NUTRITION OBESITY RESEARCH CENTER

DECEMBER 2016

PENNINGTON BIOMEDICAL RESEARCH CENTER LSU

TRAINING UPDATE

Our NIDDK T32 postdoctoral training grant entitled "Obesity from Genes to Man" filled its remaining two fellow slots for this grant cycle. We successfully recruited Nicole Fearnbach, PhD from the Department of Nutritional Sciences at Penn State University who plans to pursue training and research in neural and cognitive mechanisms underlying ingestive behavioral working with Drs. Owen Carmichael and Corby Martin. We also recruited Kara Marlatt, PhD, MPH from the departments of Exercise Physiology and Public Health at the University of Minnesota and she plans to pursue training and translational research in obesity and diabetes in the laboratories of Drs. Eric Ravussin and Leanne Redman. This brings us to a total of 27 fellows admitted to our Obesity T32 (14 in basic science, 10 in clinical science and 2 in population science). We will apply for our third competitive five year renewal in May of 2017.

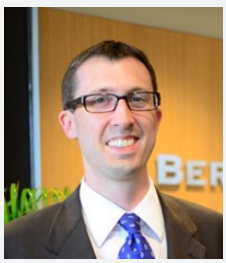
IN THIS ISSUE:

NEW AWARD FOR PILOT AND FEASIBILITY STUDIES

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. Below is the most recent P&F winner.

Effect of carnitine supplementation on liver mitochondria fatty acid processing

Owen Carmichael, PhD



Lipid over supply in bodily organs causes or worsens insulin resistance via multiple mechanisms involving the accumulation of lipids in multiple tissues. Agents that lessen accumulation of these lipids and their by-products are highly sought after, because they could be beneficial in the treatment or prevention of insulin resistance— the key step leading to type 2 diabetes. Carnitine is a nutritional supplement that is available over the counter and has the potential to reduce lipid over supply. It does so by enhancing mitochondrial fatty acid processing (MFAP): the ability of the mitochondria in cells to import and export fatty acids in an efficient manner. Prior data from Pennington Biomedical suggests that giving insulin-resistant mice carnitine enhances their MFAP, including import to, and export from, the mitochondria. But to date, data in humans on the efficacy of carnitine supplementation has been inconsistent. It is not clear whether carnitine supplementation in humans effectively raises levels of stored carnitine in organs such as the skeletal muscle, nor is it clear that carnitine supplementation improves MFAP.

This project will overcome this limitation by using state of the art stable isotope and magnetic resonance spectroscopy (MRS) techniques to characterize stored carnitine and MFAP before and after oral carnitine supplementation in 16 insulin-resistant adults. Before and after 14 days of oral carnitine supplementation or placebo, a novel MRS exam will measure skeletal muscle carnitine concentration, and a fructose challenge during infusion of 13C-lableled acetate will measure liver de novo lipogenenesis (DNL) as a measure of MFAP. Data from this study could clarify whether the appropriate next step for carnitine supplementation is to examine its effects in a more targeted population (i.e., those with confirmed low levels of stored carnitine at baseline, and/or confirmed MFAP deficit at baseline), reformulate it to enhance its storage, or better understand biological pathways preventing it from enhancing MFAP. This data is critical to acquire before launching a larger-scale clinical trial of carnitine supplementation as a preventive strategy against insulin resistance and type 2 diabetes.

NEWS FROM THE HUMAN PHENOTYPING CORE

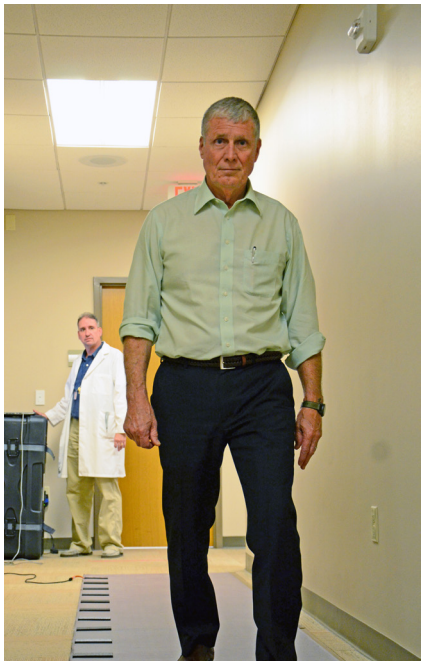
Heart Health, Brain Health, and Finding a Treatment for Alzheimer's Disease

Alzheimer's disease (AD) is currently the 6th leading cause of death in the United States, and the incidence of AD over the next 35 years is expected to triple from 5 million cases to 15 million cases*. Currently there are no treatments or disease modifying medications for AD. The number one risk factor for AD is advancing age, with 1 in 7 individuals over the age of 65 having AD*. A seemingly endless number of risk factors for AD have been described including genetic risk factors, chronic medical conditions, and lifestyle choices.

A number of advances in AD have occurred in the last 10 years, and one of the most notable is our understanding of how a number of chronic medical conditions appear to increase the risk of developing AD. For example, hypertension (high blood pressure) is associated with increased risk for developing AD later in life, particularly when hypertension begins in someone at 40-50 years of age. It should not be surprising that hypertension promotes AD and other neuropathological conditions when it is well established that hypertension promotes pathology and dysfunction in other organs such as heart or kidneys.

Another interesting thing to come out of AD research in the last 10 years is that we are beginning to learn that AD is probably not one single disease, but rather is likely a composite of multiple disorders. In this model there will likely eventually be the designation of AD type 1, AD type 2, etc. These designations will represent the known differences in AD patients in terms of how fast the disease progresses, how many behavioral disturbances arise in individuals over the course of the disease, how individuals respond to medications, and the age at which AD becomes manifest. Interestingly, we are starting to learn that the different "forms" of AD are likely to be caused by differences in the amount of brain pathology caused by hypertension and related conditions. For example, diabetes with hypertension is likely to cause a certain profile of vascular disease in the brain that is distinct from an individual who exhibits hypercholesterolemia (high cholesterol). It is very likely in the near future that the presence of hypertension, diabetes, and hypercholesterolemia (and other cardiovascular risk factors) will be an integral part of the diagnosis and management of dementia in the elderly.

Clinical trials which use a placebo control and randomize individuals to either treatment or placebo groups have demonstrated that the regulation of hypertension through medication and/or exercise has many beneficial effects including lowering overall morbidity, increasing lifespan, decreasing the frequency of heart attacks, and preserving kidney function. Many of these same studies include ancillary projects to look at brain function in individuals receiving either placebo or intervention for hypertension and found evidence for the tight regulation of blood pressure improving cognitive function and delaying the development of dementia in the elderly*. Despite the exciting nature of the results from these ancillary studies, they have had little impact on our detailed understanding of the cause and effect relationship between hypertension and dementia. This paucity in our understanding of this clinical research area is because these ancillary cognition studies were add on studies to a clinical trial focused on cardiovascular function, mortality, or kidney function. This means the ancillary studies lacked the proper clinical measures, statistical power, and other study design features necessary for accurately understanding definitive cause and effect relationships.



In October of 2016 the American Heart Association released a statement on the importance of accurately determining once and for all the relationship between hypertension and dementia risk in the elderly**. In the same month an NIH funded study was initiated at the Pennington Biomedical Research Center examining the links between hypertension and dementia risk in non-demented elderly individuals. This study is being done at 4 different sites including Pennington Biomedical, University of Texas Southwestern, Kansas University, and Washington University (www.clinicaltrials.gov/ct2/show/NCT02913664). The goal of this risk reduction for Alzheimer's disease (rrAD) study was to place elderly individuals (60-80 years of age) who

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have hypertension (including those currently taking hypertensive medications) and place them on different 2 year interventions involving exercise, medication management of hypertension, or exercise plus medication. Individuals interested in learning more about the rrAD study are encouraged to contact the Pennington Biomedical Research Center at 225 763 2973 or the dementia@pbrc.edu email.

**Alzheimer's Association 2016 Facts and Figures. Alzheimer's & Dementia 2015; 11(3)332: 1-88.*

***Costantino Iadecola, Kristine Yaffe, José Biller, Lisa C. Bratzke, Frank M. Faraci, Philip B. Gorelick, Martha Gulati, Hooman Kamel, David S. Knopman, Lenore J. Launer, Jane S. Saczynski, Sudha Seshadri, Adina Zeki Al Hazzouri and on behalf of the American Heart Association Council on Hypertension; Council on Clinical Cardiology; Council on Cardiovascular Disease in the Young; Council on Cardiovascular and Stroke Nursing; Council on Quality of Care and Outcomes Research; and Stroke Council Hypertension. 2016;68:000-000. DOI: 10.1161/HYP.0000000000000053*

NEWS FROM THE MOLECULAR MECHANISM CORE

The mission of the NORC Molecular Mechanism Core is to serve as bridge between the Animal Phenotyping Core and the Human Phenotyping Core. The two components of the Molecular Mechanism Core - Genomics and Bioimaging – together provide the technologies to conduct investigations on the molecular, cellular, and histological level.

The NORC Molecular Mechanisms Core will be playing a vital role in a recent NIH award to Dr. Heike Munzberg-Gruening entitled "Genetically-based Neuro-modulation of Adipose Tissue Functions" which is within the NIH Common Fund's Stimulating Peripheral Activity to Relieve Conditions (SPARC) program. This 2-year, $1.2 million grant aims to evaluate the feasibility of using neurotrophic viral vectors to selectively transfect neurons of the sympathetic nervous system that innervate peripheral adipose depots. Initial aims of the project will make use of the Bioimaging Core to generate anatomical and functional maps of the mouse peripheral autonomic nerves that innervate both white and brown fat tissue. These data will also guide tissue acquisition via laser microdisection for molecular analyses. In parallel, the Genomics Core will assist to generate gene expression profiles of these adipose tissue-specific sympathetic neurons, with the notion that genes with specific expression profiles may serve as future tools for targeting future interventions.

In order for the Bioimaging Core to generate images of whole adipose or overview images of intact mouse spinal column, the tissues are processed and ultimately cleared using a protocol called iDISCO. Generally, immunofluorescent images of tissues are collected by staining thin sections of material with specific antibodies that recognize, for example, tyrosine hydroxylase (TH, an enzyme present in nerves of the peripheral nervous system). By examining many slices of stained material from a fat pad or region of a spine, once can begin to generate a map of where these neurons are in three-dimensional space. By staining whole pieces of tissue and then rendering that tissue transparent by treatment with solvents to remove lipid and normalize the refractive index of the tissue we can generate 3D images of the TH-positive nerves and maintain easily identifiable landmarks such as the spinal cord and ribs by using the core's Leica SP5 confocal microscope. Offline rendering software allows for the generation of virtual cross-sections through the imaged tissue as well as animations to help convey the exact locations of these targeted nerves in a larger context.

SPARC: Genetically-Based Neuro-Modulation of Adipose Tissue Functions

Sponsor: NIH Office of Strategic Coordination – The Common Fund

Investigators: Heike Muenzberg-Gruening, Ph.D. (PI), Hans-Rudolf Berthoud, Ph.D. (Co-Investigator), Sangho Yu, Ph.D. (Collaborator), J. Michael Salbaum, Ph.D. (Collaborator), David Burk, Ph.D. (Collaborator), Jacqueline Stephens, Ph.D. (Collaborator), Robert Noland, Ph.D. (Collaborator), Randall Mynatt, Ph.D. (Collaborator)

Summary: This proposal will generate anatomical and functional maps of the peripheral autonomic nerves innervating fat tissue that is based on the genetic makeup of neurons. These maps will be verified in human tissue and aim to improve the functional precision of electrical stimulation

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protocols to improve conditions in humans. They can ultimately be applied to many other end organs and will enhance the molecular-genetic toolbox to gain a comprehensive understanding of the functional specificity of the autonomic nervous system.

Use of NORC Resources: Bioimaging Core confocal microscope, laser microdissection system, and image analysis workstation, Genomic Core SAGE RNA sequencing and qPCR platforms.

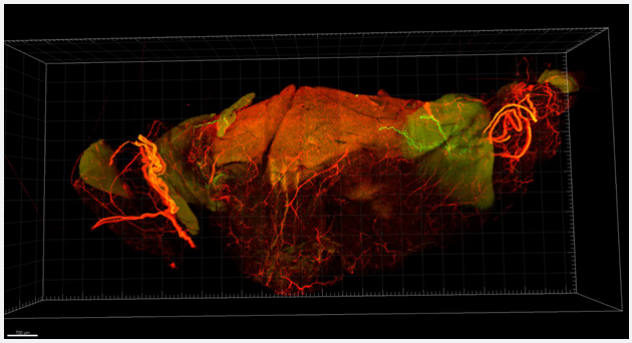


Figure X: Whole brown adipose fat pad from a mouse stained for tyrosine hydroxylase and anti-GFP. Scale bar in the lower left is 700 um.

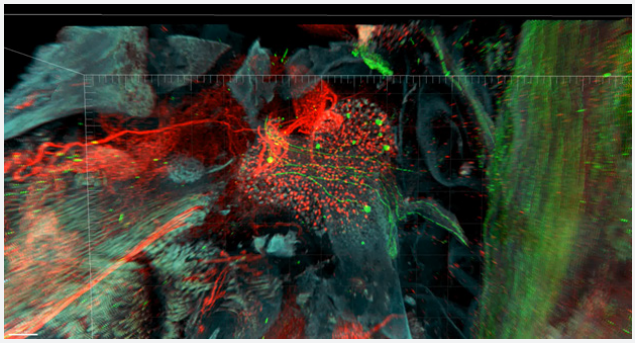


Figure Y: Center of image shows a dorsal root ganglion that contains green stained Thy1-positive neurons as well as red stained (TH-positive nerves). The green fibers running from top to bottom in the right side of the image are within the spinal cord and the cyan-colored material is autofluorescence from bone.

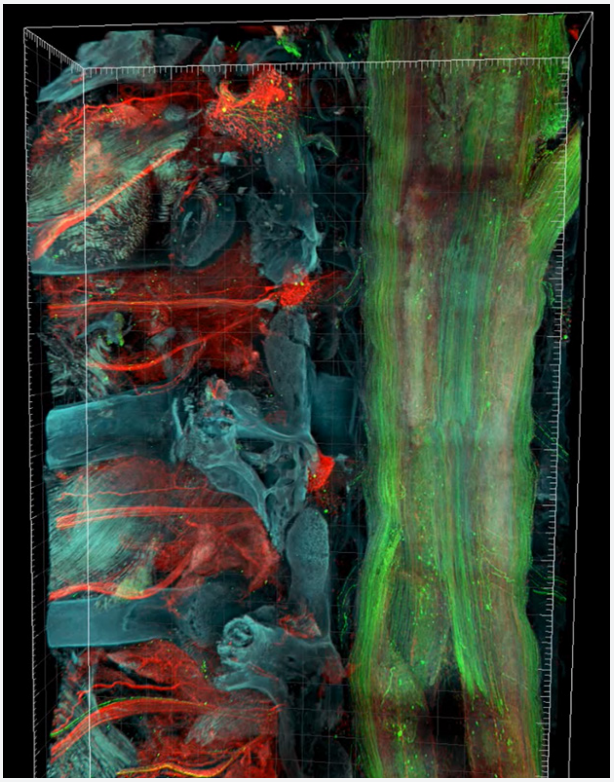
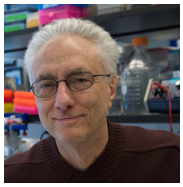


Figure Z: The dorsal root ganglion from FigY is at the top center of this lower magnification scan of the intact spinal cord and associated ribs and chain ganglia. By continuing to optimize our staining and clearing protocol we hope to image larger and larger whole tissue samples from mouse models.

EXTERNAL ADVISORY BOARD

Featured are the members of our NORC External Advisory Board. We would like to express our gratitude and acknowledge the contributions of our board members. Their advice and feedback are invaluable to the operation and strategic planning of the center.

Holly Ingraham, Ph.D.
Professor and Associate Vice-Chair,
University of California San Francisco



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