NUTRITION OBESITY RESEARCH CENTER

DECEMBER 2017

PENNINGTON BIOMEDICAL RESEARCH CENTER LSU

IN THIS ISSUE:

DR. ERIC RAVUSSIN - LIFETIME ACHIEVEMENT AWARD

Dr. Eric Ravussin, Ph.D., FTOS, was presented the 2017 Friends of Albert (Mickey) Stunkard Lifetime Achievement Award on November 1st during The Obesity Society Conference in Washington D.C.

The award is given in remembrance of Dr. Albert (Mickey) Stunkard and is designed to recognize people who, like Mickey, have made a lifetime of outstanding contributions to the field of obesity in terms of scholarship, mentorship and education.

ANIMAL MODELS AND PHENOTYPING CORE

The AMPC is pleased to announce additional equipment and upgrades which should be helpful to NORC users with an interest in feeding behavior and metabolism.

Sable Systems Promethion

Through a combination of institutional support and an NIH S10 equipment award, we were able to procure a Sable Systems Promethion Multiplexed Respirometry System. The Promethion System is an advanced, next-generation indirect calorimetry system, which allows investigators to simultaneously and continuously assess oxygen consumption, energy expenditure, respiratory quotient, activity, wheel running, food intake, water intake and body weight in a free-living, home cage environment. The metabolic analysis of mouse models is a key goal for the Core, and the Promethion System will markedly increase our capacity, thereby relieving existing bottlenecks and improving wait times. The system will also increase our capability, as the Promethion provides capabilities that were unavailable with our existing equipment. The Promethion system is now available for our users, and we expect the new equipment to have a significant impact on research productivity moving forward.

The Promethion provides the continuous assessment of energy expenditure, RQ, food and water intake, and in real time.

Upgrade to The BioDAQ System

The BioDAQ is a system for automatic assessment of ingestive behavior. The BioDAQ allows the continuous, automated measurement of food and fluid intake over time, while also providing a more sophisticated meal pattern analysis: meal size, meal number, intermeal interval, etc. Our original BioDAQ system was outdated, but through generous institutional support we have been able to upgrade to a 24 cage system with the most modern software and hardware design. This system can also be converted to 12 cages of two-choice assessment, where the

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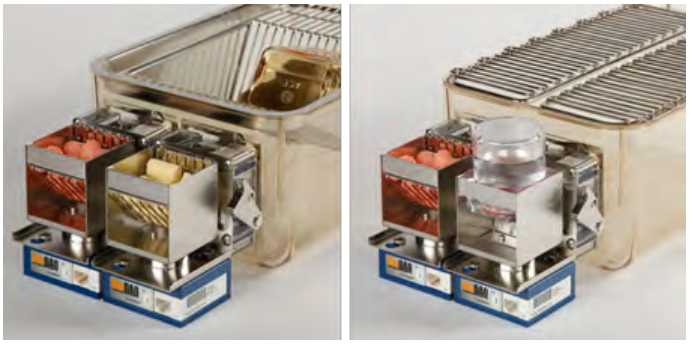
animals can choose between either two solid foods or a solid food and a liquid. Therefore, scientists can both assess feeding patterns in a single food experiment or compare meal patterns and choice between two diets simultaneously. The upgraded system will be much more powerful than our original system and should expand the type of ingestive behavior experiments that can be conducted in the core. We anticipate the upgraded BioDAQ to be available for use in early 2018.



The upgraded BioDAQ will allow assessment of feeding behavior with two food/fluid sources.

HUMAN PHENOTYPING CORE

MoTrPAC: Molecular Transducers of Physical Activity Consortium

Pennington Biomedical is one of six clinical centers across the country awarded funding by the NIH to study the benefits of exercise on health, specifically the molecular mechanisms behind these benefits. MoTrPAC is a national research consortium designed to discover and perform preliminary characterization of the range of molecular transducers (the 'molecular map') that underlie the effects of physical activity in humans.

The MoTrPAC Consortium is made up of the following:

- 7 Clinical Centers (1 Pediatric), consisting of 12 Clinical Sites
- Pennington Biomedical is one of the clinical centers within the Consortium
- 3 Animal sites
- 8 Chemical Analysis Sites
- Consortium Coordinating Center and the Bioinformatics Center: 4 institutions

To achieve the consortium's main goal of assessing molecular changes that occur in response to physical activity we will conduct a discovery randomized clinical trial. Pennington Biomedical will recruit sedentary and highly fit populations to participate in MoTrPAC locally. We expect to enroll up to 450 participants. The NORC-supported Human Phenotyping Core (Energy Balance and Behavioral) will be instrumental in executing the trial as designed via the protocol. MoTrPAC will encompass many units and facilities throughout Pennington Biomedical including but not limited to: Outpatient, Inpatient, Clinical Chemistry, Pharmacy, Imaging, Intervention, Exercise Testing, Metabolic Kitchen, and Data Management. Procedures during screening and follow-up visits for this study include anthropometrics, blood pressure, blood draws, questionnaires, physical activity and sleep monitoring via accelerometer, EKG, medical history and physical exam, whole-body DXA scan, muscle and adipose biopsies, stool collection, and exercise testing.

Pennington Biomedical MoTrPAC Investigators:

Eric Ravussin, PhD, Contact PI, is the Associate Executive Director for Clinical Science at Pennington Biomedical Research Center (Pennington Biomedical). He will be responsible for the overall scientific and administrative leadership of the MoTrPAC research project. He will assume the coordination of all components of the study and of the data analysis, publication, and other dissemination activities.

Tuomo Rankinen, PhD, Co-PI and Investigator, is an associate professor at the Human Genomics Laboratory, Pennington Biomedical. With Dr. Ravussin, he will organize all the necessary meetings for the smooth conduct of the study.

Neil Johannsen, PhD, Assistant Professor, Co-Investigator, will oversee the physical activity intervention and exercise testing teams and will be responsible for the management and integrity of all exercise data. He will also be responsible for the training and oversight of all the PA interventionists.

Robert Newton, PhD, Assistant Professor, Co-Investigator, will oversee the recruitment of subjects and specifically provide expertise as it pertains to enrolling and retaining minority populations.

Timothy S. Church, MD, PhD, MPH, Investigator, is the former Director of the Preventive Medicine Laboratory and current adjunct professor at Pennington and will be advising on the physical activity intervention as well as provide expertise as to the recruitment/retention of participants and management

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of the overall clinical trial. In addition, Dr. Church will provide valuable insight as to the management and analysis of study data.

William D. Johnson, PhD, Co-Investigator, is the Director of the Biostatistics Core at Pennington. He supervised the design of analysis plan for the proposed research and will also provide expertise in analysis of data, report writing, and presentation of results to the respective committees set up by the consortium.

Kishore Gadde, MD, Medical Investigator, will be responsible for medical screening of all participants at the onset of the study and clear them for the study. He will also provide medical supervision during the trial to assure overall safety of the participants. In addition, Dr. Gadde will provide valuable insight as to the management and analysis of study data.

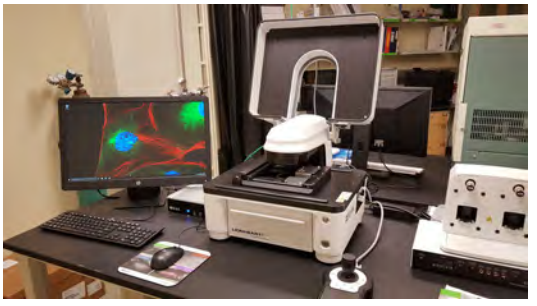
Future Impact:
Due to the complexity and breadth of the MoTrPAC trial, we anticipate several ancillary funding applications to be submitted to various funding agencies to expand upon the scientific exploration of the parent trial. To date, one ancillary project has been submitted to NIDDK by Drs. Corby Martin and Paul Laurienti and collaborators with a focus on psychological, behavioral and neurocognitive outcomes. With additional ancillary awards received, NORC supported core involvement will be expanded beyond the current scope of the parent trial.

Study Summary:
Despite the abundance of data to support physical activity (PA) as a key lifestyle factor that can prevent, delay, or reverse the onset of a variety of health-related conditions, the specific underlying physiological mechanisms and pathways by which this occurs are not fully established. The main goal of the Molecular Transducers of Physical Activity Consortium (MoTrPAC) is to assess molecular changes that occur in response to physical activity. The overarching goal is to assemble a comprehensive map of the molecular changes that occur in response to exercise in participants across the lifespan. This will be accomplished by characterizing the molecular responses to acute bouts of endurance or resistance PA in phenotypically well-described sedentary and highly fit individuals at baseline and after endurance or resistance exercise interventions in the sedentary group. The resulting molecular map is a resource that will enable the scientific community to accelerate both mechanistic research and clinical trials on the health benefits of exercise. In addition to the molecular map, other objectives include relating the exercise-induced molecular changes to the benefits of physical activity.

Approximately 3000 study participants will be enrolled in either an exercise intervention or control for a period of approximately 12 weeks. A total of 2,400 sedentary, healthy adult men and women will be randomized to control or exercise intervention in the form of endurance exercise or resistance exercise at a frequency of 4 sessions per week. The entire exercise intervention is conducted on-site and all sessions are supervised by trained and certified exercise interventionists. In addition, 300 highly fit endurance and resistance exercise participants will be exposed to an acute bout of exercise specific to their mode of training as comparison groups.

CELL BIOLOGY AND BIOIMAGING CORE

The Cell Biology and Bioimaging Core has recently installed a new fixed and live cell imaging platform – the BioTek Lionheart FX. This compact inverted imaging system, located in the main imaging suite (L4076), is equipped with phase contrast objectives, LED illumination for fluorescence, humidity and temperature control, and a dual-reagent injector for conducting fast kinetic assays on live cells. The system is capable of imaging cells or tissues mounted or grown in a wide variety of formats from T75 flasks to 1536-well plates. The system runs BioTek's newest image analysis software, Gen5Prime, which facilitates image stitching, image masking for automated analysis pipelines, and z-projection creation. The system will be available for training and subsequent unassisted use via the CBBC's iLab reservation system. If you have any questions about this new imaging system, please get in touch with Dr. David Burk @ david.burk@PenningtonBiomedical.edu.



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Labware Supported:

- 6- to 1536-well plates, microscope slides, petri dishes, T25 & T75 culture flasks, chambered slides

Environmental Control:

- Incubation to 40 °C, 0 – 20% CO2 and 1 – 19% O2 control, humidity control

X/Y Stage Resolution:

- 0.1 micron resolution

Imaging Models:

- Fluorescence, brightfield, color brightfield, phase contrast

Light Source/Fluorescence:

- High power LEDs for DAPI, FITC, TxRed, and Cy5

Camera:

- 16-bit gray scale, Sony CCD, 1.25 megapixel

Objectives:

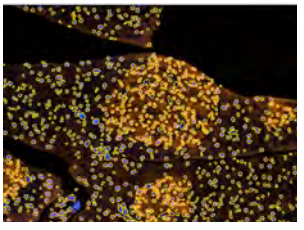
- 4, 10, 20, and 40x phase contrast for imaging slides and multi-well plates. Optional high NA dry and oil objectives could be added.

Autofocus:

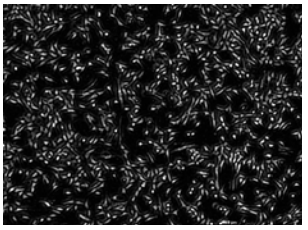
- Contrast/Image based and Laser autofocus

Reagent Injection System:

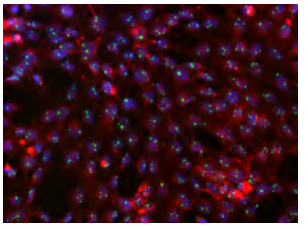
- 2 syringe pumps, supports 6- to 384- well plates, petri dishes, and chambered slides. Aligned tip for fast kinetic assays. Dispenses 5 – 1000 uL in 1 uL increments.



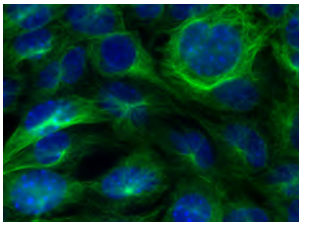
Gen5 software can automatically segment and identify objects (in this case, nuclei) for feature extraction and phenotypic analysis. Here, nuclei from a paraffin section of pancreas are located for quantification of nuclear-localized PDX-1 (orange stain).



Using a specialized 4x objective, unstained live cells can be accurately identified for quantification of cell counts and other metrics automatically. The graph (HighContrastCellCount) shows the increase in GT1-7 cell number over a period of almost 14 hours.



Mouse GT1-7 cells were cultured on ibidi chamber slides, stained for fibrillarin (green puncta in nuclei), DAPI, and membrane (red, wheat germ agglutinin). The sample was imaged using a 20x phase contrast objective.



Mouse GT1-7 cells were cultured in an adjacent well of the ibidi chamber slide but stained with mouse anti-tubulin antibodies (green) and counterstained with DAPI. The image was taken with a 60x oil-immersion objective.

2017 PILOT & FEASIBILITY AWARDS

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 are updates from our five P&F winners in 2017.

The effect of physical activity on in vivo and in vitro mitochondrial capacity in pregnant women

Nick Broskey, PhD, Pennington Biomedical Postdoc

Mitochondria are responsible for the creation of energy within virtually every cell in the body and are said to play a role in health and disease. We inherit our mitochondria solely from our mother. My NORC P&F, “The effect of physical activity on in vivo and in vitro mitochondrial capacity in pregnant women”, will test the hypothesis that women who exercise during pregnancy will have better functioning mitochondria compared to mothers who remain in a sedentary lifestyle throughout pregnancy. In turn, infants who inherit their mitochondria from exercising mothers will have higher mitochondrial function than infants who inherit their mitochondria from sedentary mothers. Mitochondrial function will be assessed in the skeletal muscle of mothers

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during pregnancy as well as in the stem cells of their infants. These stem cells, which are grown from the umbilical cord, are used as a model of fetal tissue because of their ability to turn into several different cell types of the infant.

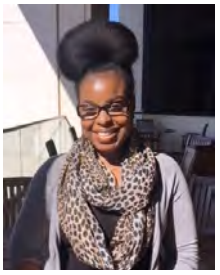
Transcriptome Changes in Skeletal Muscle During HuR Inhibition

Jaycob Warfel, PhD, Pennington Biomedical Postdoctoral Researcher

RNA is extensively used within cells as an information carrier molecule. Regulation of cellular RNA levels is essential for correct metabolic function as it dictates which RNA molecules are available to transmit information. HuR is an ubiquitously expressed protein that plays a major role in regulating RNA levels in many different cell types. Previous studies by our group indicated that metabolically unhealthy individuals display changes in HuR regulated RNA in skeletal muscle. We therefore created a murine model lacking the HuR protein in skeletal muscle cells and have found clear dysregulation of cellular RNA levels in this model. Further, these mice quickly become more insulin resistant and obese than their control littermates. With the help of the Pennington Nutrition Obesity Research Center pilot and feasibility funding, we aim to gain further insight into the extent of changes to the RNA expression patterns in the absence of HuR; and to determine the way in which these changes affect metabolite preference in skeletal muscle. We will assess these changes in both murine and human skeletal muscle in order to determine the translatable nature of metabolic dysfunction in an HuR depleted state. The results of these studies will then be used to secure future funding for projects emphasizing the importance of tissue specific RNA regulation on metabolic health.

Establishment of the ^{2}H-labeling protocol to assess *in vivo* adipose tissue dynamics at Pennington Biomedical

Ursula White, PhD, Pennington Biomedical Assistant Professor



Adipose tissue is an essential organ that is necessary to maintain whole-body energy homeostasis; and disruption of adipose function contributes to metabolic dysfunction. Adipose expansion, or adipogenesis, occurs to accommodate dynamic changes in energy balance and is characterized by enlargement of existing adipocyte size (hypertrophy) and increasing pre-adipocyte and adipocyte number (hyperplasia). Evidence suggests that the manner and magnitude of AT expansion (i.e. hypertrophy vs. hyperplasia) can be an important determinant of metabolic health.
Early studies proposed that adipose cell number in humans is established during adolescence and remains fixed during adulthood. However, recent data from our laboratory and others has shown that adipocytes are constantly formed and replaced during adulthood. A method has been developed to measure in vivo adipose kinetics and turnover rates, which is a substantive departure from previous indirect and in vitro approaches. This technique involves drinking deuterium (2H)-labeled water to increase body water 2H enrichment. The 2H from the heavy water is incorporated into the DNA of the dividing cells, as well as the lipid component, providing measures of cell formation, lipid synthesis, and adipose turnover.

We recently conducted an NIH-funded R01 clinical study to assess in vivo adipogenesis (i.e. new cell formation) in women with obesity using an 8-week incorporation of deuterium (2H), from drinking 2H20, into the DNA of adipose cells ("Apple & Pear" study). However, we currently implement the 2H-labeling protocol in collaboration with Dr. Marc Hellerstein at University of California at Berkeley, who is responsible for a majority of the sample analysis and data interpretation.

This P&F grant will enroll overweight women who are either sedentary (achieve <75 minutes of moderate-intensity or <37 minutes of vigorous-intensity aerobic physical activity per week) or very physically active (achieve > 225 minutes of moderate-intensity or >112 minutes of vigorous-intensity aerobic physical activity per week) to measure in vivo adipose cell formation in the subcutaneous abdominal and femoral adipose tissue depots. The primary aim of the study is to collect 2H-enriched adipose specimens that will be utilized to establish the innovative 2H-labeling methodology at Pennington Biomedical Research Center. In addition, the study will examine distinct populations of women (sedentary vs. physically active). Though broad adaptations and remodeling of adipose tissue have been associated with the health-related benefits of exercise, no studies have examined in vivo adipose cell kinetics in sedentary versus exercising individuals.

Overall, the 2H-labeling technique is the only in vivo approach that can be applied to assess adipose kinetics in both rodents and humans and is practical to examine the physiology of other metabolic tissues, in addition to adipose

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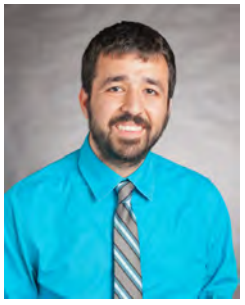
tissue. Hence, the development of this protocol would be a valuable asset to Pennington Biomedical to enhance the technical capacity to conduct future highly innovative and sophisticated research studies.

Apathy, unintentional weight loss, and cognitive decline late in life

Matthew Calamia, PhD, Pennington Biomedical
Adjunct Assistant Professor



Elderly individuals younger than age 90 years who are either overweight or underweight are at elevated risk for a variety of negative health outcomes, including higher rates of cognitive and functional decline, slower recovery from injury, and early mortality. However, while much research has focused on contributors to excess weight and the mechanisms leading from excess weight to outcomes such as cognitive decline (e.g., cerebrovascular changes and inflammation), contributors to underweight and mechanisms leading from underweight to adverse health outcomes late in life are much less well understood.

One factor associated with unintentional weight loss is apathy, a neuropsychiatric condition that becomes more prevalent late in life and consists of a sustained reduction in goal-directed behavior. Apathy may be associated with a dampened desire to seek the rewarding properties of food, thus leading to weight loss through a reduction in daily energy intake; however, no studies have explicitly examined this hypothesis nor have studies examined the degree to which unintentional weight loss is associated with specific behavioral or neural correlates late in the lifespan.

This study takes a first step toward clarifying the role of apathy in late-life weight loss though the use of multidimensional self-report and informant-report measures to assess apathy symptoms, the NIH Toolbox to assess cognition, the Remote Food Photography Method and SmartIntake® app to assess energy and nutrient intake, and the functional neuroimaging to assess food reward related brain activity. The study will leverage an existing longitudinal study, the Louisiana Aging Brain Study (LABrainS), to recruit two groups of older adults who have exhibited differing trajectories of BMI change over the previous several years: one group will be weight-stable, and the other will show a trend of significant weight loss that is not intentional. It is hypothesized that, compared to the weight stable group, individuals in the unintentional weight loss group will exhibit greater apathy, less daily energy and nutrient intake, poorer brain structural and functional health, and poorer cognitive function.

Findings of elevated apathy in the unintentional weight loss group, as well as strong associations between elevated apathy and diminished food cue reactivity and food intake in the unintentional weight loss group, would lead to follow-up grants for interventions that target apathy among elderly individuals using pharmacological or psychological approaches, and assess intervention effects on food intake, weight, and distal outcomes such as cognition. Diminished food cue reactivity in apathetic weight-losers could lead to follow-up funding that targets brain reactivity in these regions using neuromodulation techniques, possibly in combination with intervention against apathy.

Dr. Owen Carmichael, Pennington Biomedical Assistant Professor, will be handling the functional neuroimaging to access food reward related brain activity in Matt Calamia's P&F Project.

Zinc finger and BTB domain containing 16 (Zbtb16) in cold-adaptive responses

Sangho Yu, PhD, Pennington Biomedical
Professor

Change in ambient temperature is a strong modulator of both food intake and energy expenditure, and the hypothalamus is central in that process. Thus, understanding hypothalamic neural circuits and mechanisms of temperature-induced adaptations in energy homeostasis has significant implication on obesity and comorbidities. Zbtb16 is upregulated by acute cold exposure in the mouse hypothalamus and thought to be important for proper metabolic responses against cold stress. This research aims to 1) delineate locations and cell types of Zbtb16 expression and further investigate conditions and kinetics of Zbtb16 induction, and to 2) characterize in vivo functions of Zbtb16 during cold exposure and other metabolic conditions.

CANCER/OBESITY PILOT & FEASIBILITY AWARDS

The Pennington Biomedical NORC and LSUHSC Cancer Center have teamed up to fund two obesity/cancer P&F projects.

Nutrition has always been considered a pillar of optimal health in general. In cancer, adequate nutrition is considered to be essential in the prevention of cancer and the recovery from the challenging therapies prescribed for this disease. More recently new findings have shown that obesity is linked with an increased risk for the development of at least 14 different cancers, further strengthening the link nutrition and cancer. The biological mechanisms linking obesity and cancer, its implications in prognosis and outcome of treatment, and the social and economic determinants of obesity in cancer patients have become areas of great interest for the National Cancer Institute.

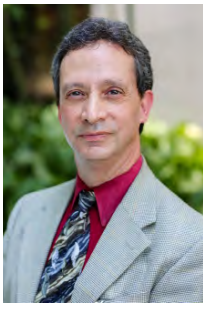
The convergence of obesity, nutrition and cancer create a unique opportunity to conduct multidisciplinary research among investigators with different but complementary backgrounds. Furthermore, the National Cancer Institute and the Cancer Moonshot Program have emphasized new initiatives on cancer prevention and novel forms of treatment (targeted therapies, and immunotherapy), by expanding the existing funding in these areas. The major objective of these studies is to provide research support to develop preliminary data and/or potential novel approaches (methods) from pairs of investigators with one investigator from LSUHSC in New Orleans (cancer expertise) and the other from Pennington Biomedical (obesity expertise).

Dr. Anne Gilmore and Dr. Maria Sanchez-Pino were each awarded funds in support of their pilot & feasibility grant proposals. Dr. Gilmore is collaborating with Agustin Garcia, MD, of LSUHSC. Dr. Sanchez-Pino, LSU Stanley S. Scott Cancer Center, is collaborating with Randy Mynatt, PhD.

Differential effects of diet on normal and malignant tissues

The risk for endometrial and breast cancer is significantly increased with obesity, and mortality from both breast and endometrial cancer is significantly higher in patients with obesity compared to normal weight. Obesity disrupts nutrient, hormone, and inflammation balance in the body. Disruption of this balance stimulates cancer cell proliferation, growth, and survival. Despite advances in our understanding of cancer biology and development of new therapies, the cornerstones of medical treatment for endometrial and breast cancer include surgery and chemotherapy, and will continue to be in the foreseeable future. Radiation and many chemotherapeutic agents such as those used to treat breast and endometrial cancer work by damaging the DNA of cells and disrupting the cell cycle thus targeting cell which divide rapidly. These treatments damage all rapidly dividing cells whether they are normal or malignant. As such, clinical toxicities observed due to the damage to normal tissues may limit the dosage of chemotherapy and radiotherapy administered and potentially limit the tumor's response to treatment. In addition, when cells don't replicate they evade the treatment effects. This is beneficial to the patient if normal cells don't replicate; however, when cancerous cells don't replicate they survive the damaging treatment and have the potential to later regrow and metastasize. Therefore it is of great importance to develop therapies that will enhance the efficacy of chemotherapy but, as importantly, limit the toxicity associated with this treatment. New therapeutic modalities which tackle the metabolic imbalance and take advantage of the different effects in tissues are needed.

Anne Gilmore, PhD
Assistant Professor,
Pennington
Biomedical



Agustin Garica, MD
Professor, LSUHSC

Fasting and the ketogenic diet are such interventions and effect normal and tumor cells differently. Studies done in animals suggest short term fasting and fasting-like diets such as the ketogenic diet protect normal cells from the toxicity of chemotherapy while maintaining its effect on cancerous cells. To our knowledge the described tissue effects of fasting on cells has been reported only in laboratory models and it is unknown if this effects can be reproduced in humans. In this collaborative project between Pennington Biomedical Research Center and Louisiana State University Health Science Center in New Orleans, women with endometrial or breast cancer and obesity will be randomized to one of three dietary interventions prior to surgery 1) usual dietary intake, 2) 72 hour fast, or 3)

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6-d ketogenic diet. Normal and cancerous tissues will be collected before and during surgery to evaluate the effect fasting or a ketogenic diet elicit on normal and cancerous cells in humans.

Effect of MDSC activation by lipids on hepatocyte ER stress, a hallmark in HCC development.

Maria Sanchez-Pino
Postdoctoral Fellow
LSU Stanley S. Scott Cancer Center

Randall Mynatt, Ph.D.
Douglas L. Manship, Sr. Endowed Professorship in Diabetes
Pennington Biomedical

Obesity has shown to increase the risk of non-alcoholic fatty liver disease (NAFLD) and progression to hepatocellular carcinoma (HCC). Although, compelling evidence has revealed that hypernutrition-related systemic alterations such as chronic low-grade inflammation and dyslipidemia are involved in cancer development, the mechanisms by which obesity promotes the progression to liver cancer is still unclear. We hypothesized that inflammatory and metabolic dysregulation promotes a microenvironment that favor the accumulation and activation of myeloid-derived suppressor cells (MDSC), thereby fostering tumor development.

Our preliminary data demonstrates that i) induction and activation of MDSC depends on lipid uptake and activation of fatty acid oxidation (FAO); ii) morbidly obese patients (Body Mass Index [MBI] ≥ 40) have a significant increase in circulating MDSC associated with high cholesterol and triglycerides, and iii) significant accumulation of MDSCs in liver is present in mice made obese with a high-fat high-sucrose diet (HF-HSD), indicating that obesity-derived factors favor MDSC development.

Previous animal and human studies have indicated that MDSCs play a role in liver inflammation and HCC; however, the molecular and cellular structures by which obesity-associated MDSC promote the progression to liver cancer are unclear, and it is the subject of this proposal. Given the increased lipids during obesity-associated NAFLD, we want to identify the lipid-dependent mechanisms that lead to the generation and activation of MDSC and how those activated cells contribute to immune-mediated hepatotoxicity by using an in vitro model of metabolic stress. This study is aimed at understanding the events that lead to the accumulation of MDSC in the liver induced by obesity and comprehend their role in the development of HCC. The results of this study will provide the foundation for the development of mechanistic and in vivo studies linking the molecular pathways triggered by obesity-associated chronic inflammation and development of HCC. Furthermore, the identification of potential targets within these cells may provide new avenues for prevention and/or treatment of patients with NAFLD or HCC.