NUTRITION OBESITY RESEARCH CENTER

NEWSLETTER

Grant #2P30DK072476 **October 2012**

ANNUAL DIRECTORS MEETING

This year's annual NORC director's meeting is being held on October 2 - 3, 2012, at LSU - Pennington Biomedical Research Center in Baton Rouge, LA. There are 12 NORCs around the United States who will be involved in this annual meeting. Each year a different NORC hosts the annual meeting; PBRC was honored as the host of this year's event.

The meeting is comprised of the 12 center directors and their executive administrators. This years annual meeting will include a presentation from Dr. Greg Germino and Dr. Mary Evans from NIH. Next on the agenda is a discussion on recurring methods across the different centers including; imaging and energy expenditure focusing on standard operating procedures, methodology, and quality control. Potential collaborative topics will be discussed, specifically one already initiated by UAB NORC (Emily Dhurandhar) known as the "Breakfast Study".

In This Issue:

NORC EAB MEMBER RECEIVES HONORARY DOCTORATE DEGREE

Dr, Rudolph Leibel

Rudolph L. Leibel, M.D., received an Honoris Causa Doctorate, or honorary doctorate degree, for his internationally recognized diabetes research at the LSU Health Sciences Center (LSUHSC) commencement ceremonies on Thursday, May 17 in New Orleans. On Friday, May 18, he was honored at the Pennington Biomedical Research Center in Baton Rouge where he will also presented his work to the faculty and research staff.

Dr. Leibel, the Christopher J. Murphy Memorial Professor of Diabetes Research at Columbia University, was nominated for the honorary degree by Pennington Biomedical and LSUHSC for his extraordinary scientific achievement as well as for his many years of service as a member of Pennington Biomedical's External Advisory Board, and his current service as chair of the institution's Nutrition and Obesity Research Center (NORC), which is funded by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

Dr. Steven Heymsfield, M.D., executive director of Pennington Biomedical, said, "Dr. Leibel has made groundbreaking contributions to our understanding of the molecular genetics and developmental process related to obesity, Type 2 diabetes and body weight control by initiating the molecular cloning of the leptin gene." His recent work includes the use of human stem cells to clarify the molecular and developmental processes related to diabetes and obesity.

Having worked in obesity research for over 25 years, Dr. Leibel has served as a physician and researcher in faculty positions at Harvard University School of Medicine, Rockefeller University, Cornell University Medical College, and subsequently at Columbia University College of Physicians and Surgeons where he has been since 1997. Dr. Leibel is a professor of Pediatrics and Medicine at Columbia University Medical Center, and head of the Division of Molecular Genetics in the Department of Pediatrics. He is also co-director of the Naomi Berrie Diabetes Center and executive director of the Berrie Program in Cellular Therapies of Diabetes, co-director of the New York Obesity Research Center and the Columbia University Diabetes and Endocrinology Research Center. He received his Doctorate of Medicine from Albert Einstein College of Medicine in 1967.

Dr. Leibel is a member of the Institute of Medicine of the National Academy of Sciences and the NIDDK Federal Advisory Council. His research is funded by National Institutes of Health, American Diabetes Association, the New York State Stem Cell Science Program, the Russell Berrie and Helmsley Foundations, and Astra Zeneca. He has published over 300 peer reviewed scientific papers, which have been cited over 13,000 times in the world scientific literature. He also serves on the editorial boards of the *Journal of Clinical Investigation, International Journal of Obesity,* and *Obesity Research*, and has received numerous awards for scientific and pioneering work in medical research.

TRAINING UPDATE

The Division of Education recently submitted a competitive renewal application on Pennington Biomedical's NIDDK sponsored T32 training grant entitled "Obesity: From Genes to Man." If approved, it would be the third five-year training cycle for Program Director Phillip Brantley, PhD, Associate Director of the NORC. The renewal application proposed to expand the number of postdoctoral trainee positions from the current four to six slots per year. The renewal application also requested the center be allowed to accept MD applicants along with PhD's. The center made the decision to include MDs due in part to the increasing medical training resources being added in close proximity to the center and the availability of graduate research training through a recently acquired translational research center grant.

A total of 18 postdoctoral trainees have participated in the training program since it began in 2003. To date, twelve faculty members of the NORC have served as primary mentors for trainees on the Obesity T32. Currently we have four regular slots and one supplemental slot filled (total of 5). Ten of the 13 trainees no longer with the program have continued a research career and are publishing. Three have faculty positions with a research focus (two more insist they are on the verge of closing the deal) and three others have faculty positions that emphasis teaching but have a research component. Three have acquired k awards, two have non-NIH grants and others have applications pending.

If the T32 renewal is approved, two to four new slots are anticipated in the spring of 2013. We are seeking applicants with less than 5 years of postdoctoral experience who have evidence of research aptitude and a strong desire to become an independent obesity researcher. Eligible applicants must be a US citizens or green card holder. Please contact Dr Brantley if you identify a strong candidate.

Along with postdoctoral training, faculty of the NORC recently served as mentors for eight summer medical students from LSU Health Sciences Center in New Orleans (LSUHSC) who participated in a newly acquired NIDDK T35 Summer Research Training Program. Students received hands on training in Pennington NORC faculty research labs, attended didactic seminars on methodology and the responsible conduct of research, and presented their projects at a special research day event at LSUHSC. The grant addresses the need for physician scientists and is designed to encourage careers in biomedical research. The program is co-directed by Dr. Paula Gregory of LSUHSC and Pennington NORC Associate Director, Dr Phillip Brantley.

NORC CORE CAPABILITIES EXPANDED

HUMAN PHENOTYPING CORE

Imaging & Body Composition Subcore

Imaging and Body Composition Subcore has acquired equipment to further expand the Center's research capabilities. This new equipment includes BOD POD and PEA POD systems and functional MRI (fMRI) components.

BOD POD and PEA POD

These systems are capable of accurately assessing body composition in infants (PEA POD) and adults (BOD POD) using air displacement plethysmography technology and will add to the Center's current capabilities for measuring body composition which include two DXA scanners and a nuclear magnetic resonance system called EchoMRI™. Dr. Leanne Redman's laboratory plans to utilize these systems in the coming months. For her "Life-Moms" study, the BOD POD and PEA POD will be used to monitor changes in body fat in overweight and obese pregnant women during pregnancy and also in the baby during the first year of life. "Life-Moms" is a national study that is a collective of seven different studies. The goal of the study is to test the benefit of lifestyle interventions for management of weight gain in expectant moms.

Functional MRI

Components for fMRI have been installed at PBRC, and quality assessments and methodology development are underway. Dr. Corby Martin's laboratory plans to pursue three lines of fMRI research once fMRI capabilities are established at the PBRC.

1. Neural substrates of macronutrient preference and restriction.

In close collaboration with colleagues from Duke University Medical Center (DUMC; Joe McClernon, Ph.D.), Dr. Martin plans to perform studies to investigate the neural substrates of macronutrient preference, as well as changes in brain activation in response to weight loss and restriction of specific macronutrients.

2. Changes in brain activation in response to exercise: Do changes in brain activation differ between compensators and non-compensators?

In some individuals, particularly women, exercise results in less weight loss than expected based on the energy expended during exercise training. It is hypothesized that this is due to people compensating for the increase in energy expenditure from exercise by increasing energy (food) intake. This phenomenon is known as compensation. Dr. Martin and Dr. Tim Church plan to quantify brain activation in response to visual food stimuli before and after exercise training They plan to test 1) changes in brain activation in response to food stimuli differs between compensators vs. non-compensators, and 2) if changes in brain activation are associated with compensation among compensators.

3. Brain networks and their relation to food intake and weight change.

Dr. Martin plans to collaborate with colleagues from Duke and possibly Wake Forest to establish the ability to map brain activation in response to food stimuli using fMRI technology. This methodology has been used to demonstrate that brain networks associated with addiction and reward are also activated in response to food stimuli. Dr. Martin plans to evaluate: 1) if activation of brain networks associated with reward and addictive behaviors differs from before to after weight loss treatment, 2) if brain network activation varies as a function of the amount of weight lost, and 3) how change in brain activation in response to the ingestion of food differs between lean and obese individuals.

HUMAN PHENOTYPING CORE, *next page*

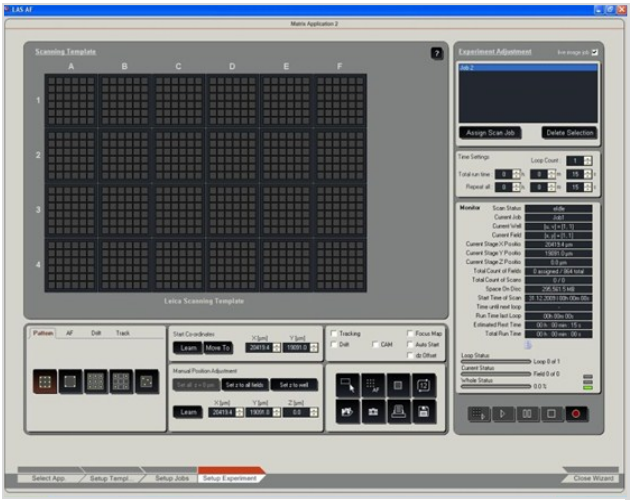
MOLECULAR MECHANISMS

Cell Biology & Bioimaging Upgrades

The Cell Biology and Bioimaging Core (CBBC) provides state of the art bioimaging and analytical instrumentation and support to facilitate data collection and analysis. We collect several types of data for researchers – images of their slides, 3D data sets of fluorescently labeled tissue, and flow cytometry data. Our users are conducting research in virtually every area covered here at Pennington, from immunological data to data for cancer research to imaging mouse embryonic developmental defects to data for obesity-related studies.

CBBC services are available to current and future Pennington PIs and their staff. The CBBC is staffed by Dr. David H. Burk, Director, Ms. D'Andreas Williams and Mr. Drury Ingram, Research Associates, Ms. Shirley Ennis, histotechnician and Ms. Marilyn Dietrich, flow technician.

The CBBC is currently scheduling an upgrade to our Leica TCS SP5 confocal. This upgrade, a result of a successfully funded Louisiana Board of Regents Enhancement grant, will allow us to replace three of the standard photomultiplier tubes (PMTs) which detect emitted photons from excited fluorophores with three HyD detectors. These new hybrid-PMTs have several advantages over the older design - specifically in the areas of quantum efficiency, high dynamic range imaging, and quantification of emitted photons. Standard PMTs have quantum efficiencies (QEs, which relates to the percent of photons that are converted into an electrical signal) of about 20% at 500 nm (such as FITC). The HyD detectors from Leica make use of technology found in GaAsP (gallium arsenide phosphide) detectors as well as conventional PMTs and have QEs in the 40-45% range as well as a significant reduction in noise. In addition, the new detectors can operate in different modes – standard, BrightR (a high dynamic range imaging mode), and photon counting. High dynamic range imaging with the HyD detectors allow users to capture bright and dim portions of a stained



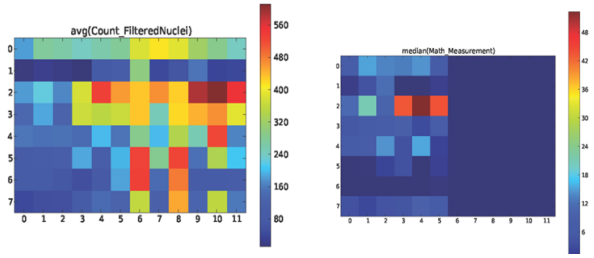
Matrix software set up to image 36 images per well for a 24-well plate.

specimen within the same gain setting – essentially an increase in the dynamic range of the detector. Photon counting mode is useful for measuring absolute number of photons emitted from each pixel scanned on a specimen and will allow very stringent quantification of signal. We hope these new detectors will be installed and operational before the end of the summer.

Another addition to the CBBC will be in the form of the Leica Matrix software module for our inverted DMI6000 microscope. This software greatly expands the capabilities of this fluorescence imaging platform in that it will enable users to rapidly and automatically image multi-well plates after a brief set-up period. For example, cells cultured on 6, 12, 24, 96, or even 384-well plates can be treated with a variety of agents, probed using fluorescent dyes and/or with fluorescently-conjugated antibodies against specific proteins and then imaged automatically on the DMI6000. Images are easily converted to a format that is recognizable by free analysis software such as CellProfiler for qualitative and quantitative measurements of metrics such as total cell number, nuclear size, lipid content, cell cycle, or virtually any other metric related to the intensity or physical characteristic of the probe of interest. We anticipate the new software will be installed before the end of May.

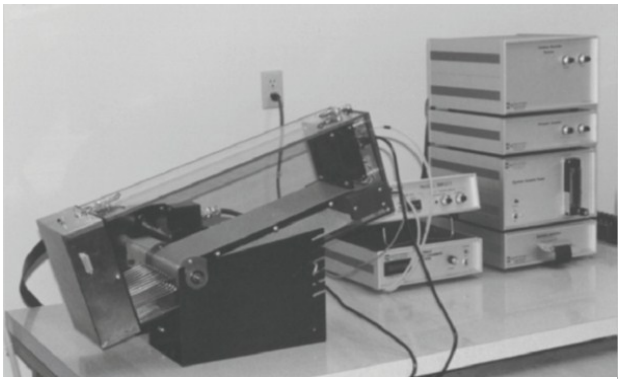
Genomics Subcore

In addition to traditional gene expression profiling methods such as microarray or robotics-assisted quantitative PCR, the Genomics Core now offers next-generation sequencing technologies for gene expression and epigenetics measurements. Sequencing is performed on an AB SOLiD 5500 XL instrument. Data processing, read quality control, and read alignment to genomic reference sequences are carried out on a Dell C6145 2x64core workstation. Analysis pipelines using GALAXY or LIFESCOPE are available, as is customized SOLIiDSAGE software for gene expression studies. Data evaluation packages include DESeq, edgeR, and SICER, as well as the genomics suite of tools in JMP Genomics. Acumenta LiteratureLab is offered for biological and biomedical annotation of results. OneNote electronic notebooks containing all experimental tracking data – experimental design, protocols, instrument run and quality control data, analysis pipelines, and results - are now offered to Investigators; secure access to these notebooks is provided via the Genomics Core SharePoint site.



(Left): Data collected from a 96-well plate was used to generate this heatmap showing mean nuclear number per image per well.

(Right): Median number of lipid containing cells per image per well in a 96-well plate. Note clear distinction between cells induced to uptake lipid on the left vs uninduced on the right (Plate maps generated in CellProfiler software).



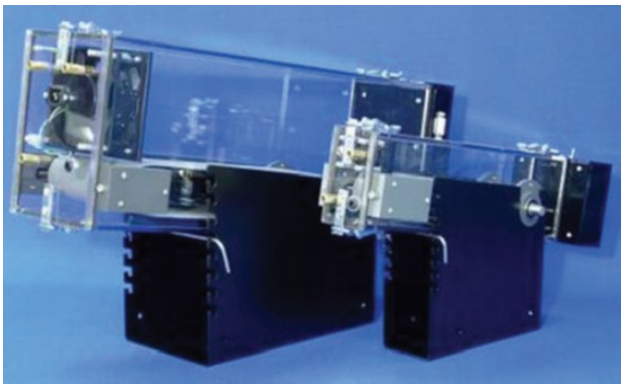
Modular Treadmills are designed for integration with the Oxymax for the measurement of VO2, VCO2, RER, Heat, and other metabolic parameters. The addition of an open-flow calorimeter to the compartment will create a complete system for the measurement of respiratory metabolic performance while exercising. The incline of the animal compartment is adjustable in 5° increments. Electrical stimulus grids are available for the exercise lane and can be activated manually by the operator.

ANIMAL MODELS AND PHENOTYPING CORE

What's New?

Using NORC funds, the Animal Models and Phenotyping Core purchased a Columbus Instruments' Modular Treadmill for mice.

The air-tight compartment allows for the connection of ventilation and gas monitoring equipment. The treadmill was connected to existing Oxymax equipment for the measurement of VO2, VCO2, RER, Heat and other metabolic parameters. The addition of an open-flow calorimeter to the compartment will create a complete system for the measurement of respiratory metabolic performance while exercising.



Columbus Instruments' Modular Treadmill encloses the animal within an isolated space which includes an exercise belt, associated pulleys, and optional stimuli all enclosed in an air-tight compartment. Air-tight versions incorporate fans for the circulation of air over the animal. The air-tight compartment allows for the connection of ventilation and gas monitoring equipment.

2011 PILOT AND FEASIBILITY UPDATES

The objective of the NORC P&F program is to encourage young investigators by providing research support to test innovative hypotheses involving nutritional programming-related research and other pilot studies related to the function of NORC.

Dr. Eric P. Plaisance
Assistant Professor
Nutritional Physiology Laboratory
Pennington Biomedical Research Center

Effects of GCN2 Kinase on Lipogenic Gene Expression Following Dietary Methionine Restriction

Restriction of the essential amino acid methionine by 80% in the diet of rodents increases lifespan to a similar extent as calorie restriction despite an increase in food consumption. The increase in food consumption is matched by an increase in energy expenditure which limits the accumulation of body fat and weight. The reduction in adiposity is associated with improvements in insulin sensitivity and reduced liver and circulating triglycerides.

Although the responses to dietary methionine restriction (MR) have been well characterized, little is known about the nutrient sensing mechanisms that detect reductions in circulating methionine and how these events are translated into the beneficial physiological and metabolic responses to the diet.

Another important unresolved question is whether restriction of other essential amino acids generates similar responses as those produced by methionine. With pilot and feasibility funding provided by the NORC, we have recently completed a study using MR in wild-type mice and mice with a whole body deletion of the general control nondepresssible 2 (GCN2) kinase. Activation of GCN2 by diets completely deficient in one or more essential amino acids leads to a series of biochemical and physiological responses designed to provide short-term adaptations to overcome the nutritional deficiency. A number of similarities and distinct differences have been observed between essential amino acid deficient diets and those produced by 80% MR. Therefore, our goal was to determine the overall role of GCN2 on the physiological and transcriptional changes produced by MR.

Our results show that GCN2 is activated and responsible, at least in part, for the increase in energy expenditure and improvements in insulin sensitivity that occur with MR. In contrast, the increase in food intake and decrease in hepatic lipid content are completely independent of GCN2 activation suggesting that alternative pathways are activated by MR. Additional studies were also proposed in mice to determine the amino acid and dose-specific effects of essential amino acid restriction.

Our results indicate that restriction of other essential amino acids such as leucine produce similar effects as MR and that a threshold of approximately 80% restriction is required to engage the physiological and metabolic responses to essential amino acid restricted diets. Next generation sequencing platforms will be used at PBRC in the coming weeks to examine the overall transcriptional responses to 80% MR. Pilot studies conducted as part of this award have recently been presented at the 2012 Experimental Biology meeting and were used as preliminary data for K01 and R01 submissions which are pending.

2011 PILOT AND FEASIBILITY UPDATES

Dr. Krisztian Stadler
Assistant Professor
Oxidative Stress and Disease Lab
Pennington Biomedical Research Center

Impact of Diet Restriction on Kidney Mitochondrial Energy Balance in Obesity

Metabolic syndrome and obesity are associated with an increased risk for diabetic complications later, including chronic kidney diseases (CKDs). CKDs eventually have devastating consequences, leading to kidney failure and dialysis. Novel data suggest that CKD can develop in obese individuals *without* diabetes as well, therefore obesity could be an independent risk factor for renal disease. Given the importance of the topic, in our NORC pilot project, we are hoping to establish a primary link between adiposity and cellular/subcellular (mitochondrial) oxidative stress in obesity-related bioenergetic, pathological and functional alterations in the kidney. With restricted HFD feeding, we mimic a situation where one still has access to food with higher fat content but in a limited manner to control body weight. Weekly food intake measurements and NMR ensure that we maintain the C57BL mice phenotype with a 60-80 % restriction of the 45 % lard food – this design allows us to study the effect of dietary lipid (lard) intake and a possible lipid overloading scenario on cellular and subcellular oxidative stress and mitochondrial energy balance in the kidney.

Our data indicates a major difference in cellular and subcellular (mitochondrial) oxidative stress processes. A dietary restriction design was able to rescue cellular, but not subcellular oxidative stress. Kidneys from HF diet fed mice showed increased levels of iNOS and nitrotyrosine as markers of cellular oxidative stress while secondary protein radical formation was accumulating and colocalizing with podocytes. Mice on 45 % kcal lard HF diet showed loss of podocytes – these cells are important part of the filtration barrier in the glomeruli. Renal mitochondria from these mice showed increased H_2O_2 release and higher basal respiration or State III respiration in the SeaHorse extracellular flux analyzer. When the diet was restricted to maintain body weight similarly to controls, mitochondria still showed increased respiration parameters, and the accumulation of long chain lipid products, suggesting an adaptation-like mechanism and a faster TCA cycle and beta oxidation. Cellular oxidative stress parameters on the other hand were normalized and podocyte loss was considerably less in these mice.

The significance of these findings is that it suggests a renal mitochondrial lipid overload situation, where – similarly to the skeletal muscle, where it was previously shown – fatty acids exceed metabolic demand, creating a perturbation in the beta oxidation cycle. This will translate to higher readouts in mitochondrial respiration, and a possible backflow of electrons through complex I, resulting in superoxide and consequently H_2O_2 release. A concerted action of superoxide and nitric oxide from cellular sources may therefore be playing an important role in podocyte loss we observed. Our data also suggest that a lipid overload may be more important than adiposity itself in mitochondrial oxidative stress, but adiposity may play a role in cellular oxidative stress through secondary mechanisms (adipose tissue inflammation related iNOS expression and nitrotyrosine formation). These processes may contribute to early renal pathology in obesity, and the development of chronic kidney disease (CKD). Emphasizing the role of lipids in the diet, and a mitochondrial intervention can be beneficial in future directions of therapies to prevent CKDs.

FOUR NEW AWARDS FOR PILOT AND FEASIBILITY STUDIES

Dr. David McDougal
Instructor of Autonomic Neurosciences at PBRC

Awarded funding as PI for his study Nutrient Detection in the Hindbrain.

McDougal shares some information on the role of the type 2 glucose transporter in the autonomic response to hypoglycemia.

According to the National Institute of Diabetes, Digestive, and Kidney disease, diabetes affects 25.8 million people in the United States, representing 8.3% of the U.S. population. It is the seventh leading cause of death as well as a major cause of heart disease and stroke. The primary intervention used to limit these complications is the control of blood glucose levels, more specifically the reduction of glucose levels towards normal levels. Common treatments aimed at countering hyperglycemia in diabetic patients often have the unintended consequence of producing hypoglycemia when the target of euglycemia is overshot. In response to hypoglycemia, the autonomic nervous system directs physiological and behavioral countermeasures which restore blood glucose to normal levels. Unfortunately, repeated bouts of hypoglycemia often lead to the loss of this critical homeostatic mechanism. This renders diabetic patients unusually susceptible to progressively severe bouts of therapeutically induced hypoglycemia. These hypoglycemic crises are a major impediment to the maintenance of healthy plasma glucose levels in these patients. Given the increasing prevalence of diabetes, it is vital to gain a better understanding of the mechanisms by which the body's response to hypoglycemia becomes aberrant in this patient population.

The type 2 glucose transporter (GLUT2) is commonly associated with the cellular detection of glucose availability. It is most well-known in regards to its involvement in pancreatic detection of hyperglycemia, which ultimately leads to the release of insulin. Yet, studies have demonstrated that GLUT2 expression by CNS astrocytes is necessary for the normal response to hypoglycemia as well. My NORC project is designed to produce a transgenic mouse model which can be used to directly test this hypothesis through the use of Cre-lox technology. More specifically, to test our hypothesis regarding astrocytic involvement in the autonomic response to hypoglycemia, we will cross mice having astrocyte specific expression of CRE with animals possessing a GLUT2 gene modified with loxP site insertions. This would result in offspring in which the GLUT2 gene would be deleted from astrocytes leaving GLUT2 expression unchanged in other cell types. Physiological assays could be used to detect deficiencies in these animals' responses to hypoglycemia and thereby test whether astrocytic expression of GLUT2 is necessary for these responses.

It is clear that the results of these experiments would greatly enhance our understanding of how the brain senses hypoglycemia, yet several biological and technical issues had to be resolved before they could be undertaken. With the assistance of NORC funding and NORC supported CORE facilities, our laboratory has made significant progress towards the resolution of these issues over the previous 14 months. We have progressed through all of the steps involved in moving from a commercially available embryonic stem cell line to a mouse line which possesses a loxP containing GLUT2 transgene. We have also made significant progress toward validation of 4 different cell specific Cre lines as well. We are currently quantifying the efficiency and specificity of Cre mediated recombination in the brainstem and hypothalamus of these mice (the brain regions most commonly associated with glucodetection). These data will be necessary to validate the results of our future studies using these Cre lines to selectively disrupt GLUT2. We expect to have completed all breeding steps of our project by early 2013 and phenotyping of these experimental animals will procede in short order. We believe these results will provide novel insights into the mechanisms of brain glucodection. A better understanding of these mechanisms is critical for further progress towards healthier maintenance of blood glucose levels in diabetic patients.

Dr. Anthony Civitarese
Assistant Professor and Director of Skeletal Muscle Physiology at PBRC

Awarded funding for his study, RNF20, a new mediator of bioenergetics and transcription in mammals.

Caloric Restriction (CR) is the most powerful manipulation known to delay the rate of aging and retard the development of age-associated diseases in rodent models and many other species. Recent data demonstrate that CR prevents metabolic disease development, increases the "health span" and survival of non-human primates and thereby establishes the transmittance of CR to human health. However, the underlying mechanism (s) of CR mode of action is unclear and very little data exist describing the mediators of gene function (transcription or epigenetic) in response to CR in non-obese, healthy humans. Our preliminary data are exciting and clearly show RNF20 expression to be up-regulated by CR in humans. RNF20 is a newly discovered E3 ubiquitin ligase that regulates DNA processing, histone function (H2B monoubiquitylation) and gene regulation. Therefore, RNF20 may be a chief regulator of the physiological and genetic aspects of CR.

We have previously provided the first evidence in non -obese humans demonstrating that CR lowers biomarkers of aging (fasting insulin and core body temperature) and reduces basal metabolic rate beyond the level expected from loss of metabolic mass. This metabolic adaptation was parallel to an induction in mitochondrial biogenesis and a decrease in DNA damage in skeletal muscle. This suggests that CR induces biogenesis of 'efficient' mitochondria as an adaptive mechanism which in turns lowers oxidative stress. Given that mitochondrial function, insulin sensitivity and metabolism are dysregulated in Type 2 Diabetes (T2D) and aging, we assessed RNF20 protein expression in the muscle of these cohorts. Importantly, RNF20 protein expression was reduced in insulin-resistant elderly individuals and subjects with T2D. Alternatively, when RNF20 was expressed in human RMS13 myoblast, we improved markers of glucose metabolism and mitochondrial efficiency. These preliminary data provide the first evidence that RNF20 can regulate human muscle metabolism in a beneficial manor. Accordingly, the NORC applications will be to establish and physiologically phenotype an RNF20-null mouse model.

Dr. Robert Dubin
Assistant Professor of The Joint Diabetes Endocrinology and Metabolism Program at PBRC

Awarded NORC P&F funding as PI for his study, *Interventions for Obesity and the Gatrointestinal Microbiota.*

Obesity, and its associated co-morbidities, continues to be one of the most pressing public health issues in this country and worldwide. While lifestyle modification is the cornerstone of treatment, weight loss and stabilization from lifestyle alone wanes over time. As there are concerns with current pharmacologic approaches to weight loss, other interventions have been investigated. Bariatric surgery is perhaps the most effective therapeutic strategy for obesity and it's metabolic consequences; notably, glucose metabolism improves within the first few days, followed by sustainable weight loss in most cases.

The mechanisms by which metabolic improvement occurs so quickly after surgery are currently unknown, but is an area of great interest. Along these lines, mounting evidence suggests that the community of microorganisms (the microbiota) in the intestinal tract significantly contribute to nutrient processing, the development of obesity and may regulate host metabolism. Our overarching hypothesis is that bariatric surgery's salutatory effects on weight-loss and metabolism are, in part, mediated through modulation of the intestinal microbiota.

***AWARDS,** next page*

For this pilot project we propose to test the **specific hypothesis** that **therapeutic interventions for extreme obesity lead to favorable alterations in the gastrointestinal microbiota.** In order to test this hypothesis and to generate preliminary data for a subsequent NIH application we will complete the following two Specific Aims:

- **Specific Aim 1**: *To determine the impact of diet and lifestyle modification on the intestinal microbiota in patients with extreme obesity (BMI>40 kg/m2).*

- **Specific Aim 2**: *To assess changes in the intestinal microbiota after bariatric surgery as compared to baseline measures.*

These objectives will be accomplished by recruiting subjects from our well-characterized *Clinical Outcomes Study in Morbidly Obese Subjects* (COSMOS) cohort, a clinical program supported by the LSU HCSD and LSUHSC School of Medicine. Stool samples pre- and post-intervention will be evaluated for ribosomal small subunit DNA-based phylogenetic characterization of the gut microbial community structure and membership using next generation deep pyrosequencing. Additionally, we will develop novel mixed microbial community transcriptomic techniques for use in this and future projects investigating the impact of the microbiota on nutrient-dependent metabolism.

By determining the early changes in the microbiota following both lifestyle and surgical interventions, which precede weight loss, this work will provide insight as to the mechanism(s) by which these treatments modulate obesity and weight-independent metabolism improves. Understanding changes that occur in the microbiota after treatment for obesity may provide valuable insight for targeting future interventions. The data generated will support an NIH Career Development Award application by Dr. Dubin.

Dr. Krisztian Stadler
Assistant Professor of Oxidative Stress and Disease Lab at PBRC

Awarded funding as PI for his study Mitochondrial lipid overload, renal pathology in a CrAT Podo -/- mouse model.

Our previous work indicates that high fat diet feeding (45 % kcal from lard) in C57BL mice leads to oxidative stress related alterations in the kidney, mainly through subcellular mechanisms. Increased mitochondrial H_2O_2 release, differences in mitochondrial respiration and increased fatty acid oxidation suggest a perturbed beta oxidation early in obesity which may relate to renal function. Moreover, restricting the high fat diet – although rescued some cellular oxidative stress markers – did not change altered mitochondrial redox patterns. This oxidative stress correlates with early renal changes such as podocyte loss and some degree of collagen deposits and fibrosis. To ultimately link a mitochondrial "overfueling" scenario as the source of redox changes related to pathology, we have chosen to create a carnitine acetyl-transferase (CrAT) Cre/Lox mouse model specifically targeted into kidney podocytes. This model could directly test the lipid overload theory in mechanistic details.

Our hypothesis is that without CrAT, mice would not be able to link the excess or partially oxidized fatty acids to carnitine and these products cannot be transported out of their renal mitochondria. Accumulation of these products can lead to perturbed beta oxidation, more highly reduced state of the electron transport chain complexes and therefore can be causative to increased mitochondrial superoxide production and oxidative stress. This model can answer how exactly the mitochondrial lipid overload relates to pathology, specifically, the loss of podocytes and disruption of the filtration barrier. (Podocyte loss was an early observation in our high fat diet model experiments). Since the floxed CrAT allele mouse has been created at PBRC, and we possess the commercial Cre ^{Podo} mice, we will be able to generate a podocyte specific model to test this hypothesis in the kidney.