NORC DIRECTOR ERIC RAVUSSIN RECEIVES PRESTIGIOUS WILLENDORF AWARD

Congratulations to NORC Director Eric Ravussin, PhD, who received the 2010 Willendorf Award from the International Association for the Study of Obesity on July 15, 2010 in Stockholm, Sweden. The prestigious Willendorf Award, given every four years, recognizes Dr. Ravussin's groundbreaking research that now spans almost three decades.

Dr. Ravussin's research focuses on the relationships between energy balance, body weight and composition, and metabolism. As noted by Pennington's Drs. George Bray, a previous recipient of the Willendorf Award, and Donna Ryan, "Dr. Ravussin is known as a creative and enthusiastic investigator who has been extremely productive during his entire career, and his publication record, mentoring ability and leadership skills are all characteristics of an eminent clinical investigator deserving of this award." ⊕

NORC CORE CAPABILITIES EXPAND

Human Phenotyping Core –
Imaging and Body Composition Subcore
William Cefalu

Bigger and Better DXA
PBRC researchers are now able to assess body fat, skeletal muscle, and bone in human subjects who weigh more than 300 pounds thanks to the recent acquisition of a new state-of-the-art GE Lunar iDXA. The iDXA, using Dual-energy X-ray absorptiometry, is clinically validated to be used with patients up to 35 cm thick and up to 450 pounds.

The purchase of the new iDXA was initiated by Dr. Steve Smith, the original director of the NORC Human Phenotyping Core. After Dr. Smith left PBRC to start a new clinical research effort in central

Important Dates for NORC Pilot & Feasibility Grants

Friday, January 28, 2011
Announcement for P&F

Friday, February 18, 2011
Online submission of Letter of Intent due

Friday, March 25, 2011
Online submission of Full Application due

Thursday, May 5, 2011
Announcement of P&F winners

HUMAN PHENOTYPING CORE, from page 1

Florida incoming PBRC Executive Director Dr. Steven Heymsfield, who has a long term interest in the development of methods for evaluating body composition, agreed with Smith's choice and championed the purchase and installation of the new iDXA.

As former Director of the Human Body Composition Laboratory and Weight Control Unit at St. Luke's-Roosevelt Hospital in Manhattan, Heymsfield and his colleagues were the first to use CT scans to explore human body composition and the relationship of liver disease and obesity as well as analyses of body fat and skeletal muscle mass among living patients. As a result of his research, new skeletal muscle mass measurement methods were developed and imaging techniques refined.

PBRC Executive Director Steven Heymsfield, MD has been instrumental in acquiring the new iDXA, recently installed in the new 80,000 sq. ft. Clinical Research building.

Other Benefits of the GE Lunar iDXA
- The iDXA's direct digital staggered array monolith detector directly affects image quality and thus image analysis and resulting data. Most other systems convert analog to digital utilizing scintillators coupled to their detectors. This can distort the image and affects image quality.
- The iDXA utilizes a staggered array which eliminates 'dead space'. Many other DXA systems utilize a linear detector array resulting in a 'dead space' which distorts the image and impacts precision.
- The iDXA offers 11.6 detectors/cm. This provides more data points and is particularly important with regard to total body composition scans as the significantly increased data points result in two times the precision of other systems on the market. This means that it takes less time to

measure true metabolic change, studies can be completed faster, and fewer subjects would be required to obtain statistical significance.
- The iDXA has the lowest possible x-ray dosage on the market for bone density. This feature allows for repeated studies, even in young children.

High-sensitivity Infrared Camera

The Imaging and Body Composition subcore has recently acquired a high-sensitivity infrared camera (FLIR Inc. Model SC5000) to monitor skin temperature changes in response to interventions such as feeding or cold exposure. PBRC's Les Kozak, Ph.D. is using the camera in the Bucket Study - *Induction of Thermogenesis by Cold Exposure Monitored by Infrared Imaging: Proof of Concept.*

According to Dr. Kozak, recent studies have shown that brown adipose tissue (BAT) activity can be induced in young individuals by cold exposure. These studies have been conducted using the state-of-the-art method of positron emission tomography in combination with computed tomography (PET/CT). While PET/CT is currently the state-of-the art technology for measuring BAT, it use is limited by the exposure of volunteers to radiation. To acquire a technique that is less expensive, non-invasive, and nonradioactive so that repeated measurements can be made in children over the course of their development, Dr. Kozak and his close colleague Dr. Koza are testing the use of infrared (IR) thermal imaging to determine BAT activity.

Brown adipose tissue represents a natural target for the modulation of energy expenditure. When activated, it requires the uptake of substrate from the circulation, mostly free fatty acids, but also glucose. Improving the detection and measurement of the amount and activity of brown fat in humans could further knowledge of what
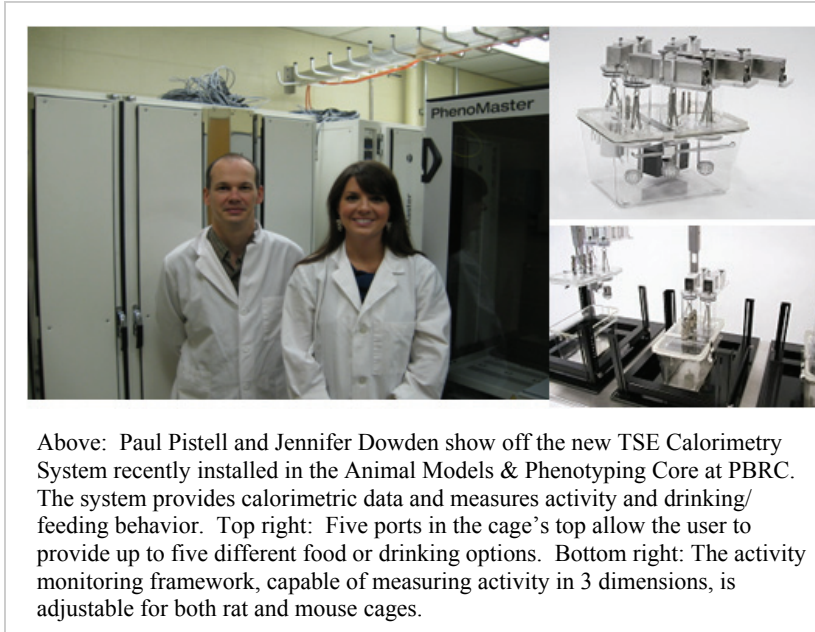
HUMAN PHENOTYPING CORE, from page 2

it does in the body and how to use it as a target for treating obesity and other metabolic disorders.

To observe the BAT, located primarily in the lower neck and supraclavicular region, baseline IR thermal imaging of the back is conducted on Bucket participants after they sit in the test room for 20-30 minutes to come to equilibrium with room temperature. In an effort to stimulate a thermogenic response in the BAT, the participants put their feet in a bucket of iced water for two minutes and the torso is scanned continuously to created a video record . The participants then undergo a seven-day cold acclimation induced by 15 minutes of daily exposure to a cold room (4°C/39° F) and are scanned again on day eight, both before and after immersing the feet in the bucket of iced water.

Above: Paul Pistell and Jennifer Dowden show off the new TSE Calorimetry System recently installed in the Animal Models & Phenotyping Core at PBRC. The system provides calorimetric data and measures activity and drinking/feeding behavior. Top right: Five ports in the cage's top allow the user to provide up to five different food or drinking options. Bottom right: The activity monitoring framework, capable of measuring activity in 3 dimensions, is adjustable for both rat and mouse cages.

In collaboration with Dr. Morteza Naraghi-Pour of the Electrical Engineering Department at LSU, the Pennington research team is quantifying the activity seen in the video of IR images with computer programs. A diagram of the back is divided into a grid and the infrared image is superimposed on the grid to try to determine where the heat changes occur. Says Kozak, "data from participant one is promising and indicates that we are measuring increases in thermogenesis but we don't yet know whether this is brown fat activity or just some other thermogenic mechanism in the individual. We currently have a grant application in to get funds to allow us to determine whether the areas of heat change coincide with a PET/CT scan, the current gold standard for picking up thermal activity in cells."

Animal Models & Phenotyping Core
Randy Mynatt

New Calorimetry System
The Animal Models & Phenotyping Core is excited to announce the arrival and installation of the new TSE Calorimetry System. Our system is set-up to handle 23 cages simultaneously. All of the cages are housed within environmental chambers (6 cages/chamber). One of the biggest advantages of the new system is the ability to use the animal's home cage by simply placing a sealed top over the cage. Using the same cage reduces the need for extensive habituation to a novel caging environment, as is the case with the existing systems. The system is capable of handling both rats and mice.

In addition to the calorimetric data, the system also measures activity as well as drinking and feeding behavior. The tops of the cages contain 5 ports which allow the user to provide up to 5 different food or drinking options for food or liquid consumption. Therefore, the system is capable of measuring food or liquid intake preference, or it can simply provide consumption data. Another advantage over the existing systems is that pelleted food can be used instead of requiring the food to be powdered.

Cell Biology & Bioimaging Core
David Burk and Barbara Kozak

New Confocal Expands Capabilities of Cell Biology and Bioimaging Core
A new state of the art Leica TCS SP5 tandem scanning multi-photon confocal has stirred excitement for the staff of the Pennington Center's Cell Biology and Bioimaging

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Core (CBBC). The new confocal is capable of very high speed live cell imaging and will save time and money for researchers.

Much like a conventional confocal, the SP5 is equipped with lasers for excitation of particular fluorophores (405, 488, 561 and 633 nm) as well as a tunable pulsed IR laser for multiphoton exciation (tunable from 680 nm to 1060 nm). The SP5 is also equipped with an AOBS (Acousto Optical Bean Splitter) which eliminates the need for standard dichroic filters and allows for increased transmission of excitation and emission light. Most importantly, the new SP5 confocal is equipped with a resonant scanner that greatly accelerates the speed at which one can collect data. The typical arrangement on a point scanning confocal can scan in the XY direction at 2800 Hz but only at a zoom of around 6.0. With the resonant scanner one can scan in XY at 16000 Hz at a zoom of 1.7 allowing for very fast frame rates (~25 fps at 512 x 512 pixels or ~250 fps at 512 x 32 pixels). By utilizing the resonant scanner one can collect large 3D sets much faster than with the standard scanner and leads to more data in less time. The resonant scanner also will allow researchers to examine the movement of fluorescently labeled objects in 3D over time instead of limiting their observations to a particular 2D plane.

In the past two years, CBBC has undergone significant expansion of its capabilities through acquisition of new instrumentation. The core provides state of the art bioimaging and analytical instrumentation and support to facilitate data collection and analysis by current and future Pennington PIs and their staff. CBBC staff is available to assist in the development of new protocols or validate existing protocols on their platforms.

CBBC consists of three divisions – imaging, histology/specimen preparation and flow cytometry. The imaging division consists of seven imaging platforms ranging from manually operated widefield microscopes for routine examinations of slides or culture plates to a state of the art multi-photon confocal capable of very high speed live cell imaging. The histology and specimen preparation section houses all the equipment needed for tissue processing, embedding, sectioning and staining of specimens as well as a new laser microdissection platform for collection of single cells or tissues of interest from fixed or fresh sectioned material. The flow cytometry division includes a four color analysis platform as well as a new high speed cell sorting system for the rapid isolation of cell populations of interest based on a particular staining profile.

CBBC staff members include Dr. David H. Burk, Director, Dr. Barbara Kozak, Associate Director, and two research associates – Ms. Courtney Cain and Mr. Drury Ingram. For additional information: http://labs.pbrc.edu/cellbiology/index.htm


The new state of the art Leica TCS SP5 tandem scanning multi-photon confocal is equipped with an easy to use interface for controlling PMT gain, zoom, z-position and a myriad of other parameters as well as an enclosure for maintaining physiological temperature, humidity and CO_2 concentrations at the stage for live cell imaging applications.
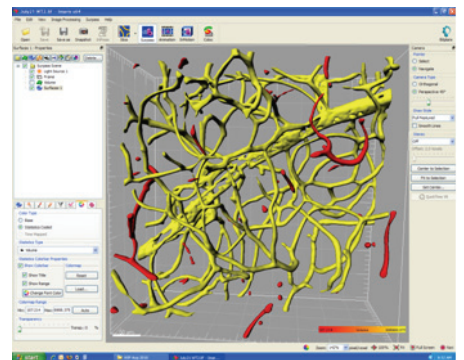
Imaging on the Leica TCS SP5 tandem scanning multi-photon confocal following the use of isolectin IB4 for labeling generates a 3D dataset from which volume data can be extracted.
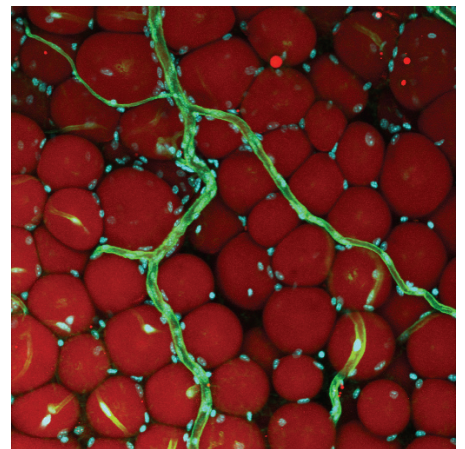
Mouse adipose tissue is labeled with Alexa 488 conjugated isolectin IB4 (capillaries in green), BODIPY (adipocytes in red) and DAPI (nuclei in cyan) then imaged on the Zeiss 510 META confocal.

Fluorescent conjugates of the isolectin, IB4, from Griffonia simplicifolia seeds provide a simple, one-step method for labeling microglial, perivascular and endothelial cells to visualize the vasculature in adipose tissue.

AN UPDATE FROM NORC MEMBER CORBY MARTIN

on his project "Remote Intervention for Diet and Exercise (RIDE)" using innovative methods developed and validated in a study funded by a 2006 NORC P&F award.

The RIDE study is nearing completion. Approximately two-thirds of the participants have completed the trial and the weight loss looks very good. People in the active weight loss group are losing substantial amounts of weight while people in the health education control group are not losing nearly as much weight.

RIDE is a P&F study that tests the efficacy of an e-health application at promoting weight loss. Data collected from participants while they live at home are sent to us automatically via wireless networks and the internet. We then evaluate those data and send the participant personalized treatment recommendations to help them eat healthier, exercise more and ultimately lose weight.

Novel technologies are used to collect three types of data with minimal patient burden. From their homes, participants weigh daily on a scale that automatically sends their data to their counselor at the Pennington Biomedical Research Center. To measure physical activity, participants wear an accelerometer that counts steps. The accelerometer fits on their shoe and every day the numbers of steps that they take is also sent to their counselor. The third piece of data is food intake information obtained from the Remote Food Photography Method (RFPM) developed and validated by our lab. The RFPM involves giving participants a smart phone to take pictures of their food selection and plate waste. These images are sent to us via the wireless network and are analyzed by dietitians trained in this method.

The RIDE e-health application offers advantages over other e-health interventions which rely on self-report data and can be used by physicians to monitor patients' health and weight loss. Having objective data in front of clinicians enables them to deliver more effective treatment by phone or email, saving time and money for themselves and their patients.

DEVELOPING A PLAN FOR CARING FOR THE SEVERELY OBESE

NORC scientists are assisting state health officials and legislators to address care for severely obese individuals insured by the Office of Group Benefits, Louisiana's managed medical insurance program for state employees and their dependents. Louisiana ranks second highest in the nation for obesity with a third of the adult population in the state being obese.

Responding to a legislative resolution, the Office of Group Benefits (OGB) is partnering with NORC researchers to develop a plan to address the challenges of severe obesity among its plan members. NORC members, Phillip Brantley (Chief, Behavioral Medicine Laboratory) and Donna Ryan (Associate Executive Director, Clinical Research) of the Pennington Biomedical Research Center (PBRC) are coordinating investigative and planning efforts aimed at producing a report to the legislature during its spring 2011 session. This effort follows earlier successful collaborations involving NORC members and the OGB.

Valerie Myers (Behavioral Medicine Laboratory at PBRC) led a study that determined long term health outcomes and costs of providing obesity surgery to a group of severely obese clients of the OGB. Donna Ryan was principal investigator on the well known LOSS Trial that examined costs and health outcomes of providing non-surgical, comprehensive medical management to severely obese OGB plan members. These and similar projects of our NORC support the mission of our center to translate basic findings into the clinical arena and ultimately into practical application.

AN UPDATE FROM NORC MEMBER CHRIS MORRISON

on his project "Maternal dietary fat independent of obesity predisposes offspring to obesity" which began as a NORC pilot grant in 2008.

Obesity represents a massive burden on the US health care system, and as such my research focuses on the physiological causes and consequences of obesity. Our NORC project centers on the maternal environment and its impact on obesity predisposition in offspring. Using animal models, we and others demonstrate that if a group of similar females are divided prior to breeding, such that half are fed a low fat (LF) diet and half a high fat (HF) diet, the subsequent offspring from the HF-fed mothers exhibit increased body weight and adiposity in adulthood. Thus there is some feature obese maternal environment that induces a persistent programming effect on the offspring, increasing their risk for obesity. Over the past year we have specifically focused on the early postnatal period, particularly on the development of key brain areas that are associated with the regulation of food intake. These neuronal populations do not appear to fully develop until the first 2-3 weeks of life, and our project tested whether offspring from obese mothers exhibit alterations in the development of these neurons. Unexpectedly, we failed to detect any difference in neuronal development, suggesting that variations in neuronal development, at least in the limited populations we assessed, do not explain the differences in obesity predisposition in the offspring.

However, a separate series of experiments did identify a surprising result. This project focused on the possibility that the maternal environment could affect multiple generations. When female offspring from HF mothers (Generation 1) were allowed to mature and have offspring of their own (Generation 2), these second generation offspring were heavier, fatter, and exhibited insulin resistance if their grandmother was on the HF diet. Thus these offspring were influenced by the diet and metabolism of their grandmother, even though they themselves were never exposed to a high fat diet. These data suggest that the programming effects of maternal obesity can induce persist effects on multiple generations. These effects are not likely to be genetic (all the animals were similar prior to being exposed to the HF diet), and thus we will begin to focus on epigenetic or developmental changes that may explain these transgenerational effects of maternal obesity.

NEW AWARDS FOR PILOT AND FEASIBILITY STUDIES

Congratulations to the following researchers. Of six applications for NORC Pilot and Feasibility grants, two were funded. 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.

Darcy Johannsen, PBRC – *Metabolic traits of adult sib-pairs discordant for intrauterine diabetes exposure*

Individuals with a family history of type 2 diabetes mellitus (T2DM) are known to be at greater risk for developing the disease, and previous studies have shown that insulin resistance, mitochondrial impairment, and intramyocellular lipid accumulation are present in these individuals at a young age and prior to onset of the disease. The susceptibility for developing T2DM is influenced strongly by genetic factors (often in combination with ideal environmental conditions that promote its development); however, epigenetic factors may also play a role. The exposure to a diabetic environment in utero represents one such epigenetic factor, caused by altered nutritional exposure (i.e., high circulating glucose concentrations) that may result in fetal imprinting of the developing

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pancreatic, hepatic, and skeletal muscle tissue, in addition to others. To date, no study has attempted to measure metabolic and physiological markers in humans uniquely exposed to a diabetic intrauterine environment while controlling for environmental and genetic variables. Here we will measure insulin sensitivity, pancreatic β-cell responsiveness, energy expenditure, substrate oxidation, abdominal adipose tissue and skeletal muscle mitochondrial function in young adult sibling pairs who were born of the same mother and father and raised together, but who are discordant for intrauterine exposure to diabetes (i.e., the mother did not have type 2 or gestational diabetes during pregnancy with the older sibling, but did with the younger sibling). Results from both sibling groups will be compared to those of a control group who have no family history of diabetes (i.e., this negative family-history group will serve as the genetic control). This will allow us, for the first time, to investigate the effects of in utero exposure (epigenetic programming), independent of genetic and environmental influences, on risk for type 2 diabetes.

Ji Suk Chang, PBRC – *Function of mitochondrial NT-PGC-1α mitochondrial DNA transcription*

Adaptive mitochondrial biogenesis and function in response to multiple physiological signals involve a complex but interconnected network of transcription factors and regulatory cofactors. While the basal mitochondrial transcription machinery is well studied, how mitochondrial transcription is modulated in response to physiological stimuli is largely unclear. Although PGC-1α plays an important role in integration of physiological signals to the wide range of transcriptional programs and in coordination of mitochondrial- and nuclear-encoded gene expression, additional key regulators would be expected to play a role in enhancing the mitochondrial gene responses. Our recent studies show that a novel PGC-1α isoform (NT-PGC-1α) and PPARγ2 are found in isolated mitochondria from mice BAT. We hypothesize that NT-PGC-1α and PPARγ2 directly regulate mitochondrial DNA transcription in mitochondria and that this direct involvement in mtDNA transcription allows more rapid and efficient adaptive responses of mitochondrial biogenesis and function. Under our proposed aims, we will determine submitochondrial localization of NT-PGC-1α and PPARγ2, association with the D-loop region of mtDNA, and the ability of mitochondrial NT-PGC-1α/PPARγ2 to enhance expression of mitochondrial-encoded genes. ⊕

Our Visiting Speakers

Part of the mission of the NORC is to drive new knowledge as well as learn from others. It is our pleasure and, ultimately to our benefit, that our colleagues take time to drop by and share with us their work. In the last months we have heard from:

Dr. Mark Mattson, Chief, Laboratory of Neurosciences, NIA, Baltimore, MD, "Dietary Energy Intake and Brain Health: A Challenging Situation"

Dr. Laurie Goodyear, Harvard Medical School, Joslin Diabetes Center, Boston, MA, "Novel Signals Regulating Glucose Transport: AMPK and Beyond"

Dr. Robert Henry, Dept. of Medicine, University of California – San Diego, "Update of Adiponectin Regulation"

Dr. David Sinclair, The Paul F. Glenn Laboratories for The Biological Mechanisms of Aging, Harvard Medical School, Department of Pathology, Boston, MA – "The Sirtuins: Ancient Survival Genes that Promote Defenses Against Diseases of Aging"

Dr. David Pettitt, Sansum Diabetes Research Institute, Santa Barbara, CA – "Long Term Effects of the Hyperglycemic Intrauterine Environment"

Dr. David Jenkins, Risk Factor Modification Centre, St. Michael's Hospital, University of Toronto, Canada – "The Glycemic Index, Dietary Portfolio and Fiber in the Treatment of Disease"