Pennington Biomedical Research Center
Clinical Nutrition Research Unit
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Website: cnru.pbrc.edu

Organization and Goals

The Pennington Biomedical Research Center (PBRC) Clinical Nutrition Research Unit (CNRU) will facilitate and promote collaborative and multidisciplinary 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. Within the chosen theme of "Nutritional Programming: Environmental and Molecular Interactions," we are targeting translational research designed to understand the metabolic and environmental factors underlying nutrition- and obesity-related health problems. The tradition of obesity research at PBRC provides an ideal academic environment to undertake interdisciplinary efforts to investigate the environmental and molecular interactions happening during epigenetic phenomena, which can influence the development of obesity and metabolic syndrome in adulthood.

The 22 NIH-funded nutrition/obesity studies and the recipients of Pilot and Feasibility (P/F) grants are using three CNRU cores. The Human Phenotyping Core provides services to measure insulin sensitivity, in-situ biochemistry (MRS), skeletal muscle metabolism, energy metabolism, body composition, as well as to administer physical activity and behavioral interventions. The Molecular Mechanisms Core provides classical genomics support and develops CpG micro arrays with adequate bioinformatics capacity, as well as cell culture and cell imaging technologies. The Animal Models and Phenotyping Core provide the required animal models, including conditional transgenic or knockout animals and state-of-the-art phenotyping. With an exceptional institutional support and an ideal academic environment, Pennington has established a strong base of obesity/nutrition research and is now poised to grow in an emerging field of Nutritional Programming through the creation of the CNRU.

The goal of the PBRC CNRU is to promote the emergence of new investigators in the field of nutrition-related disease and, more specifically, in the effect of environment and molecular interactions in nutritional programming.

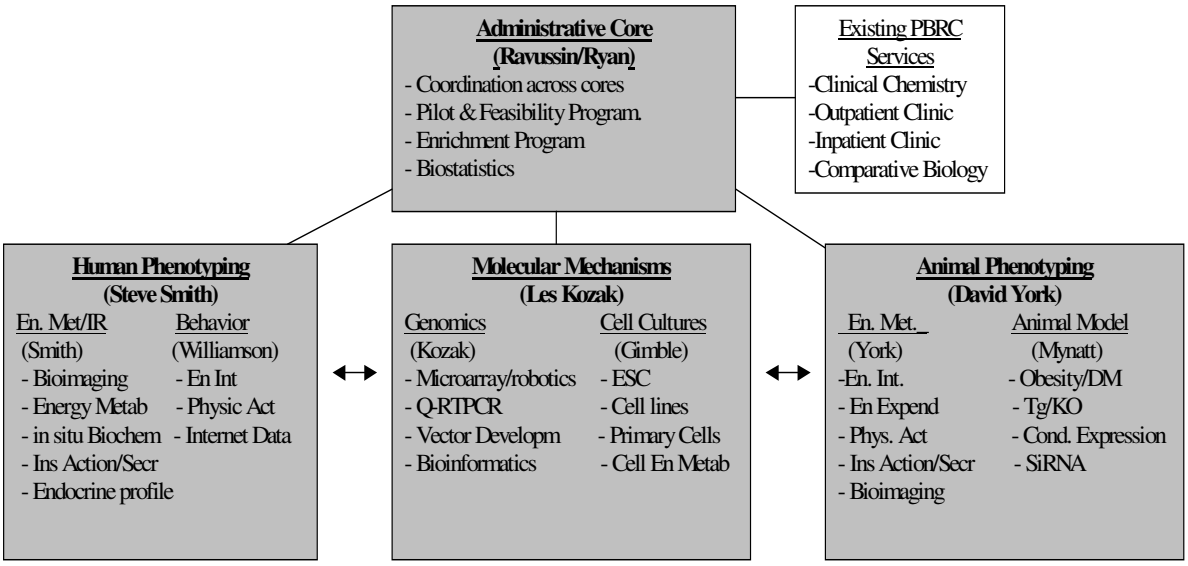
As stated by the World Health Organization, "Obesity is a chronic disease prevalent in both developed and developing countries and affecting children as well as adults." Indeed, it is now so common that it is replacing the more traditional public health concerns, including under-nutrition and infectious diseases, as one of the most significant contributors to ill health. Even if the evidence in support of a genetic component to the development of obesity is overwhelming, the output from the genetic obesity research has been rather disappointing over the past 2 decades. Barker et al. have investigated the fetal origin of adult disease hypothesis. Based on their work, low birth weight may impact the lifelong metabolic functions of the organism and represent a significant risk factor for the development of metabolic diseases in adulthood. However, others have pointed out that the risk of developing obesity and type 2 diabetes is also a risk factor for infants born at the upper end of the birth weight curve, i.e., the relationship of weight to the risk of type 2 diabetes is U-shaped. Convincing basic science and clinical data are now indicating that an interaction between gene and early life environmental conditions are

important in the development of obesity and the different facets of obesity. The major goal of the PBRC CNRU is therefore to study early life environmental factors which can predispose individuals to later life diseases.

The specific aims of the Pennington CNRU are:

- Enhancement of communication, cooperation, and collaboration among the scientific members of the CNRU at Pennington;
- Creation of multidisciplinary interaction and projects among the Pennington scientists;
- Exploration of new ideas and fresh avenues of discovery through the CNRU pilot/feasibility study mechanism; and
- Translation of nutritional research findings and enhanced understanding of research methods in the primary care setting.

Core Laboratories



As can be seen on the organizational chart above, the Pennington CNRU has been developed around three major scientific cores overseen by an administrative core.

Administrative Core: Eric Ravussin, Ph.D., Director; Donna Ryan, M.D., Associate Director; Darlene Marquis, Executive Administrator

External Advisory Board Members:

Rudy Leibel, M.D., Chair, Co-Director Research, College of Physicians & Surgeons, Columbia University, New York, NY

David Kelley, M.D., Professor of Medicine, Dept. Endocrinology and Metabolism, University of Pittsburgh School of Medicine, Pittsburgh, PA

Brad Lowell, M.D., Ph.D., Associate Professor, Endocrinology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA

Dan Kelly, M.D., Professor of Medicine, Pediatrics, and Molecular Biology & Pharmacology, Director, Center for Cardiovascular Research, Washington University, Saint Louis, MO

Human Phenotyping Core: Steve Smith, M.D., Director; Don Williamson, Ph.D., Associate Director

Molecular Mechanisms Core: Leslie Kozak, Ph.D., Director; Jeff Gimble, M.D., Ph.D., Associate Director

Animal Models and Phenotyping Core: David York, Ph.D., Director; Randy Mynatt, Ph.D., Associate Director

Pilot and Feasibility Studies

While preparing our CNRU applications, we developed a Pilot and Feasibility (P/F) grant program funded by the institution around the theme of nutritional programming. Out of 10 applications, four were retained for funding. A brief description of the aims of these four applications is provided below.

Maternal Diet and Epigenetic Programming *In Utero*. Ken Eilertsen, Ph.D. The Fetal Origins of Adult Disease (FOAD) hypothesis, often referred to as the Barker Hypothesis, posits that specific diseases in the adult, e.g. coronary heart disease, high blood pressure, and type II diabetes, are the result of the fetus' adaptation *in utero* to maternal under-nutrition, or malnutrition. The Barker Hypothesis likely extends to the preimplantation stage, since reducing protein intake during this time in the rat is linked with such long-term changes. The hypothesis of this proposal is that maternal under-nutrition during preimplantation development in mice results in offspring that have CpG island methylation patterns distinct from offspring whose mothers were not malnourished. We will investigate the *in utero* effect of a low-protein diet (LPD) during preimplantation development on DNA methylation patterns in near term pups using a CpG island microarray.

Whole-body Effects of an Up-regulation of Stearoyl-CoA Desaturase-1 in Muscle: A Transgenic Mouse Approach. Matt Hulver, Ph.D. Insulin resistance, a defining feature of type 2 diabetes mellitus (T2DM), is observed in association with obesity and the accumulation of intracellular lipids in non-adipocyte tissues. Increased intramuscular triacylglycerol (IMTG) content is thought to exacerbate insulin resistance (IR) by serving as a reservoir of fatty acids (FA) for the synthesis of putative insulin desensitizing lipid metabolites, including long chain fatty acyl-CoAs, diacylglycerols, and ceramides. Recently, the principal investigator (PI) reported that IR in skeletal muscle from obese subjects is associated with increased IMTG and fatty acyl CoAs and decreased FA oxidation. Additionally, incubated muscle strips from obese subjects preferentially directed FA towards storage (as neutral lipids) and away from mitochondrial β-oxidation. The lipogenic enzyme, stearoyl-CoA desaturase-1 (SCD1) was elevated in skeletal muscle from obese humans. SCD1 expression levels correlated positively with rates of IMTG synthesis measured *in vitro* in incubated rectus abdominus muscle strips. Additionally, elevated IMTG synthesis, reduced FA oxidation, and high SCD1 gene/protein expressions were evident in cultured primary myocytes obtained from obese compared to nonobese humans. Thus, the metabolic phenotype observed in isolated muscle strips was fully retained in a muscle cell culture system. The primary objective of this P/F grant application is to develop a transgenic mouse with muscle-specific over expression of SCD1 and assess the feasibility of using this model to study whole-body physiology as it is affected by muscle specific SCD1 upregulation.

NTPGC1 Enhances the Transcriptional Activity of PEPCK by Binding the Repressor of PGC1. Aaron Adamson, Ph.D. The aim of this study is to prove that NTPGC1 enhances the transcriptional activity of PEPCK by binding the repressor of PGC1. NTPGC-1α increases gluconeogenesis by competing with PGC-1α for binding to a repressor, thus relieving inhibition of PGC-1α and enhancing transcriptional activity of PEPCK. Our hypothesis will be evaluated through a complementary series of *in vivo* and *in vitro* experiments with primary cultures of isolated hepatocytes to address the following components of the larger aim:

1. What are the signaling components which regulate the relative expression of the PGC-1α isoforms in the fed and fasted state?
2. How do changes in the expression ratio of the two isoforms influence transcriptional activation of PEPCK?
3. How do glucagon, cAMP, and other downstream signals regulate interaction of the repressor with the two isoforms of PGC-1α?

The combined *in vivo* and *in vitro* approach will provide mechanistic insights into the details of gluconeogenic control during fasting. The critical unanswered question this proposal addresses is how this newly identified splice variant functions to regulate the transcriptional activity of full length PGC-1α. These studies will improve our fundamental understanding of gluconeogenic regulation and, in doing so, will provide new insights into the aberrant activation of gluconeogenesis in the metabolic syndrome and diabetes mellitus.

Stochastic and/or Environmental Effects on DNA Methylation and Epigenetics Can Influence the Obesity Phenotype. Robert Koza, Ph.D. Although little is known regarding the contribution of epigenetics to obesity, these environmentally induced or stochastic non-Mendelian modifications to the genome have been shown to cause dramatic effects in gene regulation, and, in some studies, profound changes in body weight. In this proposal, we plan to investigate the interaction between epigenetics, genetics, and the environment in the regulation of body weight using a well-defined mouse model. Inbred C57BL/6J mice, although genetically identical, show a large degree of variation in bodyweight after fed an obesogenic diet. Gene expression studies comparing adipose tissue of low weight gaining (LWG) and high weight gaining (HWG) mice that were fed a high fat diet identified mesoderm specific transcript (MEST) as having a high correlation to bodyweight and adiposity. We have demonstrated no significant differences in methylation patterns in a region encompassing the MEST promoter and 5' end of exon 1 in LWG compared to HWG mice, although hyper-methylation of this region has been shown to repress transcriptional activity. Since MEST is only one possible epigenetically modified gene associated with differences in adiposity in C57BL/6J mice, and its regulation could involve interactions with other differentially methylated genes or be the result of global changes in methylation, I propose to use CpG island microarray analysis to identify somatically heritable differences in CpG methylation between LWG and HWG mice, and animals with low and high adipose tissue MEST expression that were fed a high fat diet. Our hypothesis is that stochastic and/or environmental factors affects DNA methylation and epigenetics can influence the obesity phenotype. The specific aim of this study is to determine differences in CpG island methylation associated with adiposity and dietary fat-induced MEST expression in C57BL/6J mice.

Upcoming P/F Studies

Late October 2005, a new round of request for application was publicized. In response, we received 12 letters of intent and 10 full applications. Like the previous year, four were selected

for funding, one as an extension of the previous year (Ken Eilertsen) and three as novel topics. Two of the new recipients are established investigators (David York and Brenda Richards) who are taking advantage of the CNRU mechanism to explore a new field of nutritional research, i.e., nutritional programming.

The 2005 – 2006 recipients of CNRU P/F grants are:

1) David York, Ph.D.: The Neuroprotective Role of Enterostatin
2) Ken Eilertsen, Ph.D.: Maternal Diet and Epigenetics
3) Brenda Richards, Ph.D.: Dietary Fat Avoidance in ACADS Mutant Mice: A New Model of Nutrient Sensing?
4) Tiffany Stewart, Ph.D.: Genetics and Binge Eating: A Case-control Pilot Study