



CLINICAL NUTRITION RESEARCH UNIT

Newsletter

<http://cnru.pbrc.edu>

January 2008

Our Findings - CNRU Produces Significant Research

This year brought with it some significant findings within the CNRU. Here are the summaries:

Discovery of a model for human epigenetic programming.

The focus of the Clinical Nutrition Research Unit at Pennington Biomedical Research Center is Nutritional Programming. This theme addresses how environmental factors, especially nutrition, impact the genome across the lifespan, thus influencing susceptibility to overweight, obesity and related co-morbidities. One area of emphasis for the CNRU is how maternal influences on the developing fetus and on the infant predispose to the development of obesity, diabetes and cardiovascular diseases in adulthood. Work from the CNRU Genomics Core led to the development of a mouse model that can serve as an experimental model for human obesity development. In this model, mice that are genetically identical, exhibit variation in weight gain and susceptibility to obesity when exposed to a diet similar to the US diet. The susceptibility to weight gain has been indirectly linked to changes in DNA due to exposure during lactation, but not during gestation, to diets rich in methyl donors. Using sophisticated microarray technologies in the CNRU Molecular Mechanisms Core, scientists demonstrated that susceptibility to obesity is strongly linked to the genes associated with cell signaling in fat tissue. The studies suggest that the critical time period for nutritional programming for susceptibility to obesity is the period when fat tissue is being generated in infancy. The model will be used for further studies to investigate these novel observations and may lead to new insights into the origins of the current epidemic of overweight and obesity.

In another model, mice are exposed to running wheels and exhibit large variability of exercise associated with variable weight gain despite having 100 % of genes being identical.

Changes in gene expression foreshadow diet-induced obesity in genetically identical mice. Koza RA, Nikonova L, Hogan J, Rim JS, Mendoza T, Faulk C, Skaf J, Kozak LP. *PLoS Genet.* 2006 May;2(5):e81.

Discovery of a novel method to measure food intake.

One of the challenges of studying overweight and other nutritional problems is the difficulty of current methods to determine the quantity and quality of food intake. Food Record and Dietary Recall methods are traditionally used, but their accuracy is hampered because

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Scientific Discussions at Executive Meetings

PBRC's Executive Committee has invited CNRU scientists to attend some of their regularly scheduled monthly committee meetings. The first two scientists to present at Executive Committee Meetings were Les Kozak who presented on the topic of non-genetic variation in development of diet induced obesity at the January meeting, and Andrew Butler who discussed ongoing research on the function of the melanocortin-3 receptor.

Gathering of Our Peers to Examine Us

Our External Advisory Board met the day after the May symposium (May 23, 2007). Three of the four members were able to be present: John Miles (replaced David Kelley), Rudy Leibel and Dan Kelly. Carolyn Miles of the NIDDK also joined us for most of the day. We received feedback from two of the members on the progress of the CNRU to help us plan the strategy for the renewal process.

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they are time-consuming and intrusive for research participants. The CNRU Human Phenotyping Core developed a novel method that is quick, doesn't intrude with the activities of the research participants and provides an accurate estimation of food quantity and quantity. Digital photographs of plates or trays before and after eating take only seconds. Analysis by trained dietitians produces results that have been validated for accuracy, compared to the traditional methods. The technique has been used in school cafeterias and in military dining halls.

Martin CK, Newton RL, Jr., Anton SD, Allen HR, Alfonso A, Han H, Stewart T, Sothorn M, Williamson DA: **Measurement of children's food intake with digital photography and the effects of second servings upon food intake.** *Eat Behav* 8:148-156, 2007

Discovery of novel action of the fat cell hormone, adiponectin.

Recent data from several laboratories clearly shows that, in individuals with type 2 diabetes, the muscle tissue has a reduced number and function of mitochondria. Mitochondria are tiny organelles that produce energy and consume energy in cells. How and why muscle cells have reduced number and function of mitochondria is not known. Recent research by Dr. Anthony Civitarese [supported by the CNRU] shows that the fat cell hormone adiponectin increases the number of mitochondria in muscle and reduces the production of harmful free radicals Civitarese et al *Cell Met* 4; 1-13, 2006). This is important because this hormone is reduced in people with type 2 diabetes and in those with a family history of type 2 diabetes pointing toward adiponectin as a target to treat diabetes. Accordingly, mitochondrial biogenesis is increased after caloric restriction.

Civitarese AE, Carling S, Heilbronn LK, Hulver MH, Ukropcova B, Deutsch WA, Smith SR, Ravussin E: **Calorie Restriction Increases Muscle Mitochondrial Biogenesis in Healthy Humans.** *PLoS Med* 4:e76, 2007

Congratulations... Let the Research Begin

Corby Martin, Ph.D., has won an R21 by the NIH entitled "Validation of innovative technology to measure the energy intake of free-living humans." Proving the value of the CNRU in advancing new research, Dr. Martin's pilot data resulted from support from the CNRU and the Center's Health and Performance Enhancement Division pilot and feasibility grants. This 3-year award totals approximately \$672K.



Corby Martin, Ph.D.

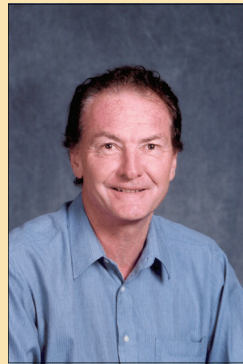
In Corby's words:

"Food intake is one of the primary culprits of weight gain, yet there are few methods to accurately measure food intake in free-living conditions. Consequently, it is difficult for scientists to study food intake, energy balance, and weight gain in free-living conditions, and clinicians have few tools to measure the food intake of patients. The "gold standard" for measuring food intake relies on the doubly labeled water method, but this method is costly, not available to most researchers and clinicians, and does not provide information on macronutrient intake. The proposed research will test the validity of the digital photography of foods method in free-living conditions. The digital photography method accurately measures food intake in cafeterias. When using this method, the plate of foods selected by an individual is photographed before the meal and plate waste is photographed after the meal. Standard portions of known quantities of the foods are also photographed and registered dietitians use these photographs to estimate food intake. The purpose of the proposed research is to develop and test innovative technology that will facilitate the collection of accurate food intake data in free-living humans using the digital photography method. Participants will take photographs of their food selection and plate waste using cell phones and these data will be transferred to the researchers over a cellular network in near "real-time." A semi-automated computer application will be developed to automatically identify the foods in these pictures and estimate the amount of food eaten based on the pictures. During the proposed project, this computer application will be developed and the reliability and validity (accuracy) of the method for estimating food intake will be tested in laboratory and free-living conditions. This research promises to significantly advance the study of energy balance and provide a useful tool to clinicians for measuring food intake."

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Congrats are also in order for **Eric Ravussin, Ph.D.**, who recently received a 5-year competitive renewal award from the NIH (NIDDK). This award is an expansion of his previous Fat Cell award and is entitled "Fat Cell Size, Overfeeding and Ectopic Fat" This 5-year award totals approximately \$2.58M.



Eric Ravussin, Ph.D.

Eric summarizes this way: "Obese people with enlarged subcutaneous abdominal adipocytes are more hyperinsulinemic and glucose intolerant than those with similar degrees of adiposity but with relatively smaller adipocytes. Independent of obesity, insulin resistance and acute insulin secretion, subjects with larger fat cells are more likely to develop type 2 diabetes than are subjects with smaller fat cells. Subjects with impaired adipogenesis cannot recruit new adipocytes to store the excess dietary fat and therefore accumulate fat in other tissues, such as skeletal muscle and liver causing insulin resistance in these tissues. Muscle lipid accumulation and the related insulin resistance are not only due to a lack of lipid storage capacity in the adipose tissue, but also to impaired fat muscle oxidation. The purpose of our competitive renewal is to characterize the morphological and metabolic characteristics of both adipose and muscle tissues (2-hit hypothesis of ectopic fat) predisposing to ectopic fat deposition and insulin resistance before and after 8 weeks of overfeeding. The overarching hypothesis is that overfeeding will significantly increase ectopic fat deposition, insulin resistance and decreased muscle oxidative capacity in individuals with hypertrophic adipocytes more than in individuals with hyperplastic adipocytes. We plan to screen 100 overweight volunteers (25<BMI<30) including fat cell size and overfeed for 8 weeks 20 with hypertrophic adipocytes and 20 with hyperplastic adipocytes."

CNRU Increases Capabilities

Now that the CNRU is picking up significant speed and accomplishments, soon we won't be able to list all the advances made possible by the grants, research programs and stimulation by the CNRU, but here a few of the latest improvements:

Molecular Mechanisms (Les Kozak/Jeff Gimble/Tom Gettys)

- Developed a new website for users of the Genomic Sub-core.
- Established new technology for high throughput purification of genomic DNA from human blood.
- Established the Bioimaging Sub-Core within the Molecular Mechanisms Core.
- Developed Quality Assurance/Quality Control validation of individual lots of human adipose derived stem cells based on differentiation and flow cytometric immunophenotype.
- Established both high throughput and single cell measurement of cell signaling through calcium mobilization.
- Established instrumentation and user support within the Bioimaging Core for quantitative flow cytometry on the BD FACScalibre.
- Established instrumentation and high level user support within the Bioimaging Core for two photon confocal microscopy on a Zeiss LSM-510meta imaging platform.
- Established instrumentation and software support for high resolution wide field imaging and data analysis.

Human Phenotyping (Steve Smith/Donald Williamson)

- Development of mitochondrial capacity (ATP_{max}) using ^{31}P MR Spectroscopy
- Development of intrahepatic and intramyocellular lipid measurements (IHL/IML) using 1H MR Spectroscopy
- Development of measurement of organ volumes and visceral adipose tissue by MRI
- Development of skeletal muscle mitochondrial enzyme assays using HPLC detection
- Development of new methods in whole-room indirect calorimetry that allow for measurement of RQ 'flexibility'
- Development of a new measure of insulin sensitivity using 6,6 2D glucose during an oGTT.
- Development of a novel method to measure food intake in free-living humans with the digital photography of foods methodology.

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Animal Model & Phenotyping (Andrew Butler/Randy Mynatt)

- Development of a “turn key” service for generating conditional knockout mice.
 - Hired a postdoctoral fellow, Jingying Zhang, PhD, who has assisted in the development of methodology generating for mice with floxed alleles. Dr Zhang has initiated a time saving recombinering strategy for introducing lox P sites into target genes.
 - The core has obtained Albino and “normal” B6 ES cells for generating knockout mice on a pure B6 background. We have produced chimeric mice carrying floxed alleles for carnitine acetyl transferase and carnitine palmitoyltransferase I.
- Hired research associate to operate and maintain metabolic and behavioral core equipment.
 - Hired a Research Associate, Marla Gomez, who has assisted with the operation of the core equipment.
- Purchased a second system for the simultaneous measurement of physical activity, food intake, and oxygen consumption.
- Purchased a Rat NMR machine.

The Way It All Starts

On the heels of the success of previous CNRU pilot and feasibility grants, come the latest round of winners for 2007/2008. We're expecting great things from these colleagues in their pilot studies:

- Deep Dixit, Ph.D. - Adipose Resident T cells and Regulation of Cellular Immune Responses
- Rob Koza, Ph.D. - Development of in vitro and in vivo models to study Mest and adipogenesis
- Christopher Morrison, Ph.D. - Maternal dietary fat independent of obesity predisposes offspring to obesity



Deep Dixit, Ph.D.



Rob Koza, Ph.D.



Chris Morrison, Ph.D.

Gathering of Our Peers To Examine “Epigenetic Mechanisms in Obesity”

One of the highlights of the CNRU sponsored events was the organization of a symposium on *Epigenetic Mechanisms in Obesity: Research & Public Health Implications, May 20 - 22, 2007*. It was funded by 5 corporate donors and a grant from the Pennington Biomedical Foundation. This symposium, attended by Dr. Carolyn Miles and many Pennington and Louisiana scientists, was chaired by:

David Barker, M.D., Ph.D.

Professor
Clinical Epidemiology
Oregon Health and Science University

Robert Waterland, Ph.D.

Assistant Professor
Departments of Pediatrics and Molecular and Human Genetics
Baylor College of Medicine
USDA Children's Nutrition Research Center

Kenneth Eilertsen, Ph.D.

Associate Professor
Nuclear Reprogramming and Epigenetics
Pennington Biomedical Research Center
Louisiana State University System



The May 2007 John S. McIlhenny Series of the Pennington Scientific Symposium: “Epigenetic Mechanisms in Obesity: Research & Public Health Implications”

CNRU Helps Center Recruitment

As the CNRU grows, it stimulates a parallel growth in our Center's nutritional research base. For example the CNRU was instrumental in the recruitments of:



Drs. Claudia Kapfen and Michael Salbaum, both with expertise in maternal biology and nutritional programming.



Dr. Deep Dixit who was influenced in his final decision by the tools that our CNRU scientific cores can provide to scientists.



Dr. Tim Church who will provide services for intervention studies and fitness testing



Dr. Conrad Earnest who is an exercise physiologist



Dr. Don Ingram who is known for his caloric restriction studies for longevity



Dr. Tom Burris an international expert in nuclear receptors

Our Visiting Speakers

Part of the mission of the CNRU is to drive new knowledge as well as learn from others who are in the driver's seat.

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:

- Deborah Muoio from Duke University Medical Center, Durham, NC, on the subject of "Mitochondrial function and insulin action in skeletal muscle;"
- Susan Fried of the University of Maryland on "Regulation of adipokine production and secretion;"
- James Kirkland of Boston University asked, "Does aging make fat go MAD?"
- and Cinzia Allegrucci of the University of Nottingham, UK, let us peer into "Human embryonic stem cells and epi-

Acknowledging CNRU in publications

Friendly reminder - In the manuscripts you submit, please add under the *Acknowledgements*:

"This work was partially supported by a CNRU Center Grant # 1P30 DK072476 entitled *Nutritional Programming: Environmental and Molecular Interactions*" sponsored by NIDDK."