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.


**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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they are time-consuming and intrusive for re-
search participants. The CNRU Human Pheno-
typing 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 die-
titians produces results that have been validated
for accuracy, compared to the traditional meth-
ods. The technique has been used in school cafe-
terias and in military dining halls.

Martin CK, Newton RL, Jr., Anton SD,
Allen HR, Alfonso A, Han H, Stewart T,
Sothern M, Williamson DA: Measure-
ment of children's food intake with digi-
tal photography and the effects of sec-
ond servings upon food intake. Eat Be-
hab 8:148-156, 2007

Discovery of novel action of the fat cell hor-
mone, adiponectin.

Recent data from several laboratories
clearly shows that, in individuals with type 2 dia-
betes, the muscle tissue has a reduced number and
function of mitochondria. Mitochondria are tiny
organelles that produce energy and consume en-
ergy 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, mitochon-
drial biogenesis is increased after caloric restric-
tion.

Civitarese AE, Carling S, Heilbronn LK,
Hulver MH, Ukropcova B, Deutsch WA,
Smith SR, Ravussin E: Calorie Restric-
tion 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 En-
hancement Division pilot and
feasibility grants. This 3-year
award totals approximately
$672K.

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 bal-
ance, and weight gain in free-living conditions, and cli-
nicians have few tools to measure the food intake of pa-
tients. The “gold standard” for measuring food intake re-
dies 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. Stan-
dard portions of known quantities of the foods are also
photographed and registered dietitians use these photo-
graphs to estimate food intake. The purpose of the pro-
posed research is to develop and test innovative technol-
ogy that will facilitate the collection of accurate food in-
take data in free-living humans using the digital photogra-
phy 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 reli-
ability and validity (accuracy) of the method for estimat-
ing food intake will be tested in laboratory and free-living
conditions. This research promises to significantly ad-
Vance the study of energy balance and provide a useful
tool to clinicians for measuring food intake.”

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CONGRATULATIONS, from page 2

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 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 those 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.”

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 FACS scalibre.
- 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 31P 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.

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:

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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 recombineering 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

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

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.”