of LRP1. ApoE4 exerts negative effects against macrophage apoptosis in the presence of LRP1 suggesting altered signaling via LRP1/apoE4 interaction compared to apoE2- and apoE3- mediated signaling events. Finally, $apoE_{cys142}$ exerts negative effects against macrophage apoptosis independent of LRP1. Taken together, the findings of this study suggest that the apoE variants act via different mechanisms, dependent and independent of LRP1, to regulate ER stress in macrophages.

A High Throughput Thallium Screen Identifies PIK3CB and PRKCZ as Regulators of Pancreatic Islet TALK-1 Channel Activity

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According to the World Health Organization, there are 347 million people worldwide who have diabetes. Patients with diabetes have impaired insulin secretion and reduced insulin sensitivity leading to disruption of glucose homeostasis. Ca^{2+} influx through voltage dependent Ca^{2+} channels is required for insulin secretion. Thus, channels that modulate the islet cell membrane potential and Ca^{2+} influx are important in maintaining glucose homeostasis. While the potassium channel, TALK-1, is only expressed in pancreatic islet cells and polymorphisms in the KCNK16 gene that encodes TALK-1 are associated with a predisposition for developing Type II Diabetes (TIID), the role of TALK-1 in islet cell function remains undetermined. Therefore, our project focused on determining how kinase regulation of TALK-1 channel activity may influence islet cell Ca^{2+} influx and ultimately hormone secretion. Utilizing a high throughput thallium screen on stable HEK cell lines expressing the TALK-1 channel we monitored TALK-1 channel activity. In an initial kinome screen, these cell lines were transfected with a library of 192 plasmids that express activated kinases. The initial thallium flux screen identified a group of TALK-1 channel activators (including ATM kinase, TSSK6, PI4K2B, and PIK3CB) as well as a group of TALK-1 channel inhibitors (including CSNK1E, PIP5K3, PIP5K1A, TK1, TESK1, PRKCZ, EPHA4, MOBK2LA). We then performed a secondary thallium screen to confirm TALK-1 channel regulation by this initial subset of kinases, which found that phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3CB) and protein kinase C-zeta (PRKCZ) activate and inhibit the channel, respectively. Interestingly, PRKCZ has been shown to stimulate insulin secretion and PIK3CB has been shown to inhibit insulin as well as glucagon secretion. This indicates that these kinases may regulate insulin secretion via TALK-1. In particular, PIK3CB plays an important role in the insulin signaling cascade. PIK3CB converts phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol(3,4,5) triphosphate (PIP3). A preliminary calcium flux assay on pancreatic islet cells indicated that increasing levels of PIP3 with PTEN and SHIP2 inhibition of phosphatases that degrade PIP3 presumably activates TALK-1, reducing calcium influx. This data suggests that the TALK-1 channel may play an important role in insulin regulation of islet hormone secretion via modulation of Ca^{2+} influx. Understanding how TALK-1 channels modulate islet hormone secretion could ultimately provide insights into potential therapeutic targets that can be utilized for treating TIID.

The Effects of Pirenzepine on Nerve Dysfunction in Type 1 Diabetic Rats

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There were an estimated 347 million diabetics worldwide in 2008 and the prevalence continues to increase. Peripheral neuropathy afflicts over half of all diabetic patients and manifests as sensory loss, pain and autonomic dysfunction. Pirenzepine (PZ) is a selective M1 antagonist used to treat peptic ulcers. We hypothesized that the anticholinergic activity of pirenzepine may slow the development of diabetic peripheral neuropathy. Nineteen rats (n=19) were divided into 3 groups: control (n=8), diabetic (n=6), and PZ treated diabetic (n=5). Rats in the PZ treated diabetic group were treated 5 times weekly with a subcutaneous injection of pirenzepine at a dosage of 5 mg/kg of body mass. Sensory functions and nerve conduction velocity (NCV) were measured at weeks 0, 4 and 8 weeks of treatment. Diabetic rats maintained persistent hyperglycemia and progressive weight loss. All rats maintained healthy motor function. Diabetic rats showed tactile allodynia, thermal hyperalgesia, and reduced motor and sensory NCV. Pirenzepine treatment attenuated paw thermal hyperalgesia and significantly reduced NCV slowing in diabetic rats. Pirenzepine corrects aspects of diabetic neuropathy without altering the underlying diabetes.

Utility of advanced MRI techniques to assess diabetic nephropathy

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Diabetic nephropathy (DN) is the most common cause of kidney failure and end-stage renal disease in the United States. Leptin receptor deficient mice (db/db) serve as an animal model of type II diabetes that develop classical pathological lesions seen in DN. Furthermore, recent studies on eNOS knockout mice (eNOS-/-) have shown these mice to display glomerular defects resembling DN. This study implemented three MRI techniques – CEST, BOLD, and ΔR2 – to determine whether these imaging methods could be effectively used to detect hemodynamic and metabolic changes seen during the development and progression of DN. A 7 Tesla MRI system and iron nanoparticle contrast agent were used to acquire renal blood volume (RBV) and renal oxygenation in one 20-week db/m, three 8-week db/db, three 20-week db/db, and two 20-week db/db eNOS-/- mice. CEST imaging was performed using a 5 sec irradiation pulse with RF offsets from -1500 to 1500 Hz. Our CEST data showed an increase in glucose in the inner medulla (IM) and papilla (P) of 8 week old db/db compared to 20 week old db/db mice. In addition, glycogen levels appeared elevated in the outer medulla (OM) of db/db mice at 8 weeks and then decreased significantly by 20 weeks. BOLD MRI showed an increase in renal cortical R2* in db/db and db/db eNOS-/- mice, suggesting decreased cortical oxygen levels in these kidneys. Minimal differences in RBV were noted. These MRI data suggest that CEST and BOLD imaging may be used as a potential diagnostic tool for DN as well as a method to track the progression of the disease.

Embryonic pituitary somatotroph development in a germline zebrafish AIP mutant generated by customized transcription activator-like effector nucleases (TALENs)

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Aryl hydrocarbon receptor interacting protein (AIP) interacts with and modulates transcriptional activity of the aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor, which mediates xenobiotic detoxification of endocrine disrupting chemicals (EDCs) including dioxins. *AIP* heterozygous mutations have been found in 20-40% of Familial Isolated Pituitary Adenoma (FIPA) families. Patients with germline AIP mutations typically have early onset growth hormone (GH)- and/or prolactin (PRL)-producing adenomas, with 20-30% familial penetrance. However, AIP is very rarely if ever mutated in sporadic pituitary tumors and it is unclear how disrupted AIP leads to pituitary tumor formation. We hypothesize that embryonic AIP gene disruption may result in altered development of the pituitary somatotroph lineage. To test this, we investigated the role of AhR/AIP signaling pathway in pituitary development and tumor formation by generating germline zebrafish with a nonsense mutation within *zAIP* exon 4, using customized transcription activator-like effector nucleases (TALENs). The goal of the current study was to perform *in vivo* analysis of the pituitary somatotroph lineage in live *zAIP*$^{+/-}$ embryos/larvae. First, genotype of F1 *zAIP*$^{+/-}$ fish was confirmed by tail DNA PCR amplification followed by DNA sequence analysis. Adult *zAIP*$^{+/-}$ zebrafish were then crossed with a transgenic zebrafish reporter line that specifically labels the pituitary GH-expressing somatotroph lineage with red fluorescent protein (RFP). Live transparent embryos/larvae enabled *in vivo* analysis of pituitary somatotrophs under the microscope. We measured pituitary RFP expression in zAIP$^{+/-}$/GH-RFP and GH-RFP siblings at 5 days post fertilization (dpf). zAIP$^{+/-}$/GH-RFP larvae demonstrated decreased pituitary RFP expression compared to GH-RFP siblings at 5 dpf. We conclude that embryonic AIP disruption lead to early pituitary somatotroph down-regulation. As differentiated embryonic and adult pituitary hormonal cell development shares common regulatory pathways, our study sheds light on the potential role of AIP in pituitary tumorigenesis.

Can Univariate, Isocaloric Modulation of Dietary Components Improve Energy Balance in Pre-Existing Obesity?

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Some dietary components, such as fiber and unsaturated fatty acids, have been associated with reduced weight gain and improved metabolic function. The mechanisms by which these salutary effects are achieved remain unclear but accumulating evidence suggests that the chemical content of food is more important than its caloric load with respect to the regulation of energy balance and metabolic function. These observations suggest that manipulation of specific nutrients could influence energy balance and metabolic regulation and thereby provide effective therapy for obesity and metabolic disease. Roux-en-Y gastric bypass (RYGB) causes rapid and dramatic improvement of the disordered metabolism and energy balance associated with obesity. RYGB also causes marked and sustained changes in gut microbial ecology with increased abundance of several specific bacterial genera, including *Akkermansia*. Induction of increased abundance of *Akkermansia* in the gut microbiota, in the absence of any surgical intervention, can lead to improved metabolic function and energy balance. Administration of a fiber-derived oligofructose prebiotic has been shown to selectively increase the abundance of *Akkermansia* and improve glucose homeostasis. In preliminary studies, other fiber-derived prebiotics, such as arabinoxylan oligosaccharides (AXOS) have also been shown to improve metabolic function and energy balance in murine models. Studies in mice have also shown that supplementation with n-3 polyunsaturated fats (PUFAs) significantly reduces diet-induced weight gain and prevents hypertrophy and inflammation of adipose tissue. However, the methods employed for these studies have not strictly controlled for dietary energy content and density. Moreover, the effect of these dietary supplements on body weight loss in animals with pre-existing obesity is not known. Therefore, we propose a study to determine whether modulation of dietary components, independent of total caloric content or density, influences metabolic function and energy balance in mice with pre-existing obesity. Identifying the differential effects of specific dietary nutrients could facilitate the search for food-based therapy for obesity and metabolic disorders. Regardless of the outcomes of this experiment, the experimental models developed should be useful in the future for examining the effects of a broad array of specific dietary components on metabolic function in a more sensitive, rigorous, and comprehensive fashion. Identification of nutrient-based therapies from these efforts would provide novel options for substitution or complementation of established treatment modalities for obesity, diabetes, and other metabolic disorders.

Proposed Title: Longitudinal Study of the Association between Diabetes and Arterial Stiffening: San Diego Population Study (SDPS)

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Previous studies conducted in a group of vascular laboratory patients being evaluated for peripheral artery disease (PAD) determined that arterial stiffening below the knee can mask the detection of PAD by artifactually increasing the measured ankle systolic blood pressure and thus increasing the ankle-brachial index (ABI). We sought to determine how much arterial stiffening occurred within the San Diego Population Study cohort when exposed to diabetes—one of the strongest risk factors for the development of PAD but also linked to peripheral arterial stiffening. We hypothesized that within the diabetic population more stiffening would occur overall and thus a higher proportion would be represented in the stiff artery cohort; however, within the group without arterial stiffening diabetics would show atherosclerosis progression by a mean decrease in the ABI. The study cohort consisted of 1103 individuals seen at baseline, who returned for a follow up visit approximately 11 years later. These individuals were analyzed by lower limb for diabetes status at follow-up (yes/no), ABI change (follow-up ABI – baseline ABI), arterial stiffening (ABI change ≥ 0.20, or not). A toe-brachial index (TBI), typically unaffected by arterial stiffening, was conducted at follow up in participants presenting with an ABI ≥1.3. T-tests were conducted for continuous variables and chi-square tests for categorical variables; regression was used to evaluate the association of diabetic status and either the mean ∆ABI or mean TBI. Overall, diabetics had a larger increase in mean ABI than non-diabetics both unadjusted (0.055 vs. 0.0297, p = 0.046) and after adjustment for age, sex, and ethnicity (0.030 vs. 0.056, p =0.043). Within the non-stiffened cohort the means were suggestive of diabetics having a greater decrease in mean ABI (-0.009 vs. 0.0067, p=0.059). However, within the stiffened artery cohort, diabetics had a greater increase in their mean ABI than non-diabetics (0.654 vs. 0.446, p =0.025) and there were more diabetics with stiffened arteries than non-diabetics (9.7% vs 5.2%, p= 0.007). After adjusting for age, sex, and ethnicity, diabetics had a lower mean TBI (0.954 vs. 0.84, p= 0.031) at follow-up than non-diabetics. In summary, diabetics are more likely to develop stiff arteries than non-diabetics which can lead to a falsely high ABI; potentially masking the presence of PAD. Thus diabetics presenting with a normal ABI may have underlying PAD, both a coronary risk factor and known cause claudication. In such persons a TBI should be conducted.

Effect of prolonged Phosphodiesterase-5 Inhibition on Energy Metabolism in African American Women with Metabolic Syndrome. A pilot study.

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African American women have the highest prevalence of obesity in the US. Obesity results from an imbalance between caloric energy intake and energy expenditure. Resting Energy Expenditure (REE) accounts for about 60-80% of 24-hour energy expenditure, and it has been shown that on average, African American women have lower REE than other groups. Studies in an animal model of obesity showed that chronic treatment with a phosphodiesterase-5 (PDE-5) inhibitor, sildenafil citrate, increases energy expenditure and reduces weight gain. To test the hypothesis that chronic treatment with a PDE-5 inhibitor increases REE and activity level in humans, 46 obese African American women were randomized to 4-week treatment with either sildenafil citrate or placebo. REE and body composition were measured at the beginning and end of the study using indirect caloriometry and a Dual Energy X-Ray Absoprtiomety (DEXA) scan, respectively. Activity level was also assessed used an ActiGraph GT3X+ activity monitor worn on the wrist. Because the study is still ongoing, all data presented is still blinded. We found a direct relationship between Fat Free Mass (FFM) and REE such that increased FFM led to increased REE (n =28). Average REE both before (1697 Kcal/Day) and after (1695 Kcal/Day) treatment was approximately the same (n=28). We also analyzed subjects for activity level using minutes spent in low intensity activity, moderate intensity activity, and vigorous intensity activity as well as Physical Activity Counts/min of device wear time. There was a tendency for an increase in low intensity activity levels on weekdays after the intervention (n=19). Further analysis in the context of assigned study drug is required for any conclusions to be drawn.

Relationship between Body Fat Distribution and Metabolic Improvements after Gastric Bypass Surgery.

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Body fat distribution is an important determinant of health risks, and is dependent upon hereditary factors and hormones. Android obesity (central or “apple-shaped”), which includes visceral fat, is correlated to increased risk of cardiovascular disease and metabolic disturbances, whereas gynoid obesity (lower body or “pear-shaped”), is associated with lower disease risk and may even be protective. We hypothesized that an improved body fat distribution after RYGB would be associated with improvements in insulin sensitivity and metabolic hormone profiles. To test this hypothesis, we utilized data from our cohort of 42 RYGB subjects (all females) collected prospectively up to 2 years after RYGB. Their android and gynoid fat measurements were compared to their metabolic counts via graphical and statistical analysis. Results showed that a decrease in AGR has a significant effect on improvements in hepatic and peripheral insulin sensitivity and a decrease in leptin levels after RYGB in addition to weight loss. Increase in adiponectin after RYGB was associated with a decrease in AGR but was not associated with weight loss. Body fat distribution before RYGB had no effect on changes in body weight, insulin sensitivity, or metabolic hormones after RYGB (all $p \geq 0.276$). Body fat distribution before RYGB had no effect on changes in body weight, insulin sensitivity, or metabolic hormones after RYGB (all $p \geq 0.276$). This data suggests that changes in AGR after RYGB are associated with changes in metabolic activity: improvements in insulin sensity and decrease in leptin levels.

Managing type 1 diabetes and preventing ketoacidosis during acute illness in children and young adults: identifying the population for a targeted intervention

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Optimal home management of type 1 diabetes (T1D) during acute illness often requires supplemental blood glucose (BG) and urine ketone testing as well as alterations in insulin administration, in order to prevent clinical deterioration and development of diabetic ketoacidosis (DKA). Despite the continuing education patients receive in clinic, the rate of admissions for diabetes-related emergencies remains unacceptably high. As part of an educational intervention for handling sick days in an attempt to decrease emergency department (ED) admissions, we collected demographic and clinical information to help identify patients who could most benefit from an intervention. We developed a short (12 questions) questionnaire to be distributed to patients 13 years and over and their parents (for all children under 18 years) during regularly scheduled clinic visits to assess baseline understanding of DKA (knowledge), the management of diabetes during acute illness (skills), and self-reported comfort and perceived knowledge and skills. Patients were then given a flow chart to bring home with an algorithm dependent upon BG and ketone levels and instructed in its use as part of the targeted DKA and sick day education. Questionnaires and demographic information were collected from 246 subjects (115 female and 131 male, age 13.9 ± 4.4 years, diabetes duration 6.2 ± 4.6 years), HbA1c 8.2 ± 1.6%. 35% of patients with a HbA1c of 10% or higher reported having been admitted to the ED in the last twelve months compared with 15% of those with a HbA1c between 8-9.9% and a mere 9% of the 6-7.9% group. A significant negative correlation ($r= 0.22$, $p=0.0009$) was found between self-reported comfort in managing diabetes during acute illness and HbA1c, with a marked decrease in comfort found in those with a HbA1c of 12% or higher. Though there was no significant difference between the mean combined knowledge and skill scores of patients who had been to the hospital (+ED) and those who had not (-ED) (+ED: 52.3%, -ED: 53.8%, $p=0.6$), there was a significant discrepancy between the parent scores in these two groups (+ED: 50.0%, -ED: 61.9%, $p=0.004$). In addition, there was decreased parent presence in the +ED group (+ED: 27 patients & 20 parents, -ED: 121 patients & 140 parents) despite similar group compositions. No significant correlations were found between the combined knowledge and skill scores and HbA1c, income, patient age, age at diagnosis, or duration of diabetes. Our results indicate that our intervention should target specific groups: those uncomfortable managing sick days, those who have high HbA1c levels, those who attend clinic without parents, and those patients requiring hospital visits for diabetes-related illness. We plan to re-administer the questionnaire 3 months after the initial intervention to assess its efficacy by seeing if patient scores and reported comfort with acute illness improve, and if reported ED visits decrease.

Characteristics of Living Kidney Donors and Factors Associated with Early Follow-Up

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Although the use of living kidney donors has increased dramatically over the past 20 years, there is still uncertainty regarding their long-term health outcomes, especially among medically complex living donors. The Organ Procurement and Transplant Network (OPTN) requires kidney transplant programs to submit living donor follow-up data through 2 years post-donation; however, this data is frequently unavailable with patients either not returning to the center for follow-up or not providing the center with follow-up information from local health-care facilities. We aimed to analyze follow-up rates and identify which variables could be used as statistical predictors of follow-up potential. After receiving IRB approval, a de-identified set of patient information was collected through retrospective chart review. Data were analyzed using chi-square, t-tests, and multivariate logistic regression models. The sample included 312 patients, of which 33% of patients eligible for a six-month follow-up visit did not return for follow-up, and this number increased to 50% at the one-year mark and 63% at the two-year mark ($p<0.001$). Older age, having a PCP, and being medically complex were associated with follow-up at month 6 (all univariate $p<0.05$) but not at years 1 or 2. While medical complexity cannot be changed, the transplant team should concentrate follow-up efforts on young donors and donors without PCPs. Possible steps forward include referring donors to PCPs and better educating younger donors on the importance of post-transplant follow-up.

Primary Care Provider, Peer Advisor, and Patient Reported Barriers to Improvement of Cardiovascular Health for Individuals Living in the Alabama Black Belt

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Despite advances in prevention, management, and treatment, cardiovascular disease (CVD) remains the leading cause of death in the United States, with Alabama among the states having the most alarming statistics. To help combat this disease, the American Heart Association created several recommendations for cardiovascular health improvement, known as “Life’s Simple 7”, involving keeping blood pressure, cholesterol, blood sugar, and weight in normal ranges, as well as lifestyle modifications including eating a healthy diet, exercising and not smoking. People who reside in Alabama’s rural Black Belt region are especially prone to poor health outcomes, including poor measures on Life’s Simple 7. Our semi-qualitative research studied the barriers to achieving Life’s Simple 7 in the Alabama Black Belt from the perspective of 3 stakeholder groups (primary care providers, peer advisors/community health workers and office staff, and patients) using the nominal group technique (NGT). The NGT is a form of information gathering used for focused problem identification, in which a facilitator solicits ideas from participants that are later added to and ranked. Results portrayed a high degree of agreement between nominal groups of each stakeholder. Peer advisors focused on barriers that they could specifically help patients with, whereas patients tended to focus on their own personal barriers. Physicians portrayed a more holistic understanding of barriers, citing both structural and personal barriers, but tended to rate all important barriers as being difficult to overcome, potentially suggesting burn-out and a degree of hopelessness in improving cardiovascular health in their patients. In contrast, the much lower scores, reflecting their perspective that barriers can be more easily overcome, in the peer advisor and patient groups suggest their receptiveness to overcoming barriers. Engaging stakeholders to provide perceptions prior to intervention development revealed important information that will be integrated

The insulin peptide, β(9:23), presented in MHC "register 3", may allow for escape of insulin-reactive T cells from thymic deletion.

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Type I diabetes (T1D) is an autoimmune disease characterized by the destruction of pancreatic islet cells primarily by $CD4^+$ and $CD8^+$ T cells that escape mechanisms of self-tolerance. During T cell development in the thymus, negative selection eliminates auto-reactive cells from the repertoire in a process known as central tolerance. This process requires the transcription factor, *autoimmune regulator* (AIRE), which promotes the expression of peripheral self-antigens within the thymus. Accordingly, *Aire*-deficient mice exhibit a breakdown in tolerance characterized by hyperactive T cells and autoimmunity in multiple organs including the pancreas. In humans with T1D and in the nonobese diabetic (NOD) mouse model of the disease, insulin is a major autoantigen detected by pathogenic T cells, and *Aire* controls insulin expression in the thymus.. It has been shown that these T cells recognize a peptide within the 9-23 region of the insulin β-chain. However, several possible MHC binding registers may be recognized. While register 1 and 2 contain more thermodynamically favorable interactions – between MHC, T cell receptor, and peptide – previous and ongoing studies have found most insulin-reactive T cell hybridomas to be register 3-specific. We therefore hypothesized that register 1 and 2-specific T cell populations are deleted in the thymus in an Aire-dependent process, while register 3-specific populations escape thymic deletion, due to low binding affinity. We found that peripheral T cells from a wild-type NOD mouse immunized with native insulin β(9:23) predominantly recognize a register 3-trapped peptide, rather than registers 1 or 2. Still, we have determined that several previously characterized insulin-specific hybridomas can also recognize registers 1 and 2. In fact, one specific 4F7 shows a strong preference for register 2 recognition. Future experiments will determine if Aire is important for the negative selection of T cells that recognize specific binding registers.

Safety net patients with diabetes experience less rapport building when providers demonstrate high computer use

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With the widespread integration of electronic health records (EHRs), the use of computers during patient visits has only become more common. Limited research on how computer use influences physician-patient communication has yielded both positive and negative effects. This study aims to determine the effects of computer use on rapport building in diverse safety-net settings. Utilizing video recordings of patient encounters in both primary and specialty settings, we conducted an observational study at a large U.S. public hospital with a basic EHR. Eligible patients included English-or Spanish-speaking adults (≥18 years old) with diabetes, rheumatoid arthritis, and/or congestive heart failure who receive primary and subspecialty care at five hospital clinics. We coded verbal communication behaviors using an adapted version of the Roter Interaction Analysis System (RIAS). The primary outcome was *rapport-building* statements (such as reassurance, concern, empathy, or partnership statements) by patients and providers. The primary predictor was high *concurrent computer use*, categorized as encounters in which >15% of total statements (provider and patient) had concurrent provider computer use. Analysts also rated *overall computer use* using a 3-item observation instrument (total possible score 0-9), categorizing high use as score ≥4. We performed regression analysis using generalized estimating equations (GEE) to account for clustering by providers, controlling for patient age and gender. This analysis focuses on encounters among patients with diabetes. To date, we have coded 15 encounters among 15 English-speaking patients and 14 providers. Among patients, 53% were women; 13% White, 20% Black/African-Americans, 13% Asian/Pacific Islander, 47% Latino/Hispanic, and 7% Multi-ethnic. In addition to diabetes, 27% have rheumatoid arthritis and 27% congestive heart failure. Although all encounters were in English, 29% prefer Spanish. Among providers, 64% were women; 64% PCPs, 14% diabetes nurse practitioners or endocrinologists, 14% cardiologists, and 7% rheumatologists. Patients were less likely to use any rapport building statements with providers who demonstrated high concurrent computer use (IRR=0.0.989; 95% CI=0.977-1.000). Specifically, both providers and patients were less likely to use emotional rapport building statements (IRR=0.968, 95% CI=0.941-0.996; IRR=0.950, 95% CI=0.907-0.996, respectively). In addition, high overall computer use was associated with less positive rapport-building by providers and patients (IRR=0.961, 95% CI=0.954-0.968; IRR=0.993, 95% CI=0.987-0.999, respectively). However, both providers (IRR=1.173, 95% CI=1.084-1.268) and patients (IRR=1.302, 95% CI=1.100-1.542) were more likely to offer personal remarks and social conversations during encounters with high overall computer use. In conclusion, preliminary analyses suggest that high computer use may be associated with less overall rapport-building, but personal conversations or “chit-chat” occur more frequently. EHR use may influence patient-provider conversations towards more biomedically-oriented agendas. Future analyses will examine the relationship between computer use and other communication outcomes, such as biomedical and psychosocial statements. Although EHRs are promoted as tools to improve efficiency and safety, it is crucial to gain a better understanding of the how computer use alters patient-provider relationships and communication.

Effects of Vitamin D Supplementation on Blood Pressure in Patients with Type 2 Diabetes Mellitus

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In the U.S., 25.8 million people suffer from Type 2 Diabetes Mellitus (T2DM), which is a major cause of heart disease, stroke, chronic renal failure, neuropathy and blindness. Hypertension affects approximately 70% of patients with T2DM, but blood pressure control reduces the risk of cardiovascular disease and diabetes complications by at least one third. An interventional study showed that supplementing vitamin D and calcium in elderly non-diabetic women with hypertension and vitamin D deficiency reduced blood pressure compared to the calcium-treated group. However, there have been no studies to assess the effects of vitamin D on hypertension in T2DM patients. We hypothesize that vitamin D supplementation over 4 months will reduce blood pressure in patients with T2DM and low vitamin D levels. To test this hypothesis we are conducting a randomized, double-blinded clinical trial of the effects of vitamin D (4000 IU/day) versus placebo over four months on blood pressure in 96 patients with T2DM, moderate hypertension, and low vitamin D levels. 81 patients have completed the study, with 2 currently enrolled. Of patients enrolled to date, there are approximately equal distributions of gender (48% male, 52% female) and race (50% Black, 45% White, and 5% other minorities). Sun exposure questionnaire reveals that in the summer, 43% of participants spend 1-2 hours and 47% of participants spend two or more hours in the sun, while in winter, the majority spend 0-1 hour in the sun. Most patients (70%) have a T2DM duration of <10 years with good disease control (mean A1c 6.9%), and metformin is the most commonly used medication. 62% have a concurrent diagnosis of hypertension and 25% a diagnosis of dyslipidemia. The mean 25(OH) vitamin D level increased from 16 ± 5 ng/mL at baseline to plateau at 26 ± 12 ng/mL after 4 months. In contrast, mean one-time blood pressure (128/78 mmHg at baseline) showed no change over the four months, while 24-hour blood pressure data have not yet been compiled. These preliminary data suggest that we have successfully recruited a study population that reflects the demographics and health characteristics of the T2DM population in St. Louis, Missouri. The vitamin D treatment dose we are using appropriately increases 25(OH) vitamin D levels as mean levels increased by 10 ng/mL with only half of patients on vitamin D. There was no change in overall blood pressure throughout the course of the study, but until groups are unblinded, we will not know blood pressure trends in treatment versus placebo groups. We hope to complete trial recruitment in the next 6-8 months, and 24-hour blood pressure monitoring data combined with detailed comorbidity data will allow analysis of the effects of vitamin D supplementation on blood pressure in T2DM patients with hypertension independently of other covariates.

Novel tandems of diabetogenic epitopes for the promotion of linked suppression by tolerogenic dendritic cells.

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BACKGROUND: Type 1 diabetes (T1D) occurs as a result of T cell-mediated destruction of pancreatic islet beta cells, leading to loss of insulin production. Tolerance-inducing dendritic cells (DCs) that can “reprogram” self-reactive T cells have a strong therapeutic potential, and require presentation of relevant epitopes in order to engage these T cells. We hypothesize that diabetogenic epitopes introduced into DCs as one single construct, made of a succession of epitopes (tandems) with or without signals that enhance MHC class II targeting, can be processed and presented to diabetogenic T cells, resulting in productive stimulation. We cloned six DNA constructs each encoding five diabetogenic peptides either in their natural form or as high affinity “mimotope” versions, with some constructs fused to a fragment of the transferrin receptor (TR) or LAMP-1 (LP) known to enhance loading of endogenously expressed antigens onto MHC class II, and produced lentiviral particles to transduce bone marrow-derived DCs from non-obese diabetic (NOD) mice. To demonstrate proper processing and presentation of the provided epitopes, we co-cultured the transduced DCs and epitope-specific CD4+ or CD8+ T cells isolated from TCR transgenic mice or obtained as a cell line, and measured T cell proliferation by FACS analysis of CFSE-labeled T cells and IFN-gamma and IL-2 secretion by ELISA. DCs were successfully transduced (47-66% GFP+) with all lentiviral vectors. Three out of five T cell populations proliferated and/or produced cytokines in the presence of transduced DCs: IGRP-reactive CD8+ T cells responded equally well to all constructs, insulin-reactive CD4+ T cell response required TR or LP for proper epitope presentation, and GAD65-reactive CD4+ T cells responded to all constructs, but more vigorously with TR or LP. In contrast, chromogranin A-reactive and IAPP-reactive CD4+ T cells failed to respond in the presence of transduced DCs although they were efficiently stimulated in our positive controls. Introduction of these tandems of diabetogenic epitopes into DCs led to the successful processing and presentation of 3 out of 5 epitopes, which is enabled for insulin and enhanced for GAD65 by the presence of TR or LP signals. We demonstrated that multiple epitopes from different antigens can be endogenously expressed as a single protein in DCs, and when co-expressed with tolerogenic and immunoregulatory products, be used to target several disease-relevant T cells at once and propagate tolerance through linked suppression.

Linkage analysis of Cystic Fibrosis Related Diabetes

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Cystic Fibrosis Related Diabetes (CFRD) is a distinct form of diabetes seen in CF patients at a prevalence higher than diabetes in an unaffected population, indicative that there is a multifactorial, non-mendelian inheritance pattern that is unmasked by the presence of the CF phenotype. A linkage analysis of the CFRD phenotype will suggest certain SNP regions to show a significant level of linkage across families. A data set of GWAS composed of affected siblings and parents, with SNPs pruned to represent linkage disequilibrium blocks using Plink, was used for the linkage analysis. Without limiting the number of SNPs, the linkage data have a higher rate of false positives. A phenotype data set was generated using R by analysis CFRD diagnosis, age of onset, blood glucose levels, transplant status, pancreatic sufficiency, and censorship. By utilizing a proportional hazard model, residual data was generated to weight the phenotype data prior to analysis using Merlin. The stringency measures applied to the phenotype remove low-confidence positive or negative CFRD diagnoses. The results are not yet conclusive. Using computational methods on GWAS data to perform linkage analyses is a powerful tool in finding variants with a known phenotype, and by increasing the quality and specificity of the phenotype data can lead to more accurate results.

Assessing Kidney Knowledge in Hospitalized Patients

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Introduction: Acute kidney injury (AKI), characterized by a sudden loss of kidney function commonly complicates hospitalization and strongly associates with mortality and long-term loss of kidney function. However, with few effective treatments for established AKI, increasing attention to preventing the longitudinal complications of the disease is warranted. An unexplored aspect of improving care following discharge is the ability of the patient to engage in the discharge plan and future care. We hypothesize that patients may have a low knowledge and literacy regarding their kidneys and kidney health, and there may be gaps in perceived and actual level of AKI knowledge amongst patients who have had a clinically significant AKI episode at the time of discharge.
Methods: To test these hypotheses, we prospectively identified adult patients hospitalized at Vanderbilt University Hospital (VUH) with AKI using the Electronic Health Record (EHR). We invited patients to participate in a questionnaire that would assess their level of kidney health knowledge. Criteria for inclusion were adults aged 18-80 with a doubling in serum creatinine level compared to their baseline or dialysis. Patients, who scored low on a "mini-cog" exam, were non-English-speaking, and were unable to provide informed consent, were excluded**.** *Results*: Average baseline serum creatinine levels were reported to be 0.85 mg/dL with an effective glomerular filtration rate (eGFR) of 89.5 mL/min/1.73m^2, and average peak serum creatinine levels of 3.54 mg/dL were measured. Survey results showed a low level of kidney health and AKI knowledge amongst patients. Despite meeting criteria for moderate to severe injury, no patients reported experiencing any kidney health problems or an AKI event. In addition, only 28.6%, 57.1%, and 14.3% of patients reported that NSAIDs, iodinated contrast, and Fleets enemas, respectively, can cause AKI. No patients reported any healthcare providers discussing kidney health or AKI with them. No gaps between perceived and actual kidney health knowledge were reported, although both were low.
Conclusion: In patients experiencing a moderate to severe AKI, awareness that AKI had occurred was low as was knowledge on how to reduce risk for future injury. These findings suggest either poor recognition that an AKI event has occurred, or a potential gap in communication between caregivers and patients. Future work to identify the potential causes for these results and methods to improve provider-patient communication is warranted.

Dopamine serves as an anti-incretin in the regulation of insulin secretion.

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A variety of external signals are involved in insulin release by pancreatic β-cells.
Classically understood as a neurotransmitter, Dopamine (DA) is thought to play a role in the incretin signal pathway of insulin release. DA, synthesized *de novo* or imported by dopamine transporter (DAT), is stored in β-cell insulin granules by the vesicular monoamine transporter type 2 (VMAT2). Insulin granules also contain dopamine 2 like receptors (D2R). During glucose stimulated insulin release (GSIS), DA and insulin are released and D2R is delivered to the cell surface where it binds DA. To test the hypothesis that DA mediates a GSIS inhibitory response in human β-cells, we analyzed protein extracts of INS1_E islets for analysis of the activation (e.g. phosphorylation) of key regulatory proteins known to propagate glucose signaling and other signal molecules which modulate insulin secretion in β-cells. We first treated INS1_E cell cultures with varied concentrations and combinations of glucose, glucagon-like peptide 1 (GLP-1), gastric inhibitory polypeptide (GIP), and DA. We analyzed cyclic adenosine monopohosphate (cAMP) levels by ELISA, calcium levels by fluorometry, and protein expression using western blot techniques. The selection of molecules was made on the basis of published evidence of their involvement in glucose, DA, and/or GLP-1R signaling either in the CNS or in beta cells. Western blot analysis and ELISA of INS1_E cell cultures provided evidence of the existence of a DA-based regulatory circuit of insulin secretion that may counterbalance the incretin system. We found that DA counteracts cAMP signaling and calcium release, thus ultimately inhibiting insulin release. We also found that the expression of key GSIS pathway proteins is significantly altered in the presence of DA. These in vitro observations that determine the particular effects of dopamine on beta cells can provide further insight on the pathogenesis of type 1 and 2 diabetes and thus provide potential for therapeutic intervention. Furthermore, due to established dopaminergic molecular imaging techniques in the brain, this study can create a platform for potential radiologic imaging of beta cells in the pancreas.

Title: All-Cause Mortality According to Troponin Levels in Hemodialysis Patients without ACS: A Systematic Review and Meta-analysis

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Cardiac troponin (cTn) subunit I and T (cTnI and cTnT) acutely elevated in patients with acute coronary syndrome (ACS) and chronically elevated in patients with Chronic Kidney Disease (CKD). These cardiovascular diseases and kidney disease are major complications in patients with diabetes. Elevated cTn is defined as a serum troponin level above the 99th percentile of a healthy reference population. Due to high variability in the assays used to measure troponin, and a lack of healthy reference population, there is no consensus on the 99th percentile value for either cTnI or cTnT. A serious complication arises as CKD patients are at significantly higher risk for developing ACS. In this case, the use of troponin as a biomarker for diagnosing ACS becomes questionable. We conducted a systematic review using MEDLINE and EMBASE through July 2013 to estimate all-cause mortality according to cTn levels in CKD patients without symptoms of ACS. 16 studies were conducted in dialysis patients and met the inclusion criteria for the preliminary analysis. Our analysis indicates that elevations in cTnI increase risk of death in dialysis patients by 2.9 fold, and elevations in cTnT increase the same risk by 3.41 fold. By using the CKD population without symptoms of ACS, we hope to identify a cutoff value of cTnI and cTnT that can be clinically implicated in treating CKD patients.