NUTRITION OBESITY RESEARCH CENTER

NEWSLETTER

formerly Clinical Nutrition Research Unit

NEW AWARDS FOR PILOT AND FEASIBILITY STUDIES

ROBERT C. NOLAND, PhD

Defining the importance of skeletal muscle peroxisomal function in regulating insulin resistance.

Excess intramuscular lipid accrual is thought to play a causal role in insulin resistance, thus strategies aimed at diminishing the cellular lipid burden offer therapeutic potential. There are currently 2 models describing how lipid overload promotes insulin resistance. The first suggests accumulation of certain lipid species, such as ceramides, directly interferes with insulin signaling (lipotoxicity theory). The second proposes that excess mitochondrial fat entry overwhelms its capacity for lipid catabolism, which results in a stress signal that impairs insulin sensitivity (mitochondrial overload theory). Both models are supported by strong experimental evidence; thus, it is likely that strategies aimed to mitigate both will be good therapeutic targets. The central hypothesis of this project is that modulation of peroxisomes provides a viable option to meet this goal as they are likely candidates that offer the potential to both reduce excess accrual of lipotoxic ceramides, while concomitantly limiting mitochondrial lipid overload.

Peroxisomes are involved in a myriad of biological processes, but their primary role is to modulate the cellular lipid environment. The presence of α, β and ω-oxidation pathways allow them to process a broad spectrum of lipids, making them invaluable to maintenance of cellular function. Of particular note, peroxisomes are required to process very long chain fatty acids (VLCFAs: ≥C22). VLCFAs represent a small fraction of the traditional lipid pool, but studies indicate C24:0 (lignoceric acid) and C24:1 (nervonic acid) are amongst the most abundant lipid species in ceramides and these VLCFA-ceramides are particularly elevated in obese human muscle. Since peroxisomes are required to catabolize VLCFAs it seems logical to predict that peroxisomal function is linked to VLCFA-ceramide turnover and a goal of this project is to address this issue. Liver and muscle from methionine restricted rats exhibit peroxisomal adaptations that coincide with contrasting ceramide accumulation. This counter-regulation is supported by preliminary studies in C2C12 myotubes where peroxisomal genes are generally decreased under conditions where ceramide synthesis and storage are promoted (exposure to ceramides or lipid-loading). Alternatively, studies using

adiponectin-null mice generally support the concept that adiponectin may play a role in coordinating peroxisomal upregulation with enhancing ceramide breakdown in liver, but these findings have yet to be extended to muscle.

Peroxisomal function is increased in obese, insulin resistant muscle; however, since peroxisomes are also consistently elevated in models where metabolic benefits are incurred (heightened aerobic capacity, exercise, methionine restriction, caloric restriction and muscle-specific CPT1b deficiency) we predict elevated peroxisomal function is a favorable adaptation. Specifically, we predict peroxisomes provide an alternative route for lipid catabolism that alleviates mitochondrial lipid overload and limits lipotoxicity. In support, studies in cultured myotubes reveal peroxisome insufficiency increases risk stemming from substrate over-

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load as both fat and glucose oxidation are reduced and cells exhibit increased lipid-induced cell death. To define the role of peroxisomes in skeletal muscle insulin resistance *in vivo* we proposed to use NORC funding to develop a muscle-specific peroxisome-deficient mouse (Pex5$^{m-/-}$). The first Pex5$^{m-/-}$ mice have been generated and we anticipate having experimental cohorts within 3 months. Studies will be designed to expose Pex5$^{m-/-}$ mice to conditions that either induce (high fat diet) or protect against (exercise, caloric restriction and muscle-specific CPT1b-deficiency) insulin resistance.

In summary, initial findings support a model where peroxisomes help maintain insulin sensitivity by limiting both mitochondrial lipid overload and lipotoxicity. We expect further results stemming from this NORC project will not only help define the role of peroxisomes in skeletal muscle biology, but will also yield valuable insight regarding whether modulation of peroxisomal function offers a logical therapeutic target to promote insulin sensitivity.

SHAWNA WICKS, PhD

Identification of myokines contributing to the anti-obesity effect of skeletal- muscle carnitine palmitoyltransferase-1 (Cpt) ablation.

Despite the proliferation of exercise and dietary regimens designed to promote weight loss, obesity is still a major challenge. While investigating the role of fat oxidation in the mitochondria using a skeletal muscle-specific knockout mouse model, we made the observation that the mice remained lean despite consumption of high or moderate fat diets. The unexpected impact on whole body fat deposition resulting from muscle-specific alterations to fat oxidation lead us to suspect myokine secretion from the muscle. Myokines are muscle proteins that are secreted into the circulation and have effects on other tissues throughout the body. Recent studies have suggested that there may be hundreds of myokines, primarily uncharacterized. A few, including FGF21 and irisin, have been shown to affect obesity and insulin resistance. In this proposal, we are examining the hypothesis that the obesity resistance of our knockout mice results from secretion of novel myokines with anti-obesiogenic action. We are utilizing proteomics (1D-LC-MS/

MS) performed by the Duke Proteomics Core to identify novel myokines; our results so far show differential expression of several proteins, including known secreted proteins and proteins with both intracellular and secretory forms and functions. We are following up on the most promising candidates identified currently, while planning additional proteomics screening using AHA labeling and click chemistry to capture newly synthesized proteins in conditioned media from primary cell culture.

STEFANY PRIMEAUX, PhD
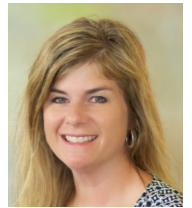
Disturbances in reproductive health are significant consequences of poor weight management and have been linked to the over-consumption of dietary fat. Identifying poorly understood mechanisms by which nutritional status and reproductive demands are interrelated is imperative for the development and advancement of therapies to improve reproductive health in response to being overweight or obese. We have recently reported that the hypothalamic peptide, QRFP (pyroglutamylated RFamide peptide), selectively increased high fat food (HFD) intake in cycling female rats and prepro-QRFP mRNA expression in the mediobasal hypothalamus was influenced by estrous phase. Our NORC pilot & feasibility grant focuses on feeding behavior and metabolic parameters associated with reproductive status in female rats. These studies are designed to test the hypothesis that reduced expression of hypothalamic QRFP will decrease energy intake and affect metabolic parameters in female rats. The first AIM will develop RNA interference (siRNA) methodology to decrease the expression of QRFP in the hypothalamus, a central location for feeding behavior. These studies will provide vital information on the use of this technique for the reduction of QRFP expression and will use a time course analysis to optimize this methodology. The second AIM of this grant is to test the hypothesis that siRNA-induced decreases in hypothalamic QRFP will decrease HFD intake and modulate metabolic parameters in female rats. These studies will lead to advances in the methodology used to significantly decrease hypothalamic QRFP expression and elucidate the functional role of QRFP on fat intake and energy metabolism in female rats.

CORE UPDATES

MOLECULAR MECHANISM CORE

The mission of the NORC Molecular Mechanism Core is to serve as the technological conduit between the Animal Phenotyping Core and the Human Phenotyping Core. The Molecular Mechanism Core provides the tools to generate biological insights as novel animal models generated in the Animal Phenotyping Core move to physiological and behavioral analysis, and offers techniques to study the mechanisms underlying findings in the Human Phenotyping Core on the molecular, cellular, and histological level. The Molecular Mechanism core has two components: Genomics and Bioimaging.

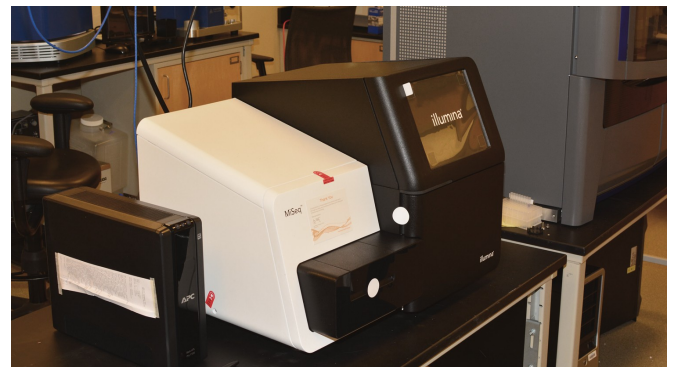## GENOMICS CORE
By Michael Salbaum

The focus in the Genomics Core has been centered on the epigenetic aspects of various biological paradigms – analysis of gene expression, and studies of the epigenome and its plasticity. The classical term for this emphasis is "Functional Genomics". Services fall into two categories according to sample size and scope of the experiment.

The first category is characterized by "*many samples - few measurements*": experiments where e.g. the expression of a few genes is measured across many samples. The main technology is quantitative real-time PCR. The Core currently houses four instruments that are further supported by a robotic pipetting setup to facilitate simultaneous measurements of up to 384 samples per run and up to 2,304 samples in a single day.

The second category is "*few samples – many measurements*". These are systems biology approaches, where the expression of every gene in the genome is measured in an unbiased way. Two experimental platforms support this category: an Illumina iScan for traditional microarray technology, and an AB SOLiD 5500XL next-generation sequencing instrument; both platforms are served by ancillary equipment. The Core also offers complete bioinformatics workflows necessary to interpret systems biology data. As the main analysis

environment, the Core utilizes a local installation of GALAXY on a Dell workstation with 2x64 cores. For gene expression measurements by next-generation sequencing, the technology of choice on the AB5500XL is 3'-expression tag sequencing (SAGE), which provides easy access to statistical depth of analysis, and takes advantage of the true sequencing quality range of the AB instrument. SOLIDSAGE processing of SAGE output generates count data, which are analyzed using software tools (*DESeq*, *edgeR*) from the R/Bioconductor repository. Both these tools are also used for novel post-hoc quality control strategies. In addition, the Core has an established bioinformatics workflow for analyzing RNA-Seq experiments. Experiments performed on the Illumina microarray platform are processed using *limma*, another R/Bioconductor tool.

The new Illumina MiSeq DNA sequencing instrument in the Genomics Core awaiting installation and official startup.

New developments in the Genomics Core are targeted towards analyses of microbiobial communities through metagenomics sequencing. One important concept that has emerged over the last few years is the essential role of microbiota for human health. Of particular interest to NORC is the relationship of gut microbiota to metabolic disorders such as obesity and diabetes. There is increasing evidence that gut microbes promote the onset of the low-grade inflammation in metabolic disorders, and that differences in gut microbiota composition, functionality, and metabolic activity distinguish lean from obese individuals, suggesting that gut 'dysbiosis' contributes to the development or programming of obesity and/or its complications. To analyze such microbial communities, the Genomics Core has recently received an Illumina MiSeq sequencing instrument. This platform not only

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provides the longer sequencing read lengths necessary for microbiota analyses, but also permits high-confidence variant calling and mutation detection in human genetics studies. With this functionality, the new instrument will serve both basic research-related and clinical candidate gene studies.

CELL BIOLOGY & BIOIMAGING CORE
By David Burk

Quantifying changes at the tissue and cellular level in animal models of metabolic disease states as well as from clinical tissue samples typically requires tedious examination of sectioned and stained material via microscopic imaging and subsequent manual segmentation of areas of interest. As part of the Cell Biology and Bioimaging Core's mission, we provide the expertise and infrastructure to produce sectioned – and in many cases – stained slides for PIs after fixed tissue drop-off to our staff. Either formalin-fixed or flash frozen material can be submitted to our histology sub-core for subsequent tissue processing, embedding, sectioning, and routine staining. More specialized stains such as trichrome, picrosirius red, and oil red O are currently offered and we are always open to expanding our capabilities based on the needs of our user base. In addition to standard stains we also are capable of performing immunohistochemical staining for a select (but expanding) list of characterized antibodies using either manual or automated methods.

By coupling our histological services with our whole slide imaging system we have been able to develop a straightforward procedure for the semi-automated analysis of adipocyte cell size and, more recently, their classification as white or brown/beige/brite from paraffin processed adipose tissue. A common method for the determination of the sizes of adipocytes from human and animal fat deposits is the Coulter counter method which requires dissociation of fresh unfixed tissue and subsequent staining/fixation with osmium tetroxide prior to sizing. Unfortunately, many researchers may not have access to fresh material or do not have the capabilities to undertake this procedure. In such cases, the examination of formalin fixed, paraffin embedded and sectioned adipose tissue can be used. Hematoxylin and eosin stained sectioned adipose tissue slides are scanned on the CBBC's Hamamatsu Nanozoomer platform. The scanned images are converted to JPEG files and batch processed in the freely available ImageJ analysis program for processing, segmentation, and analysis. The quality of the resultant data is highly dependent on the quality of the sectioned material – tears and compression artefacts can introduce errors in the measurements so care must be taken in the collection and sectioning of the adipose tissue.

In cases where one wishes to classify adipocytes in sectioned material as either white or brown/beige/brite, we have developed a simple staining procedure followed by an analysis protocol to semi-automate this process. Sectioned adipose tissue is stained using imunofluorescent methods in conjunction with a fluorescently-labeled wheat germ agglutinin to demonstrate lipid membranes and cellular membrane, respectively. These fluorescently labeled slides can then be scanned, regions exported, and another freely available analysis platform (CellProfiler) used to segment the labeled adipocytes into WAT or BAT categories.

Adipose cell sizing workflow: Raw image processed with ImageJ, binary threshold applied, and individual adipocytes identified. Data can be exported for further analysis and presentation.

There are advantages and disadvantages to using histological approaches such as these. Advantages include the ability to do cell sizing and characterization on archived formalin-fixed tissue samples as well as the minimal technical and
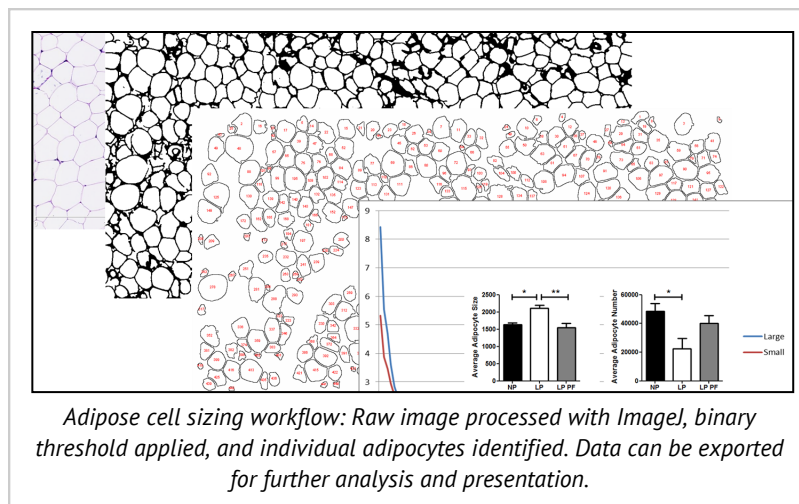
CELL BIOLOGY & BIOIMAGING CORE, from page 3

infrastructure needs. Some disadvantages include the necessity for well-fixed and subsequently intact sections for the image analysis – damaged tissue and broken cell membranes result in the need for more stringent image analysis thresholds to 'throw out' broken cells or compressed areas. Additionally, the examination of two-dimensional sections introduces some potential bias as adipose tissue is not a completely homogenous tissue – variability exists within the fat pads in terms of local size variations and WAT/BAT characteristics. It would be prudent to examine several sections from disparate regions of a pad to generate more realistic profiles of the cell size. Lastly, whole slide analysis requires the manipulation of very large images and requires considerable computing power – our current protocol involves the analysis of whole slides at a 5x magnification to balance the need for large area examination with computing response. If one needs to examine objects below 20 microns,

analysis of 10 or 20x image data would be required but would necessitate pre-selection of ROIs.
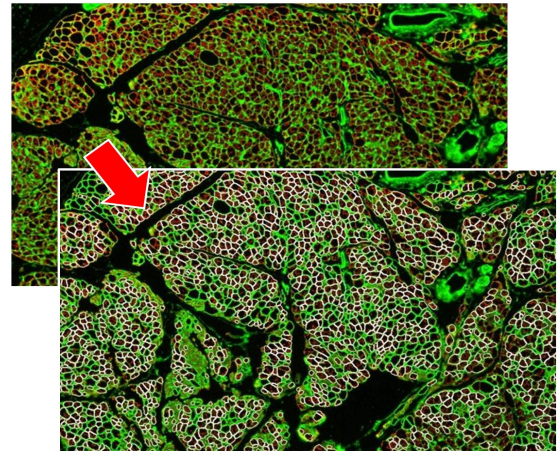
Classification of WAT/BAT using immunofluorescent staining, digital slide scanning, and automated classification with CellProfiler.

HUMAN PHENOTYPING CORE
Food Photography & Computer Imaging: The Use of Innovative Technologies in Pennington Biomedical Studies
By Candice Myers
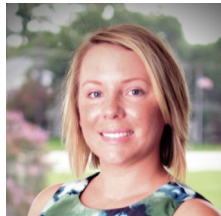
Energy intake is an integral component of the energy balance equation. To put it more simply: what people eat matters! Knowing what and how much people eat, or 'food intake,' is a necessary component of interventions to improve health and to help people manage body weight. To measure what people eat, two pieces of complementary technology have been developed to first collect and then analyze food intake data. These technologies are the result of collaboration between PBRC researchers, including Ray Allen, PhD and the Ingestive Behavior Laboratory, and engineering faculty and graduate students at Louisiana State University, including Bahadir Gunturk, PhD, Robert DiBiano, and Manal Abdelwahab, PhD. The first piece of technology is a smartphone application, or app, for iPhones and Droid phones. The SmartIntake© app captures food intake while people are in their natural environment using the smartphone's built-in camera to take pictures of foods before and after meals. The photos of foods are then analyzed with the help of a software program to estimate energy and nutrient intake, which is the second piece of technology developed by the research group.

SmartIntake© is currently being used in a number studies at PBRC to collect food intake data by study participants, and is also being used in a collaboration with Baylor College of Medicine in a project led by Theresa Nicklas, PhD. By leveraging the ubiquity of smartphones to quickly and simply take pictures of food, this app greatly improves ease-of-use for study participants and quality of food intake data for researchers. Indeed, the technology has greatly streamlined data collection procedures. A focus of the research group is the continual development and improvement of these technology-assisted methodologies to reduce participant and study personnel burden. Further, understanding what people eat is important for diet quality, weight management, and health and these innovative technologies are advancing research and interventions that aim to improve people's health.
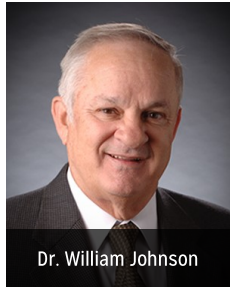
INTERNAL ADVISORY BOARD

Featured are the members of our NORC Internal Advisory Board. We would like to express our gratitude and acknowledge the contributions of our board members. They advise the Executive Committee in decision making and strategic planning, provide instruction and mentorship for our enrichment programs, and offer peer review support to our pilot and feasibility grant initiatives.
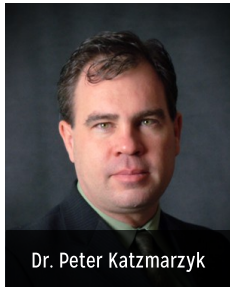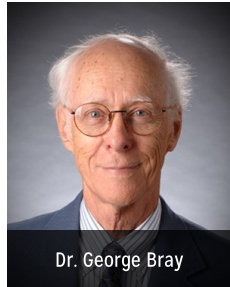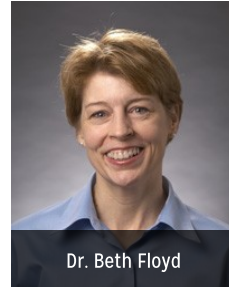
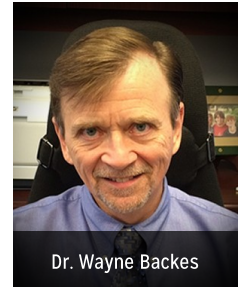Dr. Peter Katzmarzyk   Dr. William Johnson   Dr. Jacqueline Stephens   Dr. George Bray   Dr. Beth Floyd   Dr. Wayne Backes

UPDATE ON TRAINING

In June 2013, the Division of Education was awarded a competitive renewal of Pennington Biomedical's NIDDK sponsored T32 training grant entitled "Obesity: From Genes to Man." It is the third five-year training cycle for Program Director Phillip Brantley, PhD, Associate Director of the NORC. The renewal provides support for four slots per year.

Since the renewal in June, three new postdoctoral fellows have entered the Pennington training program.

Molly Matthews-Ewald, PhD, joined the Behavioral Medicine Lab with an interest in secondary and tertiary prevention of overweight and obesity through behavioral interventions.

Monica Klempel-Donchenko, PhD, became a member of the Reproductive Endocrinology and Women's Health Lab and is currently researching gestational weight gain, post-partum weight loss, and maternal/fetal energy expenditure.

New to the Central Leptin Signaling Lab, **Emily Qualls Creekmore, PhD** is looking at the role of hypothalamic neuropeptides in the motivation for nutrient specific food intake.

Molly Matthews-Ewald, PhD
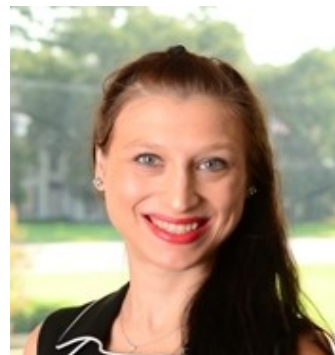
Monica Klempel-Donchenko, PhD

To fill the 4th slot, we are currently seeking an applicant with less than 5 years of postdoctoral experience who has evidence of research aptitude and a strong desire to become an independent obesity researcher. Eligible applicants must be a US citizen or green card holder. Please contact Dr. Brantley if you identify a strong candidate.

A total of 22 postdoctoral trainees have participated in the training program since it began in 2003. To date, faculty members of the NORC have served as primary or secondary mentors for trainees on the Obesity T32. Currently we have three regular slots and one supplemental slot filled (total of 4). Sixteen of the 18 trainees no longer with the program have continued a research career and are publishing. Nine have faculty positions with a research focus and three others have faculty positions that emphasis teaching but have a research component. Three have acquired k awards, four have non-NIH grants and others have applications pending.

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, from page 6

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.

OPTIMAL CLINICAL MANAGEMENT & TREATMENT OF CHILDHOOD OBESITY & TRANSLATION TO THE PUBLIC HEALTH CONTEXT

A Pennington Scientific Symposium was held October 28-29, 2013 on "Optimal Clinical Management and Treatment of Childhood Obesity and Translation to the Public Health Context." The Scientific Program Committee for this symposium included NORC members Claude Bouchard, PhD, Catherine Champagne, PhD, RD, Daniel Hsia, MD, Peter Katzmarzyk, PhD, Leanne Redman, PhD and Amanda Staiano, PhD. Fifteen distinguished scientists presented on drivers of pediatric obesity including genetics, adipose tissue development and epigenetic effects. Sessions were also devoted to a review of lifestyle, surgical and pharmacological treatment options for childhood obesity. A summary article of this NORC enrichment event is being prepared for publication.