

NUTRITION OBESITY RESEARCH CENTER

MAY 2016



NORC FOCUS - NUTRITION AND METABOLIC HEALTH FROM YOUNG TO OLD AGE

Since its inception 10 years ago, the Pennington Biomedical Research Center NORC elected to emphasize a theme important for the origins of chronic disease, i.e. "Nutritional Programming: Environmental and Molecular Interactions." This focus was based on emerging interests in epigenetic phenomena, such as how environmental events in utero and in early life can influence the risk for the development of obesity and metabolic syndrome in later adulthood via mechanisms such as gene methylations and demethylations or histone modifications. Despite this specific theme of research, the Pennington Biomedical NORC mission has always been "to facilitate and promote collaborative and multi-disciplinary interactions that will foster new research ideas and enhance the translation of basic nutritional research findings into the clinical arena and ultimately into practical application" in the field of nutrition research.

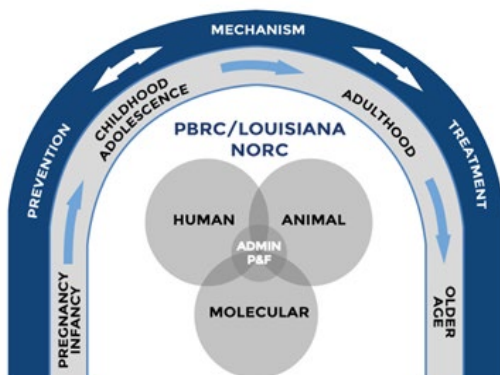
In our new renewal application, we have expanded the breadth of our NORC research beyond our initial theme of "nutritional programming" and we are now poised to emphasize the more general theme of "nutrition and metabolic health from young to old age." We plan to progressively develop research teams focusing on three critical periods of the life cycle (see diagram):

- Maternal/Infant nutritional status and metabolic consequences;
- Pediatric and adulthood obesity and metabolic health;
- Nutritional status at older age to preserve physical and cognitive functionality.

These three research foci will lead to the formation of Research Teams to address big research questions from the basic science

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NUTRITION, OBESITY AND METABOLIC HEALTH THROUGH THE LIFESPAN



RENEWED FOR ANOTHER 5 YEARS!

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NORC 2016-2017 PILOT & FEASIBILITY ANNOUNCEMENT

INITIAL EMAIL

ANNOUNCEMENT May 6, 2016

LETTER OF INTENT

Due: May 28, 2016

<http://norcfunding.pbrc.edu>

(Mentor needed for Postdocs and Instructors)

FULL APPLICATION

Due: July 22, 2016

<http://norcfunding.pbrc.edu>

TRAINING UPDATE

Our NIDDK T32 postdoctoral training grant entitled "Obesity from Genes to Man" anticipates two openings, one in June and another in October, 2016. Up to three years of sponsored research training and support for PhD and MD trainees is provided. US citizenship or permanent residence certification (green card) is required.

Visit our website at www.pbrc.edu/t32obesity and apply at <https://lsusystemcareers.lsu.edu/>.



level, to clinical investigation, and finally to the population in a translational way. Our three proposed research focus areas are:

1) Maternal/Infant nutritional status and metabolic consequences.

The initial focus on “nutritional programming” was chosen 12 years ago based on emerging interests in epigenetic phenomena such as how environmental events in utero and in early life can influence the risk of obesity and metabolic syndrome in later life. This focus will remain important to the NORC members because of the trans-generational transmission explaining, in part, the striking increase in obesity and its complications in children over the past two decades. As an example, there is emerging concern about the impact of the procedures of In Vitro Fertilization (IVF; 1% of births each year in the US are IVF babies) on the metabolic health of babies conceived by IVF. Large epidemiological studies derived from the U.S. Centers for Disease Control and Prevention (CDC) Assisted Reproductive Technology database clearly demonstrate that children conceived by IVF have a significantly increased risk of prematurity, low birth weight, and being born small for gestational age. The impact of these outcomes on the future health of the IVF offspring is poorly understood. As examples, such a research team could address the following research questions in a translational manner:

- Role of breastmilk and infant formula feeding on establishment of infant microbiome profiles?
- Influence of early life feeding on mechanisms of energy expenditure including BAT activation?
- Roles of maternal nutrition during pregnancy (e.g. protein intake, nutritional supplements) on epigenetic markers for obesity and type 2 diabetes risk?

2) Pediatric and adulthood obesity and metabolic health.

A large proportion of obesity and its associated diseases is attributable to lifestyle factors and can therefore be improved by interventions including behavioral and/or pharmacological approaches. Since its creation, Pennington Biomedical basic scientists and physiologists have made important contributions to understanding the physiology of weight regulation and have collaborated with clinicians to develop nutritional and physical activity programs as a modifier of obesity associated metabolic disorders. Pennington Biomedical behavioral scientists have expanded frontiers in lifestyle intervention through mobile health applications. Our NORC funding is therefore poised to continue its contribution to better understand the molecular mechanisms of weight gain, to test such

discoveries in a clinical setting, and importantly, to develop treatment and prevention programs to help the population.

As examples, such a research team could address the following research questions in a translational manner:

- How to better prevent and treat obesity in childhood and adolescence?
- Cross-talk between adipose and skeletal muscle (insulin sensitivity) in response to weight change?
- Role of metabolic flexibility in health and disease?
- Translation of intervention approaches to the clinical arena and to the population?
- Role of intermittent hypoxia on insulin sensitivity?

3) Nutritional status at older age to preserve physical and cognitive functionality.

Life expectancy is increasing dramatically around the globe. However, obesity and Type 2 diabetes mellitus adversely affect the health of individuals as they age; rates of mortality, microvascular complications, and age-related health events and hospitalization have been increasing and physical function and quality of life have diminished. These factors are real threats to public health in late life. Importantly, nutritional and activity programs have to be developed to protect against the progressive loss of cognitive and physical functionality. Lifestyle interventions on body composition, metabolic health as well as functionality need to be validated in order to preserve optimum quality of life at an older age.

As an example, such a research team could address the following research questions in a translational manner:

- How to manage excess body weight in the elderly while maintaining lean body mass?
- Dietary and exercise interventions to improve functionality and manage body weight in the elderly?
- Pharmacotherapy and lifestyle change for the preservation of physical and cognitive function in the elderly?
- Identification of predictors and biomarkers of age-related cognitive and physical decline?
- Development of interventions to target these risk factors?

With the sharpening of focus areas and the creation of related research teams, the Pennington Biomedical NORC represents a multiplier to reach additional resources to address important unresolved questions regarding the mechanisms, the prevention and the treatment of obesity and therefore of metabolic health throughout the lifespan.

NEW AWARDS 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 are updates from our two P&F winners.

Regulation of Pancreatic Islet β -cell Growth and Function by the IL-1 Receptor

Jason Collier, PhD



Diabetes mellitus increases risk for diseases of the eyes, kidneys, and cardiovascular system and is also a major cause of death worldwide. Pre-clinical studies and clinical trials have revealed interleukin-1beta (IL-1beta) as a major mediator of inflammation contributing to insulin resistance, beta-cell dysfunction, and Type 2 Diabetes Mellitus

(T2DM). However, the site of action remains unknown. The beta-cell is the major cell type located within the pancreatic islets and is responsible for production and secretion of the hormone insulin. Islets isolated from genetically obese mouse and rat models display elevated production of IL-1beta, which signals via the IL-1 receptor (IL-1R). Because the majority of

studies investigating IL-1R signaling in pancreatic islets have been conducted using isolated tissues, we have generated a novel mouse model with pancreatic islet beta-cell targeted deletion of the IL-1R. We hypothesize that IL-1R signaling pathways in pancreatic beta-cells promotes inflammatory responses that support initial beta-cell mass expansion, while chronic exposure to IL-1R activating ligands eventually results in a decline in insulin secretion. This hypothesis is consistent with phenotypes observed in obese mouse, rat, and human tissue samples. Completion of these studies will reveal whether IL-1R in pancreatic islet beta-cells is a major contributor to inflammation-associated proliferation responses that control beta-cell growth during obesity and insulin resistance, and also whether pathways linked to IL-1R signaling are part of the eventual decline in beta-cell function that ultimately leads to diabetes.

Effects of adipocyte STAT5 deletion on mammary fat pad morphology and mammary gland development and function

Carrie Elks, Ph.D.



The mammary gland is unique in that most of its development occurs postnatally. The fat tissue surrounding the mammary gland is required for its proper development, and mammary adipocytes undergo coordinated crosstalk with mammary epithelial cells during all stages of development. During

lactation, mammary adipocytes are completely remodeled and the fat stored inside the cells is used as a milk component or as an energy source for milk production. In order for the fat to be properly partitioned during lactation, the adipocyte must undergo severe lipid depletion and suppression of lipid and glucose uptake.

The transcription factor, signal transducer and activator of transcription 5 (STAT5), plays a critical role in adipocyte lipid metabolism. We and others have shown that STAT5 regulates adipocyte development and adipocyte gene expression. While there is a known requirement for STAT5 for the normal development of mammary epithelium, its role in the mammary adipocyte is unknown.

Our goals are: 1) to evaluate the contribution of the mammary adipocyte to the development and function of the mammary gland, and 2) to determine the effects of adipocyte STAT5 deletion on lactation, milk composition and on the nutritional status of offspring. We will examine milk production and milk composition in lactating mice and we will place their offspring on chow or high-fat diets for six months to assess their susceptibility to obesity and insulin resistance. If STAT5 deletion promotes lipid retention in adipocytes, the efficacy of or amount of lipid contribution to the milk may be affected, which will thereby affect the nutritional status and growth of offspring by limiting the amount of energy contributed to the milk in the form of lipid.

In summary, results from this project will enhance our understanding of the role of mammary adipose tissue as a paracrine effector of mammary gland development and milk production. Even more important are the links these studies may reveal between maternal mammary gland lipid metabolism, milk composition, and the future obesity risk of offspring.

NEWS FROM THE HUMAN PHENOTYPING CORE

Pediatric Obesity & Health Behavior Laboratory

The Pediatric Obesity and Health Behavior Laboratory, under the direction of Assistant Professor and LA CaTS Roadmap Scholar Dr. Amanda Staiano, launched several new studies to investigate the influence of technology on children's physical activity and weight status. Many of these studies rely on NORC resources, specifically equipment and resources housed in Pennington Biomedical's Translational Research Clinic for Children (TReCC). See a snapshot of key studies below.

GameSquad: Gaming Technology to Encourage Healthy Weight and Activity in Youth (2015-17)

Sponsor: American Heart Association (15GRNT24480070)

Investigators: Amanda Staiano, Ph.D. (PI), Robert Newton Jr., Ph.D., Daniel Hsia, M.D. (MI)

Summary: The goal of this randomized controlled trial is to test the efficacy of exergaming, i.e. video gaming that involves physical activity, to reduce adiposity in overweight and obese children. The study is a 6-month, 2-arm randomized controlled trial among 46 overweight/obese children (aged 10 to 12 years) assigned to: 1) 3 hours/week of in-home exergaming or 2) a control group. An innovative aspect is the inclusion of a fitness trainer who will regularly videochat with the participant and virtually monitor gameplay.

Use of NORC Resources: Age-specific exercise and exergaming equipment, activity assessment via accelerometers and heart rate monitors, behavioral assessments, design and implementation of population-specific exercise.



Pause and Play: Physical Activity and Screen-Time Regulations in Child Care Centers (2015-17)

Partner Organization: Mayor's Healthy City Initiative

Sponsor: NIH National Institute on Minority Health and Health Disparities (U54MD008602), Gulf States Health Policy Center

Investigators: Amanda Staiano, Ph.D. (PI), Andrew Allen (Director of the Mayor's Healthy City Initiative; Co-PI), Corby Martin, Ph.D.

Summary: Together with the Mayor's Healthy City Initiative, this study aims to: 1) examine the physical activity and screen-time environment of licensed childcare centers before and after the enactment of new state regulations; 2) examine the physical activity and screen-time behaviors of children enrolled in childcare centers before and after the enactment of new state regulations; and 3) establish community strategies to improve young children's health behaviors.

Use of NORC Resources: Activity assessment via accelerometers and heart rate monitors, behavioral and self-report assessments.



Our Lifestyles, Our Lives: Obesity Treatment and Physical Activity Promotion for Underserved Children (2014-16)

Partner Organization: Our Lady of the Lake Children's Hospital

Sponsor: American Council on Exercise; Franciscan Missionaries of Our Lady Foundation

Investigators: Amanda Staiano, Ph.D. (PI), Patrice Tyson, M.D. (MI), Savarra Mantzor, M.D., Daniel Hsia, M.D., Peter Katzmarzyk, Ph.D., Robert Newton Jr., Ph.D.

Summary: The project aims to evaluate and adapt a 10-week pediatric obesity program to increase the efficacy of achieving clinically significant weight loss in overweight and obese children. The primary outcome is change in body mass index (BMI) z-score. Secondary outcome variables include improvements in pedometer-assessed physical activity, quality of life, and physical activity enjoyment.

Use of NORC Resources: Behavioral and self-report assessments, design and implementation of population-specific exercise interventions and diet interventions.



Klub Kinect: Social Exergaming for Healthy Weight in Adolescent Girls (2014-15)

Investigators: Amanda Staiano, Ph.D. (PI), Peter Katzmarzyk, Ph.D., Robert Newton Jr., Ph.D., Daniel Hsia, M.D. (MI)

Summary: This study aimed to determine the feasibility of recruiting, enrolling, and following overweight and obese adolescent girls in a 12-week exergaming program versus self-directed care. We also assessed changes in body weight, body fat, visceral adiposity, cardiovascular risk factors, physical activity, peer support, health-related quality of life, and self-efficacy towards exercise.

Use of NORC Resources: MRI, DXA, age-specific exercise and exergaming equipment, activity assessment via accelerometers and heart rate monitors, observation rooms for behavioral analysis, behavioral and self-report assessments, design and implementation of population-specific exercise interventions.



TIGER Kids: Effects of obesity on physical activity, sedentary behavior, and cardiometabolic health in White and African American children and adolescents (2016-19)

Sponsor: United States Department of Agriculture (3092-51000-056-04A)

Investigators: Amanda Staiano, Ph.D. (PI), Peter Katzmarzyk, Ph.D., Catherine Champagne, Ph.D., Robert Newton Jr., Ph.D., Stephanie Broyles, Ph.D., Daniel Hsia, M.D. (MI)

Summary: Our interdisciplinary team will establish a prospective pediatric cohort to identify intervention targets based on the location, timing, barriers, and facilitators of current physical activity and sedentary behavior in a child's day. We will conduct a prospective examination of 340 African American and White girls and boys aged 10 to 16 years, including 50% who are classified as severely obese. We will use state-of-the-art technology including accelerometry to quantify physical activity, magnetic resonance imaging to quantify fat accumulation, and global positioning system (GPS) information and ecological momentary assessment to identify environmental and socio-emotional barriers and facilitators.

Use of NORC Resources: MRI, DXA, activity assessment via accelerometers and heart rate monitors, observation rooms for behavioral analysis of activity, behavioral and self-report assessments.



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.

GENOMICS CORE

The focus in the Genomics Core is on “Functional Genomics” and more specifically on the epigenetic aspects of various biological paradigms – analysis of gene expression, and studies of the epigenome and its plasticity. Gene expression studies are either conducted by quantitative real-time PCR, or by next-generation sequencing. PCR-based experiments typically examine a limited number of genes for expression in many samples; for this purpose, the core currently maintains four AB7900 instruments that are further supported by a robotic pipetting setup to facilitate simultaneous measurements of up to 384 samples per run and up to 2,304 samples in a single day. Sequencing technology is offered for experiments on the systems biology level, such as measuring the expression of every gene in the genome in an unbiased way, or determining the genome-wide distribution of epigenetic marks on DNA. Sequencing approaches are complemented by a bioinformatics workflow designed to facilitate the biological interpretation of expression or epigenomic data sets.

A new development in the Genomics Core is marked by the recent arrival of a Bio-Rad Digital Droplet PCR Instrument. Initially procured by Dr. Jason Collier through a successful grant application, Dr. Collier has made the instrument available to all investigators at Pennington Biomedical by placing it in the Genomics Core. The core will provide instrument maintenance, and facilitate investigator access to the technology; Richard Carmouche and Dr. Claudia Kruger have received training on the instrument.

The main usage of this instrument is in the quantitative detection of specific nucleic acid targets of very low abundance – e.g. in the analysis of genes that are expressed at very low level, or in studies involving cell-free circulating DNA. This sensitivity is achieved by conducting the polymerase chain amplification reaction in droplets of 1 nanoliter volume, where reaction conditions can be optimized to reliably amplify a single target molecule. The biomedical



application driving the acquisition of this instrument is the detection of pancreatic beta-cell death in diabetes based on the presence of un-methylated DNA from the insulin gene locus in cell-free circulating DNA. This measurement permits recognizing beta-cell death before it becomes apparent on the physiological level, and such an early recognition may improve chances for success for any subsequent therapy.

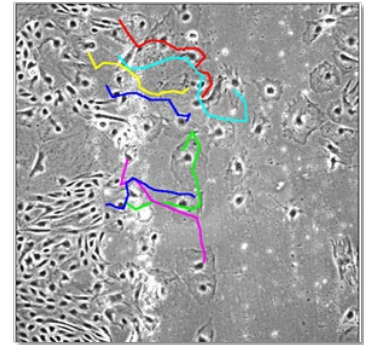
The Genomics Core also offers technology to analyze the composition of gut microbiome, the community of commensal organisms in the digestive tract. There is increasing evidence that gut microbes can promote the onset of the low-grade inflammation in metabolic disorders, and that gut ‘dysbiosis’ can contribute to the development of obesity and/or its complications. To determine the composition of gut microbiota, the core can perform 16S metagenomics sequencing, a technology to recognize taxonomical units based on characteristics in the sequence of the gene encoding the 16S ribosomal RNA. Sequencing is performed on an Illumina MiSeq instrument, and the core provides the necessary bioinformatics to analyze such data sets.

BIOIMAGING CORE

While genomics methods can help identify molecules that are crucial for a specific biological or pathophysiological mechanism, the question where those molecules act requires a different set of technologies. These are available in the Bioimaging Core. The Bioimaging Core provides histological services such as tissue processing, embedding, sectioning, and staining that are combined with state-of-the-art imaging as well as image processing tools to visualize critical molecules in cells or tissues, or to measure important biological processes. One example for this technological approach is the semi-automated analysis of fat tissue, with particular emphasis on the classification of adipocytes. Using histological staining or immunofluorescence together with the Hamamatsu NanoZoomer whole slide imaging system, the core can assist with determination of adipocyte size of from human and animal fat deposits, or detect and classify the conversion of white adipocytes into brown/beige/brite fat cells. Automated slide scanning facilitates high throughput, and computational processing permits elimination of human observer bias, thereby offering a much more sound basis for

subsequent statistical evaluation of specific parameters. Together, these workflows elevate classical histology from an observational to a quantitative level.

One technology that has received more attention in the recent past is live-cell imaging on the Leica TIRF DM6000 imaging station. This instrument is capable of providing temperature and atmospheric control, and therefore allows culturing of live cells on the stage of the microscope for continuous observation. Software control over the x-y movement of the stage permits repeated and reproducible inspection of many different cells during the culture, and documentation of cells at specified time intervals can be used to create time-lapse data sets. These 'movies' can then be used to extract quantitative data on the behavior of cells: migratory behavior, total migration distance, trajectory, migration velocity, and acceleration or deceleration patterns can all be determined from such data sets. Current applications of this technology in the Bioimaging Core are e.g. the analysis of kidney cells in genetically manipulated mice, or the migration properties of nascent mesodermal cells in a research project on the etiology of neural tube defects in diabetic pregnancies.



The Bioimaging Core continues to provide high-power imaging procedures on a Leica SP5 confocal microscope, laser microdissection capabilities, as well as fluorescence-activated cell sorting methods. For all technologies, the core offers not only direct hands-on expertise, but also training to investigators and their laboratory staff with respect to (i) choice of the experimental platform that is most appropriate to a desired experiment, (i) procedures to operate each piece of equipment, as well as (iii) software use and informatics workflows for correct data processing and biological interpretation.

INTERNAL ADVISORY BOARD

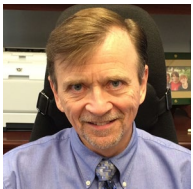
Featured are the members of our NORC Internal Advisory Board. We would like to express our gratitude to these members and acknowledge their contributions. They advise the executive committee in decision making and strategic planning, provide instruction and mentorship for our enrichment programs, and offer peer review support to our pilot and feasibility grant initiatives.



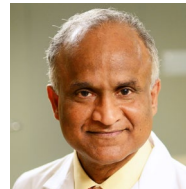
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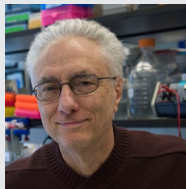


EXTERNAL ADVISORY BOARD

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.



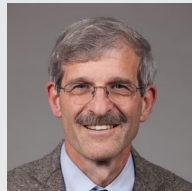
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