

Washington University Nutrition Obesity Research Center

Start Date: 1999

Status: Ongoing

Source of NIH Support: NIDDK

Website: <http://chn.im.wustl.edu/cnru/framedcnru/maincnruhome.htm>

Organization and Goals

The Washington University (WU) Nutrition Obesity Research Center (NORC) provides a centralized research infrastructure that supports and stimulates cost-effective and high-quality basic and translational research, collaborations among investigators, community outreach, career development and training, and clinical activities in nutrition and obesity. It is our intention to continue to attract both new and established investigators to the field, and to continue to bring state-of-the-art methods in molecular biology and clinical investigation to NORC investigators. The Washington University NORC is focused on three major interactive themes: 1) Obesity: Pathophysiology, Complications, and Therapeutics; 2) Nutrient Metabolism in Health and Disease; and 3) Growth, Development, and Aging.

In addition to the NIH support of the NORC, several other key factors, including institutional recognition and support, generous donations from philanthropic organizations, and creative management by NORC leadership, have transformed the original NORC into the intellectual and physical home for nutrition and obesity at Washington University School of Medicine (WUSM) and made the NORC a unique resource for interdisciplinary and translational nutrition and obesity research, clinical care, and training/enrichment. Accordingly, the NORC has had a major impact on the medical center and has: 1) increased the number of Washington University faculty involved in nutrition and obesity research by attracting both established and young investigators; 2) stimulated the recruitment of new outside faculty interested in nutrition and obesity research careers; 3) stimulated productive collaborations among investigators, including interactions between basic and clinical science investigators and between clinical science and community investigators; 4) supported and mentored young investigators; 5) increased the resources available to support NORC activities by developing cost-effective collaborations among other institutional centers and obtaining considerable support from Washington University and philanthropic organizations; and 6) developed innovative new programs to stimulate new directions in nutrition and obesity research, and to address nutrition and obesity issues in the community.

Organizational Structure of the NORC Within Washington University

The NORC is administratively based within the Department of Medicine, a reflection of Dr. Klein's primary appointment there, but it serves the entire School of Medicine. The NORC director reports directly to the Dean and Vice Chancellor for Medical Affairs (Dr. Larry Shapiro), who reports to the Chancellor of the University (Dr. Mark Wrighton) and the Board of Trustees. The NORC also interacts with other Centers and Core Laboratories at the School of Medicine to provide the most efficient and comprehensive services to NORC investigators. The NORC Biomedical Research Core Laboratories were designed specifically to provide needed services for nutrition and obesity research that complement, not duplicate, existing facilities.

Furthermore, many of the directors or associate directors of the existing or proposed Centers that have themes or functions related to NORC activities are members of the NORC Executive Committee or the Internal Advisory Committee, which facilitates interactions between these Centers, stimulates research collaborations, and avoids duplication of services. In addition, the NORC is a member of our Council of NIH Center Directors. This Council, which consists of the principal investigators of NIH-supported Centers, was recently established by the Institute for Clinical and Translational Science (ICTS, which is funded by the NIH Clinical and Translational Science Awards). The Council meets biannually to coordinate efforts around issues of common interest, to avoid duplication, and to develop joint strategic and infrastructure initiatives around research and research training.

External Advisory Committee

The External Advisory Committee 1) provides advice and direction to the director, associate directors, and executive committee on the function and activities of the NORC; 2) serves as external Pilot and Feasibility project reviewers; and 3) participates in our research and education program.

External Advisory Group Members:

Naji Abumrad, M.D., John L. Sawyers Professor of Surgery, Chair, Department of Surgery, Vanderbilt University

Dennis Bier, M.D., Professor of Pediatrics, Director, Children's Nutrition Research Center, Baylor College of Medicine

Jay Horton, M.D., Dr. Robert C. and Veronica Atkins Chair in Obesity and Diabetes Research, Associate Professor, University of Texas Southwestern Medical Center

Steven Zeisel, M.D., Professor and Chair of the Department of Nutrition, Professor of Pediatrics, University of North Carolina at Chapel Hill

Overview of the Ongoing Major Research Activities Within Each NORC Theme

Obesity: Pathophysiology, Complications, and Therapeutics. The largest group of NORC investigators (72 members) is studying some aspect of obesity. There is considerable diversity in research programs within this group (basic and clinical departments, Physics Department, School of Social Work, and Program in Physical Therapy), which provides a foundation for unique interactions between disciplines, facilitated by the NORC Cores and the research seminars. The interest and expertise include:

- Genetics (Borecki, Love-Gregory, Permutt, Polonsky)
- Microbiome (Gordon, Crawford)
- Organ system dysfunction, including i) nonalcoholic fatty liver disease and liver metabolism (Klein, Fabbrini, Finck, Hall, Chambers, Anderson, Brunt, Davidson, Chen); ii) cardiac dysfunction (Gropler, Peterson, Davila-Roman, Duncan, Zheng, Holland, Singh, Zheng); iii) atherosclerosis (Semenkovich, Hruska, Muslin, Ory, and Towler); iv) beta cell function

- (Polonsky, Turk, McDaniel, Permutt); v) insulin resistance (Gross, Shoghi, Muslin, Mueckler, Holloszy, Fisher); and vi) adipose tissue inflammation (Colonna, Klein)
- Adipocyte physiology (Abumrad, Wolins, Su)
 - Brain function and behavior (Sheline, Hershey, Lustman, Lenze, Newcomer, Stein, Wilfley)
 - Taste and sensory perception (Holy, Pepino de Gruev)
 - Diseases that cause obesity and metabolic dysfunction (human immunodeficiency virus [HIV]: Yarasheski, Hruz, Cade, and Reeds; schizophrenia: Newcomer; polycystic ovarian syndrome: Mittendorfer; Moley, Jungheim)
 - Diet and exercise therapy in humans (Klein, Holloszy, Racette, White, Stein, Wilfley, Sullivan)

The newly established Washington University Institute for Public Health (IPH) brings an important dimension in community engagement to the NORC. The IPH is co-directed by Graham Colditz, M.D., Dr.P.H., who is known for his expertise in diet and lifestyle interventions to prevent and modify chronic disease such as obesity, diabetes, and cancer. Several IPH investigators have become NORC members (Colditz, Haire-Joshu, Brownson, Wolins, Drake). There is a natural synergy between the NORC and the IPH, which will be more fully developed in the next funding cycle.

Growth, Development, and Aging. Twenty-seven NORC investigators are studying this broad theme. This group of investigators can be divided into several categories. One group is studying the very young: fetal development and embryonic substrate metabolism, (Moley, Jungheim, Riley, Jay) and neonatal pulmonary function (Hamvas, Cole, Patterson). This latter group is studying pulmonary surfactant production and dietary factors that might enhance pulmonary surfactant production in neonates; the group benefitted greatly from the CSR Core, which developed a novel method for measuring surfactant production *in vivo* in infants by using stable isotope tracers. A second group is studying growth, development, and regeneration (Rudnick, Goldsmith, Heuckeroth, Rubin, Levin, Warner). The third group is studying nutritional factors associated with aging and complications of aging, including bone remodeling and osteoporosis (Civitelli, Teitelbaum, Towler, Hildebolt), muscle function and frailty (Mittendorfer, Yarasheski, Binder), effect of calorie restriction on aging (Holloszy, Fontana, Imai, Klein), and Alzheimer's disease (Han, Holtzman, Bateman, Yarasheski, Gross). The Biomolecular Analysis (BMA) Core has been particularly critical for the studies in Alzheimer's disease because the Core developed methods for measuring intracellular metabolites and for assessing amyloid- β production in the cerebrospinal fluid, by using stable isotope tracer methods.

Nutrient Metabolism in Health and Disease. Almost all of our investigators are conducting studies within the umbrella of nutrient metabolism. Therefore, our research base is collaborating with each other based on metabolic interests and disease states. The largest groups are those studying different aspects of lipid metabolism (n=34; adipose tissue, fatty acid, cholesterol, and lipoprotein) and carbohydrate metabolism (n=34). NORC investigators are involved in many aspects of carbohydrate and lipid metabolism including normal physiology and the pathophysiology of insulin resistance and abnormalities in glucose and fatty acid metabolism associated with obesity and diabetes. Investigators are also studying the effect of amino acids on cell function and whole-body and muscle protein metabolism (Yarasheski, Mittendorfer, Smith, Patterson). This theme also includes vitamin and mineral research and their role in bone metabolism, development, and intestinal growth.

Core Laboratories

Administrative Core

Samuel Klein, M.D., Director; David Alpers, M.D., Associate Director; Nada Abumrad, Ph.D., Associate Director; Stephanie Paton, NORC Administrator.

The Administrative Core supervises all NORC activities with oversight by the Executive Committee and input from the Internal and External Advisory Committees. The members of the Executive Committee are Samuel Klein, Nada Abumrad, David Alpers, Mark Manary, Bruce Patterson, Kenneth Polonsky, Clay Semenkovich, John Turk, and Monique Williams. The diverse interests and expertise of the Executive Committee make them particularly well suited to monitor and manage the varied interests and activities of the NORC. The leadership of the NORC is also strengthened by the synergistic abilities of the Director (S. Klein: clinical and translational investigator) and Associate Directors (N. Abumrad, basic science investigator; D. Alpers, experienced mentor and teacher with accomplishments in both basic and clinical science research). This group has developed a productive working relationship and has collaborated on research projects, grant applications, and publications. The day-to-day operations of the NORC are performed by the NORC administrator, supervised by the director or, in his absence, the associate directors. The close physical proximity of the offices of the administrator, director, and associate directors (within a few yards of each other) enhances communication and facilitates regular interactions to discuss NORC issues.

Biomedical Research Core Laboratories

The NORC supports four Core Laboratories: 1) Clinical Science Research (CSR) Core, 2) Animal Model Research (AMR) Core, 3) BMA Core, and 4) Adipocyte Biology and Molecular Nutrition (ABMN) Core. The Cores provide research support and training to established investigators who are members of the NORC, to trainee members of the NORC, and to Pilot and Feasibility Award recipients. We expect that contact with experienced investigators, who serve as Core directors, and access to state-of-the-art research technology will improve the quality of current research and stimulate new and innovative nutrition research. The Cores are designed to facilitate both clinical and basic research.

Clinical Science Research Core

Bettina Mittendorfer, Ph.D., Core Director; Bruce Patterson, Ph.D., Core Associate Director

Purpose:

Provide: 1) a cohesive organizational structure that facilitates the performance of complex and diverse clinical research activities, 2) training for investigators and their staff who are new to the field, and 3) clinical research services that make it possible for basic researchers to test hypotheses in human subjects.

Services:

Drs. Mittendorfer and Patterson and CSR Core personnel will assist investigators in conducting complex clinical metabolic nutrition/obesity-related research in human subjects. They will work with each investigator to ensure that the most appropriate research techniques

and services are chosen for optimal productivity and reliability and assist in training young investigators and those who are new to clinical research. Several new services have been added and others have been enhanced significantly since the last competitive renewal, in response to direct feedback from the NORC research base and suggestions from the NORC Executive Committee and Internal and External Advisory Committees.

1. Clinical Studies Performance Sub-core (Director: Bettina Mittendorfer)

The complexity of clinical research is intimidating for many investigators. The new *Clinical Studies Performance Sub-core* will reduce this barrier, by providing personal guidance and act as a supportive interface between the investigator and the use of CSR Core services. This feature of the CSR CORE will facilitate the involvement of basic and clinical scientists, and particularly young investigators, in clinical and translational nutrition/obesity research, and should encourage new collaborations between basic and clinical investigators by linking research groups through use of the CSR CORE. Specifically, the *Clinical Studies Performance Sub-core* will provide:

- Subject recruitment
- Metabolic study design and performance
- Infusate preparation
- Muscle and adipose tissue acquisition
- Body composition analyses
- Aerobic fitness testing
- Indirect calorimetry
- Lifestyle intervention
- Cardiovascular assessments

2. Metabolic Kinetics Sub-core (Director: Bruce Patterson)

The *Metabolic Kinetics Sub-core* will assist investigators (in collaboration with the *Clinical Studies Performance Sub-core*) in designing and executing study protocols aimed at evaluating substrate kinetics by using stable isotope labeled tracer and non-tracer approaches, processing blood and tissue samples for tracer analysis, and assisting in data analysis and interpretation. The following services will be provided:

- Metabolic kinetic study design consultation
- Sample processing, derivatization, and gas chromatography-mass spectrometry (GC-MS) analyses
- Quantitative GC analysis of fatty acid profiles
- Kinetic analysis and mathematical modeling of metabolic kinetics

3. Training and Education

Training and education are important Core missions. Accordingly, the CSR CORE provides excellent training opportunities for students, fellows, and faculty and their research associates. This includes, as needed, one-on-one education, consultations, and hands-on training in any of the areas covered by the CSR CORE. Previously, Dr. Patterson coordinated

an annual didactic course consisting of weekly 1-hour sessions entitled “Methods of *In Vivo* Metabolic Research” that focused on the application of stable isotope labeled tracers and mathematical modeling to studies of metabolic kinetics. This course has been available free of charge to students, research fellows, and NORC investigators and their staffs, and has been attended by approximately 35 persons including research fellows, young faculty, and established faculty. In response to the growing needs of NORC investigators, Drs. Mittendorfer and Patterson will expand this course during the next funding cycle, to be renamed “Nutrition- and Obesity-Related Clinical Research,” which will add topics for other services provided by the CSR CORE such as conducting clinical research, body composition analyses, indirect calorimetry, and cardiovascular assessments. This series brings together research fellows and faculty from diverse backgrounds, including collaborators from basic science laboratories, to learn how clinical studies are conducted in human subjects.

Animal Model Research Core

Clay Semenkovich, M.D., Core Director

Purpose:

Provide well-characterized mouse models, services critical for evaluation of mouse phenotypes, and training required for testing hypotheses related to nutrition and obesity in animal models.

Services:

1. Providing genetically engineered mice from small breeding colonies for nutrition-related research
2. Performing biochemical assays of serum
3. Performing biochemical assays of tissues
4. Providing facilities and reagents for assaying messenger RNAs (mRNAs) of genes relevant to nutrition and obesity research
5. Maintaining facilities for and providing instruction in assessing atherosclerosis in mice
6. Assaying metabolic parameters including metabolic rate and food intake
7. Determining blood pressure in mice
8. Glucose tolerance and insulin tolerance testing in mice
9. Hyperinsulinemic/euglycemic clamp procedure in mice
10. Determination of body composition
11. Training/consultation services dealing with establishing mouse colonies and phenotyping animals
12. Immunocytochemistry of mouse tissues

Biomolecular Analysis Core

John Turk, M.D., Ph.D., Core Director; Kevin Yarasheski, Ph.D., Core Associate Director

Purpose:

Offer a comprehensive range of mass spectrometry (MS) analyses for the structural characterization and quantification of nutrition-related biomolecules for NORC investigators. The predominant BMA Core objective is to provide means for NORC investigators to gain maximum benefit from the power of MS with a focus on three areas of need: 1) to apply the

sensitivity and specificity of MS to detect and quantify low abundance biomolecules, 2) to develop innovative MS techniques for detection and structural characterization of nutrition- or obesity-related biomolecules, and 3) to provide MS training for students and fellows in nutrition or obesity research. Because MS analyses are labor intensive and require specialized skills, it is often more economical and practical for BMA Core staff to perform MS analyses. In cases where sustained demand for analyses or development of analytical techniques is anticipated, members of investigators' laboratories are trained to perform appropriate MS procedures.

Services:

1. Electrospray ionization tandem mass spectrometry (ESI/MS/MS) analyses of complex lipids (glycerolipids, sphingolipids, and their subclasses), for qualitative structural identification or for quantification with internal standards
2. Collaborative assistance in preparing samples for ESI/MS and instruction in extraction, purification, and, when needed, derivatization of complex lipid species from biological materials
3. GC-MS analyses of fatty acids and their metabolites in various ionization modes for qualitative identification or for isotope dilution quantification with heavy isotope-labeled standards
4. Collaborative assistance and instruction in sample preparation for GC-MS
5. Advice in obtaining heavy isotope-labeled internal standards for small organic molecules by purchase or synthesis for use in quantification of target analytes
6. Assistance with identification of unknown molecules from biological extracts by MS methods and interpretation of mass spectra
7. GC-MS analyses of sterols and their oxidation products in various ionization modes for qualitative structural identification or for quantification in conjunction with internal standards
8. GC-MS analyses of derivatized amino acids and their oxidation products for qualitative structural identification or for quantification
9. GC-MS analyses of polyols and of other carbohydrates for quantification
10. Liquid chromatography electrospray ionization tandem mass spectrometry (LC/ESI/MS/MS) of peptides from protein digests to identify proteins and their post-translational modifications
11. Matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI/TOF/MS) analyses of peptide mixtures from enzymatic digests to identify proteins and their post-translational modifications
12. Training in the operation of MS systems for GC-MS, IRMS, ESI/MS, LC/ESI/MS, ESI/MS/MS, LC/ESI/MS/MS, and MALDI/TOF/MS systems, for NORC investigators, their technical staff, and their trainees
13. Maintaining functional MS systems for GC-MS, IRMS, ESI/MS, LC/ESI/MS, ESI/MS/MS, LC/ESI/MS/MS, and MALDI/TOF/MS systems for use by NORC investigators, their technical staff, and their trainees
14. Instruction in the principles of various modes of MS and their applicability to analyses of biological samples and in the preparation of samples for MS analyses
15. Organizing Mass Spectrometry Discussion Group seminars for presentation and discussion of NORC investigators' data in studies requiring use of BMA Core

instruments pertaining to the pathogenesis, complications, or treatment of nutrition- and obesity-related disorders and physiologic events

16. Training postdoctoral fellows, students, and technical assistants in principles of MS, applying MS to nutrition-related problems, and operating MS systems in the BMA Core. An MS Discussion Group meets biweekly for presentations by NORC investigators and trainees on the seventh floor Southwest Tower, one floor below the BMA Core instruments, staff offices, and laboratories. A number of extramural scientists or their trainees have visited the BMA Core for various periods for instruction in aspects of MS. Another training resource is the MS facility website, which contains tutorials on principles of MS and its biomedical applications and on complex lipid structure determination by ESI/MS/MS.

Adipocyte Biology and Molecular Nutrition Core

Nada Abumrad, Ph.D., Core Director

Purpose:

The Core provides NORC researchers with access to specific equipment (e.g., a tissue culture facility, a transfection system, a high resolution respirometer for assessing the metabolic state of biopsy samples, cells or isolated mitochondria, a Counter Multisizer for adipocyte sizing) and expertise that are difficult to assemble by individual investigators and that can present a barrier to those new to this field. The equipment and training available through the ABMN Core facilitate and enhance nutrition-related studies and maximize resource use for NORC investigators.

Services:

1. Access to highly specialized facilities and equipment (e.g., a tissue culture facility, a transfection system, a high resolution respirometer for assessing the metabolic state of biopsy samples, cells or isolated mitochondria, a Counter Multisizer for adipocyte sizing) and technical expertise for its operation
2. Assistance with specialized assays to evaluate: (i) adipocyte metabolic activity (e.g., glucose transport, glucose incorporation into glycogen, fatty acid transport, fatty acid incorporation into major lipid fractions, fatty acid oxidation, adipocyte lipolysis); (ii) adipose tissue morphology; (iii) adipose tissue macrophage content; and (iv) adipocyte gene and protein expression
3. Training for investigators, their staff, and research fellows in these areas of research, both in the form of one-on-one hands-on instruction as well as through seminars

Pilot and Feasibility Studies

Recently Funded Projects

Fasting Induced Changes to Ischemia and Reperfusion Injury in the Steatotic Liver

P&F Investigator: Christopher Anderson, M.D., Assistant Professor of Surgery

Funding Period: 10/01/08–09/30/10

The hypothesis to test is that steatotic livers are more susceptible to cold ischemia and reperfusion injury (CIR) due to increased endoplasmic reticulum (ER) stress, and the findings to

date suggest that this may be altered by short-term fasting via a mechanism not related to alterations in insulin resistance or changes in hepatic lipid metabolism. This project has employed a preclinical model of steatotic rat liver transplantation to study how ER stress contributes to allograft injury in transplanted steatotic livers. Specific Aim 1: Determine the effect of fasting induced changes in insulin signaling and hepatic lipid metabolism on the ER stress response to CIR in the steatotic liver. Specific Aim 2: Determine the effect of systemic donor inflammation, hepatic lipid content, and donor fasting time on the magnitude of acute ER stress and early allograft inflammation following CIR in human liver transplantation.

Adipose Lipid Droplet Proteins as Markers for Lipid Overload

P&F Investigator: Nathan E Wolins, Ph.D., Research Instructor of Medicine

Funding Period: 10/01/08–09/30/10

As obesity has increased so has the prevalence of metabolic disease including type 2 diabetes, nonalcoholic fatty liver disease, and cardiomyopathy. These diseases are associated with fat outside of adipose tissue—ectopic fat, especially in muscle and liver. Ectopic fat correlates strongly and directly with metabolic derangements and disease. Paradoxically, endurance training increases intra-myocyte fat and insulin sensitivity, and is protective against metabolic disease. Dr. Wolins proposed that the answer to this paradox is in the proteins surrounding this fat. Proteins delineate the fat/cytosol interface and have been shown to recruit cytosolic proteins. Hence, fat coat proteins form a scaffold for aqueous cytosol structures to attach. Both cell-based and whole-animal studies have shown that PAT family proteins (Perilipin 1, Adipophilin, TIP-47/perilipin 3) exert control over the fat they enclose. Dr. Wolins proposed to determine how levels of adipose fat coat proteins and corresponding mRNAs are affected by changes in adiposity and changes in insulin sensitivity, and how they differ between fat depots. He also proposed to identify a profile of fat coat proteins and corresponding mRNAs that correlates with other markers of metabolic disease.

Endoplasmic Reticulum Stress in HIV-Metabolic Syndrome

P&F Investigator: Dominic Reeds, M.D., Assistant Professor of Medicine

Funding Period: 10/01/09–09/30/10

Highly active antiretroviral therapy (HAART) has lowered deaths due to infectious causes in people living with HIV, but greater than 50% of patients develop metabolic abnormalities after initiation of therapy. These metabolic changes, known collectively as HIV-metabolic syndrome (HIV-MS), include insulin resistance, hypertriglyceridemia, accumulation of visceral fat, and subcutaneous lipoatrophy in the limbs and face. The pathogenesis of HIV-MS is poorly understood, but antiretroviral medications, in particular protease inhibitors (PIs), are implicated. Studies conducted *in vitro* and primarily in tumor cell line models show that several PIs induce endoplasmic reticulum (ER) stress. However, there is little information regarding whether PIs induce ER stress in critical insulin target tissues *in vivo*. In obese humans, ER stress is strongly associated with insulin resistance and dyslipidemia, and may cause cellular autophagy. Animal models of insulin resistance have shown that treatment of ER stress with the chemical chaperones taurodeoxycholic acid (TUDCA) and phenylbutyrate (PBA) ameliorates ER stress and improves insulin resistance and dyslipidemia. Specific Aim 1: To determine whether treatment of HIV+ subjects with PI-based or non-PI based HAART induces markers of ER stress in adipose tissue and skeletal muscle. Specific Aim 2: To evaluate the efficacy of chemical chaperones that alleviate ER stress in treatment of PI-induced insulin resistance in mice. In preliminary studies Dr. Reeds has shown that the initiation of HAART rapidly induces insulin

resistance and dyslipidemia. Adipose tissue and skeletal muscle samples obtained from these subjects will be used for specific aim 1. In body composition studies he also showed that PI use is associated with reduced appendicular and subcutaneous fat. In HepG2 cells he has found that the PIs nelfinavir and ritonavir induce ER stress. Thus, Dr. Reeds will examine the expression of ER stress genes and components of the insulin signaling cascade in human biopsy specimens. For Aim 2 he will use continuous release pellets of PIs in male C57BL6 mice and examine the same endpoints as for Aim 1. In addition, he will perform measurements of insulin resistance *in vivo* using the challenge of intraperitoneal glucose or insulin. Predicted results for specific aim 1: It is predicted that many markers of the ER stress response will be increased in adipose tissue and/or skeletal muscle of patients with HIV who receive PI-based HAART. These measures are not likely to be affected by HIV infection alone. Further, it is predicted that the increase in these ER stress markers will be associated with negative changes in insulin sensitivity and insulin signaling protein phosphorylation. Predicted results for specific aim 2: Based on previous work in rodents, PI treatment in mice is predicted to induce insulin resistance and glucose intolerance coincident with a marked induction in ER stress markers. Dr. Reeds also predicts that TUDCA will ameliorate ER stress and improve glucose tolerance in PI-treated mice.

The Effects of Obesity and Saturated Fatty Acids on Peri-Implantation

P&F Investigator: Joan Riley, Ph.D., Assistant Professor of Obstetrics & Gynecology-Research Operations

Funding Period: 10/01/09–09/30/10

The objective of this proposal is to determine whether obesity and elevated free fatty acid concentrations affect placental development in a manner similar to that seen in pregnancies complicated by preeclampsia. It is hypothesized that both obesity and elevated free fatty acid levels will inhibit blastocyst proliferation and enhance cell death in trophoblast, embryonic cell mass, or both, thereby limiting blastocyst expansion and decidual invasion to yield poor placentation. The specific aims of this proposal are: 1) to determine if palmitic acid affects blastocyst proliferation, survival, expansion, and invasion *in vitro* and whether the effects of palmitic acid can be recapitulated *in vivo* by placing mice on a diet high in saturated fatty acids, and 2) to determine whether the mechanism by which palmitic acid and a high fat diet induce adverse effects on peri-implantation events involves the production of reactive oxygen species and/or the mammalian target of rapamycin (mTOR) pathway.

Caloric Restriction, Sleep Loss, and Metabolism

P&F Investigator: Paul Shaw, Ph.D., Assistant Professor of Neurology

Funding Period: 10/01/09–09/30/10

Test the hypothesis that genes activated by caloric restriction will negate/mitigate against the metabolic consequences of insufficient sleep. Dr. Shaw has developed a model of human insomnia in the genetically tractable organism *Drosophila melanogaster*. These *insomnia-like* flies (*ins-l*) exhibit short-sleep as well as increased triglyceride stores, free fatty acid levels, and cholesterol levels. Thus, he will activate genes associated with nutrient sensing in *ins-l* flies to reverse the metabolic consequences of sleep loss, and use genetics to understand the molecular link between sleep and metabolism. He has successfully introgressed *ins-l* with three GeneSwitch GAL4 drivers: 1) *ins-l^{Gsw-elav-GAL4}*, which expresses in all neurons; 2) *ins-l^{Gsw-32-GAL4}*, which expresses in fat bodies; and 3) *ins-l^{Gsw106-GAL4}*, which express in the head fat body. Dr. Shaw will evaluate the effects of two genes that are antagonistic with respect to insulin signaling (*UAS-TOR* and *UAS-Tsc1,2*) and two genes that are antagonistic for lipid storage (*UAS-Lsd2* and

the adipose triglyceride lipase *brummer* (*UAS-bmm*). He will also evaluate the effects of increase in Sir2 activity, given its established role in metabolism and aging. Sleep duration and metabolism will be evaluated in young (5 days to 10 days old) and old (20 days to 25 days old) flies maintained on a standard diet (1X) or a calorically restricted diet (0.4X). To further assess the functional relevance of these manipulations, Dr. Shaw will evaluate plasticity-induced sleep in 20-day-old animals. Finally, lifespan will be evaluated in all genotypes and in *ins-l/ins-l* flies under both dietary conditions. The potentially most informative outcome would be a decrease of organismal triglycerides through tissue-specific expression of genes that protect healthy flies from the effects of sleep loss (e.g., *UAS-TSC,1,2* and *UAS-bmm*) but not for their antagonistic counterparts.

Funding Derived From Previous Pilot and Feasibility Studies

- **Vitamin A deficiency and intestinal motility disorders.** Robert Heuckeroth, M.D., Ph.D. Funding: NIH/NIDDK R01 DK087715, 04/01/2010–03/31/2015.
- **A proof-of-concept clinical trial of xenin-25 in the treatment of type 2 diabetes.** Burton Wice, Ph.D. Funding: NIH/NIDDK RC1 DK086163, 09/30/2009–08/31/2011.
- **Calorie restriction and gene expression in humans.** Luigi Fontana, M.D., Ph.D. Funding: American Federation of Aging Research Foundation BIG award, 07/01/2009–06/30/2011.
- **Genetics-InFormatics Trial (GIFT) of warfarin to prevent DVT.** Brian Gage, M.D. Funding: NIH/NHLBI R01 HL097036, 09/10/2009–05/31/2014.
- **Direct effects of antiretroviral therapy on cardiac energy homeostasis.** Paul Hruz, M.D., Ph.D. Funding: NIH/NHLBI R01 HL092798, 09/20/2009–05/31/2014.
- **Diagnostic and therapeutic applications of a novel plasma metabolite, Nicotinamide Mononucleotide (NMN), for age-associated metabolic complications in humans.** Shin-Ichiro Imai, M.D., Ph.D. Funding: Longer Life Foundation. 09/01/2008–08/31/2010.
- **Biomarkers and pathogenesis of multiple sclerosis: from mouse to human.** Dorothy Cross, M.D. Funding: NIH P01 NS059560, 09/25/2008–07/31/2013.
- **Lipin 1 in the regulation of hepatic lipid metabolism.** Brian Finck, Ph.D. Funding: NIH/NIDDK R01 DK078187, 01/01/2008–11/30/2013.
- **Insulin resistance and myocardial glucose metabolism in pediatric heart failure.** Patrick Jay, M.D., Ph.D. Funding: NIH/NHLBI R01 HL092798, 09/20/09–05/31/2014
- **CD36 variants on cardiovascular risk factors.** Latisha Love-Gregory, Ph.D. Funding: American Heart Association 07/01/2010–06/30/2013
- **Fatty acid alpha oxidation in adipocytes.** Xiong Su, Ph.D. Funding: NIH/NIDDK R21 DK082951 03/01/2009–02/28/2011

Scientific Advances and Accomplishments

The Washington University NORC has demonstrated considerable success since its initiation in 1999, particularly during the last 5 years, and has transformed nutrition and obesity research, training, and clinical activities at WU. The following accomplishments have been achieved:

1. Developed an intellectual and physical home for nutrition and obesity at WU that is recognized for excellence throughout the institution

2. More than doubled the research base by facilitating the recruitment of new faculty to WU and attracting existing faculty to nutrition and obesity
3. Increased total funding for nutrition/obesity research
4. Increased the number of publications from our research base and nearly doubled the number that acknowledge support from the NORC
5. Provided cost-effective, state-of-the-art, sophisticated, and practical Core Laboratory research services and training targeted specifically to meet the needs of NORC investigators
6. Stimulated outstanding interdisciplinary clinical and translational collaborative research
7. Initiated collaborations with other NIH-funded WU Centers, particularly the Diabetes Research and Training Center (DRTC) and the ICTS, to leverage resources across Centers and thereby reduce research costs while expanding services to NORC investigators
8. Developed a new nutrition/obesity community outreach program that provides enrichment and clinical services, and is generating pilot data for extramural grant applications
9. Developed a unique Pilot and Feasibility program that provides career mentoring in addition to research funding to young investigators
10. Developed a Junior Investigator Group to stimulate interaction, support, and camaraderie among young investigators
11. Developed a joint NORC, DRTC, and Digestive Diseases Research Core Center annual young investigator research day for Pilot and Feasibility Awardees, which will provide a forum for recognition and interaction
12. Developed a novel, web-based training program in obesity for medical students that is being piloted at WUSM for potential distribution throughout the country, if successful
13. Provided high-quality interactive research seminars that are attended by faculty and trainees throughout the university and that have stimulated new research ideas and interdisciplinary collaborations
14. Obtained considerable institutional and philanthropic support for NORC functions and activities

The specific examples of research accomplishments and collaborations listed below would not have been possible without the use of the NORC laboratories and resources.

Women's Health

Fatty Acid Metabolism. An increased release of free fatty acids (FFA) into plasma likely contributes to the metabolic complications associated with obesity. Dr. Mittendorfer evaluated the interrelationships between body fat, sex, and plasma FFA kinetics in 106 lean, overweight, and obese non-diabetic subjects. There were no differences between men and women in the

relationship between fat mass (FM) and total FFA Rate of appearance (Ra), but FFA Ra in relationship to fat-free mass was greater in women than in men because women have more body fat; total FFA Ra increased linearly with increasing FM and FFA Ra per kg FM decreased in a curvilinear fashion with increasing FM. Her data demonstrate that total body fat, not sex, is an important modulator of the rate of FFA release into plasma. Although increased adiposity is associated with a decrease in fatty acid release in relationship to FM, this downregulation is unable to completely compensate for the increase in FM, so total FFA Ra and FFA Ra with respect to FFM are greater in women than in men.

Mittendorfer B, Magkos F, Fabbrini E, Mohammed BS, Klein S. Relationship between body fat mass and free fatty acid kinetics in men and women. *Obesity* 17:1872–1877, 2009. NIHMSID207072.

Muscle Mass. Men have more muscle than women but most studies evaluating sex differences in muscle protein metabolism have been unable to discern sexual dimorphism in basal muscle protein turnover rates in young and middle-aged adults. It has been hypothesized that the anabolic response to nutritional stimuli (i.e., amino acids and insulin) would be greater in young/middle-aged men than women. Drs. Mittendorfer, Mohammed, and Reeds measured the rates of muscle protein synthesis (MPS) in 16 healthy individuals after an overnight fast and during a hyperinsulinemic-hyperaminoacidemic-euglycemic clamp. The rates of MPS were not different between men and women. During the clamp, the rate of MPS increased by approximately 50% with no difference in the increases from basal values between men and women. There were also no differences between men and women in the basal concentrations of muscle phosphorylated AktSer473, AktThr308, mTORSer2448, and p70s6kThr389 or in the hyperinsulinemia-hyperaminoacidemia induced increases in phosphorylation of those signaling elements ($p \geq 0.25$). These data demonstrate that there are no major differences in the rate of MPS and its intracellular control during basal conditions and during hyperinsulinemia-hyperaminoacidemia between young adult men and women.

Smith GI, Atherton P, Reeds DN, Mohammed BS, Jaffery H, Rankin D, Rennie MJ, Mittendorfer B. No major sex differences in muscle protein synthesis rates in the postabsorptive state and during hyperinsulinemia-hyperaminoacidemia in middle-aged adults. *J Appl Physiol.* 2009;107(4):1308–1315. PMC2763845.

Minority Health

CD36 is a membrane receptor that recognizes long chain fatty acids (FA), cholesterol, and oxidized lipoproteins. In rodents, CD36 has been implicated in atherosclerosis and linked to abnormalities of lipid metabolism. This protein also impacts survival to malaria and the influence of natural selection has resulted in high CD36 genetic variability in populations of African descent. Drs. Love-Gregory, a junior NORC investigator and NORC Pilot and Feasibility Awardee, and Dr. Abumrad evaluated the impact of selected high-density lipoprotein (HDL)-associated single-nucleotide polymorphisms (SNPs) on CD36 expression and the relationship between CD36 expression and HDL and very low-density lipoprotein (VLDL) serum concentrations in 104 unrelated African American subjects. Their results show that genetic variability at the CD36 locus affects CD36 expression, which influences serum lipid

concentrations; lower CD36 is associated with a protective metabolic profile. These data demonstrate the potential effect of genetics on cardiovascular disease risk in African Americans.

Love-Gregory L, Schappe T, Sherva R, Qi J-S, McCrea J, Klein S, Connelly MA, Abumrad NA. Common CD36 SNPs reduce protein expression and promote a protective atherogenic profile. *Human Molecular Genetics* (in press).

AIDS

Cellular fatty acid metabolism. Infection with HIV and treatment with HIV PI-based HAART is associated with dysregulated fatty acid and lipid metabolism. Drs. Yarasheski and Ramanadham found that HIV PIs directly suppress fatty acid oxidation in murine skeletal muscle cells, and that this may be related to decreases in cytosolic- and mitochondrial-associated fatty acid transporters.

Richmond SR, Carper MJ, Lei X, Zhang S, Yarasheski KE, Ramanadham S. HIV-protease inhibitors suppress skeletal muscle fatty acid oxidation by reducing CD36 and CPT-I fatty acid transporters. *Biochim Biophys Acta* 1801:559–566, 2010. PMC2838954.

Inflammatory Bowel Disease

Clay Semenkovich, M.D., has characterized the first physiologically relevant ligand for the nuclear receptor peroxisome proliferated-activated receptor-alpha (PPAR α). This work made extensive use of Core services and was done in collaboration with John Turk, a NORC investigator and BMA Core Director. Previously, these investigators made the observation that inactivation of de novo lipogenesis carried out by fatty acid synthase in mouse liver resulted in a phenotype that resembled PPAR α deficiency (fatty liver and hypoglycemia) that was rescued by administration of a PPAR α activator. This finding suggested that fatty acid synthase was contributing to the generation of an endogenous ligand for this nuclear receptor. In a series of experiments, this group characterized the lipids associated with PPAR α in the presence and absence of FAS in mouse liver, discovered that the phosphatidylcholine species 16:0/18:1-GPC is associated with the receptor in the presence of FAS, and then demonstrated the bioactivity of this complex lipid in mice. This work has translational potential since phosphatidylcholine is already being used as an anti-inflammatory agent in humans with inflammatory bowel disease. These investigators are now extending this work to address relevant issues in obesity and fatty liver in an attempt to develop novel nutritional approaches to these disorders.

Chakravarthy MV, Lodhi IJ, Yin L, Malapaka RR, Xu HE, Turk J, Semenkovich CF. Identification of a physiologically relevant endogenous ligand for PPAR α in liver. *Cell* 138(3):476–488, 2009. PMC2725194.

Obesity

Nonalcoholic Fatty Liver Disease (NAFLD). Dr. Fabbrini, a junior NORC investigator, in collaboration with two senior NORC investigators (Drs. Abumrad and Klein) evaluated the importance of increased either intrahepatic triglyceride or intraabdominal fat on metabolic dysfunction. Visceral adipose tissue (VAT) is an important risk factor for obesity-related metabolic disorders. Therefore, a reduction in VAT has become a key goal in obesity management. However, VAT is correlated with intrahepatic triglyceride (IHTG) content, so it is

possible that IHTG, not VAT, is the primary determinant of metabolic disease. The independent contribution of IHTG and VAT to metabolic function was determined by evaluating groups of obese subjects, who differed in IHTG content (high or normal) but matched on VAT volume, or differed in VAT volume (high or low) but matched on IHTG content. Stable isotope tracer techniques and the euglycemic-hyperinsulinemic clamp procedure were used to assess insulin sensitivity and VLDL-triglyceride (VLDL-TG) secretion rate. Tissue biopsies were obtained to evaluate cellular factors involved in ectopic triglyceride accumulation. Hepatic, adipose tissue, and muscle insulin sensitivity were lower, whereas VLDL-TG secretion rate was almost double in subjects with higher than normal IHTG content, matched on VAT. No differences in insulin sensitivity or VLDL-TG secretion were observed between subjects with different VAT volumes, matched on IHTG content. Adipose tissue CD36 expression was lower, whereas skeletal muscle CD36 expression was higher, in subjects with higher than normal IHTG. These data demonstrate that IHTG, not VAT, is a better marker of the metabolic derangements associated with obesity. Furthermore, alterations in tissue fatty acid transport are likely involved in the pathogenesis of ectopic triglyceride accumulation by redirecting plasma fatty acid uptake from adipose tissue toward other tissues.

Fabbrini E, Mohammed BS, Magkos F, Pietka T, Abumrad N, Klein S. Intrahepatic triglyceride, not visceral fat, is linked with metabolic complications of obesity. *Proc Nat Acad Sci* 106:15430–15435, 2009. PMC2741268.

Cardiovascular Structure and Function. Obesity is associated with adverse cardiac and vascular structural and functional alterations. Dr. Davila-Roman and other NORC investigators evaluated the effect of moderate weight reduction and subsequent partial weight regain on cardiovascular structure and function. Cardiac and vascular ultrasound studies were performed in 60 obese subjects before and at 3, 6, 12, and 24 months after starting a dietary weight loss program in which subjects lost approximately 9% of their body weight by 6 months and then regained weight thereafter. Decreases in weight were associated with decreases in left ventricular (LV) mass and carotid intima-media thickness as well as significant improvement in LV diastolic and systolic function. Partial weight regain diminished the maximal observed beneficial effects of weight loss; however, cardiovascular parameters measured at 2 years still showed a net benefit compared with baseline. These data demonstrate that diet-induced moderate weight loss in obese subjects is associated with beneficial changes in cardiovascular structure and function. Subsequent weight regain is associated with partial loss of these beneficial effects.

de las Fuentes L, Waggoner AD, Mohammed BS, Stein R, Miller B, Foster G, Wyatt H, Klein S, Davila-Roman VG. Effect of moderate diet-induced weight loss and regain on cardiovascular structure and function. *J Amer Coll Cardiol* 2009;54(25):2376–2381. PMC2818984.

Diabetes. The E23K variant in the Kir6.2 subunit of the ATP-sensitive K(+) channel (K(ATP) channel) is associated with an increased risk of type 2 diabetes. Dr. Polonsky and colleagues evaluated insulin secretion and action in subjects with the variant who had normal glucose tolerance. Their data show that the insulin secretory responses to oral and intravenous glucose were reduced by approximately 40% in glucose-tolerant subjects homozygous for E23K. The hyperinsulinemic-euglycemic clamp was used to find that hepatic insulin sensitivity is approximately 40% greater in subjects with the E23K variant, and these subjects demonstrated increased insulin sensitivity after oral glucose. These findings suggest a direct molecular

explanation for reduced insulin secretion in these subjects. The E23K variant leads to overactivity of the K(ATP) channel, resulting in reduced insulin secretion. Initially, insulin sensitivity is enhanced, thereby maintaining normal glucose tolerance. Presumably, over time, as insulin secretion falls further or insulin resistance develops, glucose levels rise resulting in type 2 diabetes.

Villareal DT, Koster JC, Robertson H, Akrouh A, Miyake K, Bell GI, Patterson BW, Nichols CG, Polonsky KS. Kir6.2 variant E23K increases ATP-sensitive K⁺ channel activity and is associated with impaired insulin release and enhanced insulin sensitivity in adults with normal glucose tolerance. *Diabetes* 2009;58:1869–1878. PMC2712777.

PPAR α Receptor. The nuclear receptor PPAR α is an important regulator of fatty acid oxidation in liver and muscle. However, the endogenous ligand for the PPAR α receptor is unknown. Drs. Semenkovich and Turk found that PPAR α -dependent gene expression is impaired with inactivation of fatty acid synthase (FAS), suggesting that FAS is involved in generation of a PPAR α ligand. They demonstrated the FAS-dependent presence of a phospholipid bound to PPAR α isolated from mouse liver. Binding was increased under conditions that induce FAS activity and displaced by systemic injection of a PPAR α agonist. Mass spectrometry identified the molecule as 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine (16:0/18:1-GPC). Knockdown of Cept1, required for phosphatidylcholine synthesis, suppressed PPAR α -dependent gene expression. Interaction of 16:0/18:1-GPC with the PPAR α ligand-binding domain and coactivator peptide motifs was comparable to PPAR α agonists, but interactions with PPAR δ were weak and none were detected with PPAR γ . Portal vein infusion of 16:0/18:1-GPC induced PPAR α -dependent gene expression and decreased hepatic steatosis. These data suggest that 16:0/18:1-GPC is a physiologically relevant endogenous PPAR α ligand.

Chakravarthy MV, Lodhi IJ, Yin L, Malapaka RR, Xu HE, Turk J, Semenkovich CF. Identification of a physiologically relevant endogenous ligand for PPAR α in liver. *Cell* 2009;138:476–488. PMC2725194.

Lipin. Lipin 1 is a bifunctional intracellular protein that regulates fatty acid metabolism in the nucleus via interactions with DNA-bound transcription factors and at the endoplasmic reticulum as a phosphatidic acid phosphohydrolase enzyme (PAP-1) to catalyze the penultimate step in triglyceride synthesis. Brian Finck, Ph.D., a junior NORC investigator and past Pilot and Feasibility Awardee, found that hepatic lipin 2 is enriched in liver, and hepatic lipin 2 protein content was markedly increased by lipin 1 deficiency, food deprivation, and obesity. Importantly, RNA interference against lipin 2 markedly reduced PAP-1 activity in hepatocytes from both wild-type and fld mice and suppressed triglyceride synthesis under conditions of high fatty acid availability. These data suggest that lipin 2 plays an important role as a hepatic PAP-1 enzyme.

Gropler MC, Harris TE, Hall AM, Wolins NE, Gross RW, Han X, Chen Z, Finck BN. Lipin 2 is a liver-enriched phosphatidate phosphohydrolase enzyme that is dynamically regulated by fasting and obesity in mice. *J Biol Chem* 2009;284:6763–6772. PMC2652272.

Gut Microbiota. Dr. Jeff Gordon has continued his exciting studies evaluating the functional importance of the gut microbiome in the pathogenesis of nutrition-related metabolic dysfunction and diseases. His studies in mice indicate that the gut microbiota promotes energy harvest and

storage from components of the diet when these components are plentiful. He has also examined how the microbiota shapes host metabolic and physiologic adaptations to periods of nutrient deprivation. Germ-free (GF) mice and mice who had received a gut microbiota transplant from conventionally raised donors were compared in the fed and fasted states by using functional genomic, biochemical, and physiologic assays. A 24-hour fast produced a marked change in gut microbial ecology. Short-chain fatty acids generated from microbial fermentation of available glycans are maintained at higher levels compared with GF controls. During fasting, a microbiota-dependent, PPAR α -regulated increase in hepatic ketogenesis occurs, and myocardial metabolism is directed to ketone body utilization. Analyses of heart rate, hydraulic work, and output, mitochondrial morphology, number, and respiration, plus ketone body, fatty acid, and glucose oxidation in isolated perfused working hearts from GF and colonized animals (combined with *in vivo* assessments of myocardial physiology) revealed that the fasted GF heart is able to sustain its performance by increasing glucose utilization, but heart weight, measured echocardiographically or as wet mass and normalized to tibial length or lean body weight, is significantly reduced in both fasted and fed mice. This myocardial-mass phenotype is completely reversed in GF mice by consumption of a ketogenic diet. Together, these results illustrate benefits provided by the gut microbiota during periods of nutrient deprivation, and emphasize the importance of further exploring the relationship between gut microbes and cardiovascular health.

Crawford PA, Crowley JR, Sambandam N, Muegge BD, Costello EK, Hamady M, Knight R, Gordon JI. Regulation of myocardial ketone body metabolism by the gut microbiota during nutrient deprivation. *Proc Natl Acad Sci USA* 2009;106:11276–11281. PMC2700149.

Pediatric Obesity. Obesity in adolescents is associated with metabolic risk factors for type 2 diabetes, particularly insulin resistance and excessive accumulation of IHTG. Drs. Stein, Mohammed and Kirk evaluated the effect of moderate (approximately 8%) weight loss on IHTG content and insulin sensitivity in obese adolescents. Weight loss caused a marked decrease in IHTG content and improved both hepatic and skeletal muscle insulin sensitivity. These results support the benefits of weight loss therapy in obese adolescents who do not have evidence of obesity-related metabolic complications during a standard medical evaluation.

Vitola BE, Deivanayagam S, Stein RI, Mohammed BS, Magkos F, Kirk EP, Klein S. Weight loss reduces liver fat and improves hepatic and skeletal muscle insulin sensitivity in obese adolescents. *Obesity (Silver Spring)* 2009;17:1744–1748. PMC2834223.

Health Promotion and/or Disease Prevention

Protein-Energy Malnutrition in Children. Dr. Manary continues to conduct clinical studies to determine optimal dietary approaches for combating protein-calorie malnutrition in impoverished Malawian children. Dr. Manary uses the Clinical Science Research Core for mass spectrometry analyses of plasma samples obtained during these studies. During the last year he and his colleagues have demonstrated that home-based therapy using ready-to-use therapeutic foods can successfully treat uncomplicated childhood malnutrition on an outpatient basis.

Connor NE, Manary MJ. Monitoring the adequacy of catch-up growth among moderately malnourished children receiving home-based therapy using mid-upper arm circumference in southern Malawi. *Matern Child Health J.* 2010; Jan 16 [Epub ahead of print].

Ndekha MJ, van Oosterhout JJ, Zijlstra EE, Manary M, Saloojee H, Manary MJ. Supplementary feeding with either ready-to-use fortified spread or corn-soy blend in wasted adults starting antiretroviral therapy in Malawi: randomised, investigator blinded, controlled trial. *BMJ*. 2009;338:1867. PMC2685879.

Anthor RE, Cole SM, Manary MJ. The use of home-based therapy with ready-to-use therapeutic food to treat malnutrition in a rural area during a food crisis. *J Am Diet Assoc*. 2009;109(3):464–467. PMC Journal – In Process.

Matilsky DK, Maleta K, Castleman T, Manary MJ. Supplementary feeding with fortified spreads results in higher recovery rates than with a corn/soy blend in moderately wasted children. *J Nutr*. 2009;139(4):773–778. NIHMSID207070.

Ashorn P, Phuka JC, Maleta K, Thakwalakwa C, Cheung YB, Briend A, Manary MJ. Undernutrition malnutrition in infants in developing countries. *Arch Pediatr Adolesc Med* 163:186, 2009. PMC Journal – In Process.

Vitamin D and Atherosclerosis. Cardiovascular disease is the leading cause of death among those with diabetes mellitus, and vitamin D deficiency is associated with an increased risk of cardiovascular disease in this population. Dr. Bernal-Mizrachi and colleagues evaluated the potential mechanism by which vitamin D deficiency mediates accelerated cardiovascular disease in patients with diabetes mellitus. Macrophages were obtained from obese, diabetic patients with vitamin D deficiency and a series of control groups with or without diabetes with and without vitamin D deficiency. Macrophages were cultured in vitamin D-deficient or 1,25-dihydroxyvitamin D(3) [1,25(OH)(2)D(3)]-supplemented media and exposed to modified LDL cholesterol. 1,25(OH)(2)D(3) suppressed foam cell formation by reducing acetylated or oxidized LDL cholesterol uptake in diabetic subjects only. Conversely, deletion of the vitamin D receptor in macrophages from diabetic patients accelerated foam cell formation induced by modified LDL. 1,25(OH)(2)D(3) downregulation of c-Jun N-terminal kinase activation reduced PPAR γ expression, suppressed CD36 expression, and prevented oxidized LDL-derived cholesterol uptake. In addition, 1,25(OH)(2)D(3) suppression of macrophage endoplasmic reticulum stress improved insulin signaling, downregulated SR-A1 expression, and prevented oxidized and acetylated LDL-derived cholesterol uptake. These results identify reduced vitamin D receptor signaling as a potential mechanism underlying increased foam cell formation and accelerated cardiovascular disease in diabetic subjects, and has potential important public health implications.

Oh J, Weng S, Felton SK, Bhandare S, Riek A, Butler B, Proctor BM, Petty M, Chen Z, Schechtman KB, Bernal-Mizrachi L, Bernal-Mizrachi C. 1,25(OH)₂ vitamin D inhibits foam cell formation and suppresses macrophage cholesterol uptake in patients with type 2 diabetes mellitus. *Circulation* 2009;120:687–698. NIHMSID207088.

Community Outreach/Science

The NORC is developing a collaboration with the newly established Washington University Institute for Public Health (IPH) to bring transdisciplinary investigators together to stimulate research and community engagement to improve population health. The IPH, which has primary offices located one block from the NORC, is co-directed by Graham Colditz, M.D., DrPH, who

is known for his expertise in diet and lifestyle interventions to prevent and modify chronic disease, such as obesity, diabetes, and cancer, and Ed Lawlor, Ph.D., Dean of the School of Social Work and graduate programs in Public Health, internationally known for his work in health policy. The IPH provides funding for faculty scholars interested in pursuing collaborative public health research in designated priority areas. One of these priority areas is obesity and poor nutrition, which disproportionately affect African Americans living in urban, low-income neighborhoods of St. Louis.

Community-based Therapy for Obese African Americans with Diabetes and Depression.

This is a complex project evaluating the potential efficacy of a community-based intervention in reducing body weight and improving glycemic control and mood in obese African American adults with type 2 diabetes and comorbid depression. The project takes advantage of the YMCA system in the St. Louis area as a partner for community-based intervention. The study brings together a multidisciplinary team of psychologists, dietitians, counselors, physical trainers, social workers, nurses, and physicians. Specifically, the project is conducting a 1-year, randomized controlled trial to evaluate the effectiveness of three interventions for this population: 1) usual care (i.e., registered dietitian visit for meal planning provided within the primary care practice setting); 2) a culturally competent lifestyle-intervention weight loss program, administered within the YMCA; and 3) the same culturally competent weight loss program provided in conjunction with collaborative care for depression, both administered within the YMCA. Effects of these interventions will be compared on clinical outcomes (body weight, glycemic control, and various health parameters), mood, and psychosocial factors. If successful, the program could be adapted and eventually disseminated nationwide.

Educational Activities/Accomplishments

The Education/Enrichment Program is divided into two components: University Enrichment Program and Community Enrichment Program. The University Enrichment Program is directed by Dr. David Alpers and involves: 1) weekly Nutrition Seminar Series, 2) regular conferences on research techniques provided by each of the Core Laboratories, 3) hands-on training in research methods by each of the Core Laboratories, 4) a mentoring program for junior faculty provided by Dr. Alpers, 5) clinical electives in nutrition support and obesity for medical students and medical residents, and 6) NORC website information on nutrition-related lectures throughout the medical center as well as funding opportunities. The Community Enrichment Program is directed by Dr. Consuelo Wilkins and involves organizing nutrition/obesity lectures and programs for the St. Louis community and providing media outreach.

Professional Education/Enrichment Program

1. **NORC Seminar Series.** A research seminar series, which usually focuses on basic or clinical nutrition- and obesity-related research, but also includes clinical nutrition presentations, is held weekly. This lecture series is coordinated with other Centers so that visiting faculty will be “shared” with appropriate groups for the mutual benefit of all parties. Therefore, the NORC often shares the cost of hosting a visiting professor with another program. Although outside speakers are available about once a month, most of the seminars are focused on junior investigators who present data from their own research or a topic that

they are considering for future research; they receive feedback from their peers and more-experienced faculty. In addition, senior WU investigators and visiting faculty are invited to present at this conference, which provides additional exposure for trainees and stimulates interactions between established investigators. All Pilot and Feasibility Award recipients are required to present their research progress at this seminar each year, accompanied by their mentor. This interaction has been particularly useful for both the young investigators and their mentors in exchanging ideas from others with different perspectives.

2. **Interdisciplinary Obesity Research Seminar Series.** This seminar series was started approximately 1 year ago in response to requests by NORC investigators. The research seminars are held weekly, and focus on current obesity-related research activities including work-in-progress, data analysis and interpretation, protocol/project development, and grant applications. The sessions usually involve an interactive presentation with detailed discussions. These conferences benefit investigators by optimizing protocols and by sharing expertise and ideas among researchers; they also stimulate collaborations among investigators from different disciplines. Some sessions in the seminar series function as journal clubs in which recent, important publications related to obesity are discussed.

3. **Core Laboratory Conferences on Research Methods**

Clinical Science Research Core. The Clinical Science Research Core continued its series of didactic lectures entitled “Methods of *In Vivo* Metabolic Research” annually during the current funding cycle. There are 20 units in the series, with each unit consisting of one to three hour-long lectures. Topics covered include stable isotope tracers, performance of isotopic tracer studies, mathematical modeling of metabolic kinetics, energy expenditure, substrate oxidation, arterio-venous balance methods, and units devoted to specific areas of metabolic research (lipolysis, glucose metabolism, gluconeogenesis, protein metabolism, VLDL-TG and apoB metabolism, lactate kinetics). The series has been attended by approximately 30 to 40 persons, including established faculty, young faculty, and research fellows. In response to the growing needs of NORC investigators, this course will be expanded during the next 5 years, to be renamed “Nutrition- and Obesity-Related Clinical Research.” The course will incorporate topics for other services provided by the Core, such as conducting clinical research, body composition analyses, indirect calorimetry, and cardiovascular assessments. This series brings together research fellows and faculty from diverse backgrounds, including collaborators from basic science laboratories, to learn how clinical studies are conducted.

BMA Core Mass Spectrometry Discussion Group. A research seminar series, which focuses on the use of mass spectrometry in basic and clinical research, is held every other week and is sponsored by the NORC BMA Core and the Biomedical Mass Spectrometry Resource. Dr. John Turk, who is the Director of the NORC BMA Core, organizes this seminar series, which includes presentations from junior and senior investigators.

4. **Hands-on Research Training.** Each Core Laboratory has a training component, which allows investigators and trainees to learn specific research techniques. This experience provides one-on-one education, consultations, and/or bench-top laboratory training.

5. **Mentoring Program for Young Investigators.** Dr. Alpers has developed a popular mentoring program for NORC P/F Award recipients. In this program, scientific progress is monitored by Dr. Alpers after the first 6 to 8 months. At this time Dr. Alpers provides comments on the work's progress, as well as reviewing the career goals and grant plans for the awardees. The majority of awardees have used Dr. Alpers as a faculty advisor to review their grants before and after submission, and to discuss their career options. We have found that Dr. Alpers' mentoring efforts are often the first time the awardees have been provided with this support, which was very important for several awardees as they worked to establish their independence. Dr. Alpers ensures that the project on which they are working will indeed lead to an independent research direction, and has made the administrative chiefs of units provide a written guarantee of such independence. In addition, awardees must present their work at the weekly NORC Seminar Series and at the annual meeting of the External Advisory Committee. At this time the External Advisory Committee has the opportunity to provide constructive advice to each applicant.

6. **Clinical Nutrition Education**

Medical School Curriculum in Obesity/Obesity Education Initiative. Most physicians in the United States do not receive adequate training or background in obesity. This major gap in medical schools and postgraduate training programs is due to a paucity of faculty who have expertise in clinical obesity as well as the limited amount of time devoted to clinical obesity training in medical school curricula. Currently, most medical schools lack any structured approach to teaching the basic skill sets necessary to manage overweight and obese patients. Dr. Klein was able to raise funds from philanthropic organizations to develop a unique interactive, computer-based course that provides the basic principles of obesity management. This course focuses on independent learning through videos of real patient-physician interactions, didactic teaching slides, and interactive sessions that highlight key educational objectives. Several NORC physicians have collaborated on the project, which is currently in final production. This initiative uses a case-based teaching approach. Four modules were developed, each approximately 1 hour in length. The first module includes a patient interaction that will allow a careful review of basic office principles for managing obese patients and the use of appropriate and effective communication strategies. Three patient cases illustrate physician-directed interventions through: 1) lifestyle intervention (diet and physical activity), 2) pharmacotherapy, and 3) bariatric surgery. All modules are sensitive to gender and race. The effects of this intervention on learner knowledge and attitudes will be assessed at the conclusion of the course, and retention can be re-assessed at subsequent intervals.

Nutrition Support Elective. The Barnes-Jewish Hospital (BJC) Nutrition Support Service offers 3-week and 6-week electives for medical residents and clinical fellows. This rotation provides direct experience in nutritional assessment and enteral and parenteral feeding of hospitalized patients with diverse illnesses, including anorexia nervosa, wasting diseases, gastrointestinal diseases, and critical illness. At present, this rotation is very popular among medical residents and there is at least one trainee physician on service each month.

Weight Management Elective. The WU Weight Management Program offers 3-week and 6-week electives for medical students, medical residents, and clinical fellows. This elective

provides direct experience in obesity management. Exposure to outpatient treatment, including group behavior modification, dietary counseling, exercise activity, and appropriate medical monitoring, is provided. In addition, those who take the elective can also become involved in the obesity surgery program and learn indications and contraindications for surgery, witness the operative procedure itself, and learn appropriate preoperative screening and postoperative follow-up procedures.

Manual of Nutritional Therapeutics. Dr. Alpers developed the first edition of the *Manual of Nutritional Therapeutics* to create a single source of information that was appropriate for medical students, medical residents, and postdoctoral fellows for these healthcare professionals. The first edition of the book appeared in 1983, published by Little, Brown, and Co. It has been very successful and the fifth edition was published in 2008 by Lippincott, Williams, and Wilkins. Drs. Alpers and Klein agreed to make the sixth edition of the *Manual* a product of the Center for Human Nutrition/NORC and to become part of the outreach program of the NORC in teaching medical professionals about nutrition. NORC faculty will be involved in writing the next edition. It will continue to be part of the Spiral Manual series of Lippincott, Williams, and Wilkins.

7. **NORC Website.** The NORC has a website that is maintained by the Administrative Core. This website provides information for all NORC activities, including resources and services, upcoming nutrition-related lectures and seminars, studies sponsored by the Pilot/Feasibility Program, grant opportunities related to the NORC theme, and announcements of relevant national meetings. A large number of research seminars and clinical lectures are offered daily at WU; some of these programs are directly related to nutrition and the themes of the NORC. The NORC monitors the many lists of Departmental and Divisional lectures and disseminates information on nutrition-related lectures by posting it on the NORC website.

Public Education/Enrichment Program

Dr. Wilkins and the NORC are partnering with the BJC HealthCare System to foster community education in nutrition. The BJC HealthCare System has established a rich network of educational activities in the greater St. Louis metropolitan area. Throughout the past funding period, the NORC community education activities have continued to develop in conjunction with the network of other programs developed by WU and BJC. Dr. Mark Manary (NORC research base investigator) has been personally involved in nutrition education for children in the community and has developed an active community enrichment program to educate and inform the St. Louis community on issues of individual and global nutrition. The individual nutrition component teaches individuals good principles and practice with respect to their personal nutrition, and teaches health educators in the community good nutritional practices as well. Dr. Wilkins provides enrichment for underserved, older adults. This is achieved through the following activities:

1. **Nutrition education in the St. Louis City public school system.** Eighth graders participate in a 2-hour interactive session given by Dr. Manary at their schools, where they learn how to make wise choices with respect to diet and to balance dietary intake with physical activity. This activity currently reaches 500 students annually.

2. **Nutrition education to St. Louis City and County public schools.** In this program, sixth graders attend a daylong seminar at the St. Louis Science Center. Students attend a 20-minute session on human nutrition given by Dr. Manary during the course of this program. This activity reaches 1,500 students annually.
3. **Parish Nurse Education Program.** Parish nurses are nurses working for community organizations (schools, churches, neighborhood centers) as health advocates and educators. There are about 250 parish nurses in St. Louis. They participate in obesity education sessions given by Dr. Manary annually to help them in their work. The Parish Nurse Association also has nutrition month and a series of educational resources that the NORC assists with for this activity.
4. **The Global Nutrition Education Program.** This program teaches grade-school students about childhood malnutrition worldwide, its prevalence, devastating effects, and efforts to alleviate it. This involves a 45-minute talk given to a classroom of primary school students. This program reached 22 schools and about 400 students in the last year.
5. **Nutrition education for underserved, older adults.** Dr. Wilkins provides a lecture on proper nutrition, obesity, and bone health to underserved, older adults living in subsidized, elderly housing in the greater St. Louis area every 2 months.
6. **Community lectures/health fairs.** BJC HealthCare system provides a variety of healthcare lectures and health fairs. Dr. Wilkins and the NORC will continue to work with BJC in providing nutrition education to the community and developing topics for dissemination.

Benefits and Interactions Resulting From the Existence of the NORC

Examples of NORC Stimulating Collaborations Between Basic Science and Clinical Investigation and Bringing New Investigators to the Nutrition Field

The presence of the NORC provides a framework for nutrition-related research at WU, stimulating collaborative research, particularly “bidirectional” clinical and translational research (basic to clinical science and clinical to basic science), providing services and expertise for research in human subjects, providing services that allow assessment of cellular mechanisms in human tissues, and stimulating and facilitating collaborative interactions between the Cores themselves. The NORC also encourages young investigators to pursue research careers in nutrition. It is recognized as an important and respected component of the medical center and has had a significant impact on our research base. The following research accomplishments highlight the importance of the NORC in enhancing collaborative nutrition/obesity research:

1. **Translation of basic to clinical research by an established investigator.** Jeff Gordon, M.D., is an established basic science investigator who has been able to translate his work in animal models to human subjects with support from NORC Core Laboratories and NORC clinical investigators. He has made a series of important discoveries that have linked the character and function of gut flora with bioenergetics and obesity. These findings grew from initial observations generated from work with the Animal Model Research (AMR) Core. Dr. Gordon has shown that alterations in gut microbiota can affect nutrient extraction in mice

leading to obesity and that this obesity phenotype can be transferred to germ-free mice (Turnbaugh PJ et al. 2006, *Nature* 444:1027–31). These observations have been translated to humans in studies that involved the Clinical Science Research (CSR) Core and collaboration with other NORC clinical investigators (Ley RE et al. 2006, *Nature* 444:1022–3). With the assistance of the AMR Core, phenotyping has been performed in mice reconstituted with human gut flora and challenged with high fat diets (Turnbaugh PJ et al. 2009, *Sci Transl Med* 1:6ra14). These findings establish a platform for testing different microbiota on different bioenergetic phenotypes, which could ultimately lead to new a paradigm for the prevention and treatment of obesity. Dr. Gordon is currently collaborating with NORC clinical investigators (Sam Klein and Luigi Fontana) to assess the effect of long-term calorie restriction and the manipulation of dietary protein intake on gut flora in humans.

2. **Translation of basic to clinical research by a young investigator.** Latisha Love-Gregory, Ph.D., is a NORC Pilot and Feasibility Awardee who studies the impact of polymorphisms in the fatty acid translocase CD36 on metabolic function. CD36 is a scavenger receptor membrane protein that facilitates the uptake of fatty acids and oxidized LDL. Polymorphisms in the gene are common in many populations, but especially in African Americans. The CSR Core recruited 300 African American adults for Dr. Love-Gregory's studies. Her work has uncovered associations between common CD36 SNPs and susceptibility to the metabolic syndrome, which provided pilot data for a successful Scientist Development Grant from the American Heart Association. In the next funding cycle she plans to use the CSR Core to obtain muscle and fat tissue to examine genetic effects on tissue specific regulation of CD36.
3. **Translation of basic to clinical research with potential translation to community practice.** Shin Imai, M.D., Ph.D., is a basic science investigator who has translated his work in cells and animal models to humans, and is now collaborating with industry for product development and potential clinical trials to test a new therapy for glucose intolerance and diabetes. This progression would not have been possible without the NORC. Data from a project funded by a NORC Pilot and Feasibility Award (2005–2006) demonstrated that nicotinamide adenine dinucleotide (NAD) biosynthesis mediated by intra- and extracellular nicotinamide phosphoribosyltransferase (iNAMPT and eNAMPT, respectively) plays a critical role in the regulation of glucose-stimulated insulin secretion (GSIS) in pancreatic beta cells (Revollo et al., *Cell Metab* 6:363–375, 2007). With support from the AMR Core and the Adipocyte Biology and the ABMN Core, Dr. Imai also demonstrated that 1) nicotinamide mononucleotide (NMN), a NAMPT reaction product and a novel plasma metabolite, can ameliorate the defects in GSIS observed in NAMPT-heterozygous mice and islets and also can augment GSIS in aged mice (Ramsey et al., *Aging Cell* 7:78–88, 2008), and 2) NMN administration ameliorates metabolic complications in diet- and age-induced diabetic mouse models (unpublished results). These results initiated a new study to explore the importance of NAMPT-mediated systemic NAD biosynthesis and the therapeutic application of NMN in obese humans with help from the NORC CSR Core and collaboration with NORC clinical investigators (Klein, Holloszy). He has found that NAMPT gene expression in tissue biopsy samples before and after weight loss, induced by gastric bypass surgery or diet therapy, is consistent with the data obtained from animal models. Dr. Imai has recently established a collaboration with a Japanese nutraceutical company, Oriental Yeast Co., to explore the efficacy of NMN therapy in the treatment of glucose intolerance and type 2 diabetes. This

company is now producing NMN for experiments in mice, with plans to advance to pilot clinical studies if results are positive.

4. **Applying molecular biology to clinical research.** Elisa Fabbrini, M.D., Ph.D., is a young clinical investigator who has benefitted by collaborations with NORC basic science investigators (Nada Abumrad and Brian Finck) and the NORC Core Laboratories, which has allowed her to bridge her clinical metabolic research studies with molecular analyses of tissue samples that provide the cellular insights into her *in vivo* findings. The NORC has supported her studies in humans by providing CSR Core, ABMN Core, and BMA Core services. Her work has demonstrated that nonalcoholic fatty liver disease is associated with multi-organ insulin resistance and increased VLDL-TG secretion rate (*Gastroenterology* 134:424, 2008, *Gastroenterology* 134:1369, 2008), and that alterations in cellular endoplasmic reticulum stress (*Diabetes* 58:693, 2009) and fatty acid trafficking (differences in organ fatty acid transport [CD36] and lipogenesis [fatty acid synthase]) (*Proc Nat Acad Sci* 106:15430, 2009) are likely involved in the pathogenesis of these metabolic derangements.
5. **Interdisciplinary research.** The NORC Research Cores have facilitated interdisciplinary research by providing a range of expertise and technical services that allow collaboration among investigators from different disciplines to address a research question. A prime example is the large multidisciplinary, collaborative research program under the general direction of Dr. Klein, which is evaluating the pathogenesis and pathophysiology of the metabolic complications of obesity (multi-organ insulin resistance dyslipidemia and nonalcoholic fatty liver disease [NAFLD]) in human subjects. This research group was established during the current NORC funding cycle and has resulted in a series of active research grants, many publications, and several pending grants. Both established (Klein, Abumrad, Finck, Mittendorfer, Patterson, Davila, Peterson, Gropler, Fontana, Colonna) and young (Sullivan, Reeds, Stein, Su, Pepino de Gruev, Fabbrini) NORC investigators are collaborating in this research activity, which has helped generate data for several successful grant applications (NIH R01 DK37948, DK033301, DK078187, WUSM and Pfizer Biomedical Agreement, Longer Life Foundation, and Barnes Jewish Hospital Foundation) that support the research projects. Furthermore, this work helped secure four NIH K-awards for young investigators (Kirk, Stein, Sullivan, Pepino de Gruev) to help them launch their independent careers in nutrition/obesity research. The research conducted in this program bridges clinical and basic science, and illustrates a strong cooperation between the CSR Core, the ABMN Core, and the BMA Core. The CSR Core is used to assist in subject recruitment, body composition analyses (DXA, MRI, and MRS), lifestyle intervention, exercise testing/training, muscle and adipose tissue collection, and substrate kinetic analyses. The ABMN Core and BMA Core assist in cell signaling, biomarker assays, and lipidomics on plasma and tissue (adipose tissue, muscle, and liver) samples. A series of integrated studies was completed that have increased our understanding of the pathogenesis and pathophysiology of NAFLD. In addition, the effect of several treatment strategies (including acute and chronic calorie restriction and exercise) on intra-hepatic triglyceride content and the metabolic abnormalities associated with NAFLD were evaluated.
6. **How the NORC has attracted established investigators from other fields to nutrition/obesity research.** David Piwnicka-Worms, M.D., Ph.D., a molecular imaging

scientist, developed an interest in the effects of nutrition and obesity on various disease states, in part because of interactions through the AMR Core. He has developed a unique bioluminescence imaging method to provide a noninvasive, specific, and highly sensitive optical readout of phagocyte-mediated myeloperoxidase (MPO) activity *in vivo*, which allows *in vivo* imaging of inflammation (Gross S et al. 2009, *Nature Med* 15:455–61). He is using this and related technologies to examine the relationships between nutrition, inflammation, and disease in animal models, and is in the process of developing an R01 application to support these studies. James Cheverud, Ph.D., a geneticist in Anatomy and Neurobiology, has used the services of the AMR Core to direct the primary focus of his research program toward identifying genetic determinants of obesity in mice. By using a quantitative trait locus approach based on crosses involving Large (LG/J) and Small (SM/J) inbred mice strains, his group discovered that an unexpected degree of body composition in mice is determined by effects on imprinting (Cheverud JM et al. 2008, *Proc Natl Acad Sci USA* 105:4253–8). Dr. Cheverud has generated a series of recombinant inbred strains based on the LG/J and SM/J cross and is in the process of fine mapping regions of the mouse genome responsible for obesity in mice as well as sequencing corresponding regions of the human genome from population studies (collaboration with Ingrid Borecki initiated by the CSR Core) to translate his animal model findings to a clinical context. Tamara Hershey, Ph.D., is a neuropsychologist who has been studying the neural basis of cognitive dysfunction in several diseases relevant to dopamine metabolism. By interactions with NORC investigators and the NORC CSR Core she became interested in the relationship between the dopamine reward system and obesity. In fact, this collaboration led to recent new R01 grant funding for Dr. Hershey as PI. The CSR Core is providing support in subject recruitment and weight loss therapy for her study.

- 7. Productive collaboration between NORC Cores.** A collaboration between Clay Semenkovich and John Turk and an extensive collaboration between the AMR Core and BMA Core resulted in the successful characterization of the first physiologically relevant ligand for the nuclear receptor PPAR α in liver (Chakravarthy MV et al. 2009, *Cell* 138:476–88). These investigators found that inactivation of *de novo* lipogenesis carried out by fatty acid synthase in mouse liver resulted in a phenotype that resembled PPAR α deficiency (fatty liver and hypoglycemia) that was rescued by administration of a PPAR α activator. This finding suggested that fatty acid synthase was contributing to the generation of an endogenous ligand for this nuclear receptor. To characterize the ligand, the BMA Core purified a tagged PPAR α molecule from livers of wild-type (WT) mice or mice with a FAS knockout (KO) specific to liver (FASKOL) to test the hypothesis that *de novo* lipid biosynthesis generates an endogenous PPAR α ligand. Using ESI/MS/MS methods of lipid analysis developed in the BMA Core and LipidQA data processing, the BMA Core identified the PPAR α -bound phospholipid species as 1-palmitoyl-2-oleoyl-*sn*-glycerol-3-phosphocholine (16:0/18:1-GPC). Portal vein infusion of 16:0/18:1-GPC in the AMR Core induced PPAR α -dependent gene expression and decreased hepatic steatosis, suggesting that 16:0/18:1-GPC is a physiologically relevant endogenous PPAR α ligand.

Increase in Research Base. The NORC research base has increased from 78 members at the beginning of the current funding cycle in 2006 to 115 members (97 full members and 18 associate members) now in 2010. This increase occurred even though 16 faculty who were full members at the start of the current funding cycle are no longer part of the research base, because

they either left WU for career advancement at other institutions (10 members), retired (1 member), or are no longer conducting nutrition-related research (5 members). The growth in NORC investigators represents adding 54 new faculty during the current funding cycle (2006–2010) because of an increase in funded WU faculty who developed research interests along the themes of the NORC and by the recruitment of new faculty with career interests in nutrition and obesity. This increase in our research base does not represent simply capturing more investigators at WU who were already doing nutrition research. The enhanced profile of nutrition and obesity within the medical center produced by the NORC, the services and intellectual expertise of the Core Laboratories, the Pilot and Feasibility Awards Program, and the influence of NORC leadership throughout the medical center have been important factors in attracting investigators from different disciplines and departments to conduct nutrition/obesity research and helped in the recruitment of new faculty from other institutions. Most of the new NORC members are conducting obesity-related research (prevention & therapy, pathophysiology and complications). Therefore, although the major research themes proposed for the next funding cycle are similar to those we have now, there has been a marked increase in the absolute and relative number of investigators who are focused on some aspect of obesity.

Examples of Collaboration from Biomedical Research Cores

Clinical Science Research (CSR) Core

Examples of facilitation of collaborations:

Collaborations Among WUSM Departments

- Drs. Holloszy and Fontana (Internal Medicine) collaborate with Dr. Racette (Program in Physical Therapy) on studies of energy expenditure with calorie restriction.
- Drs. Villareal, Klein, and Mittendorfer (Internal Medicine) collaborated with Dr. Sinacore (Program in Physical Therapy) on studies evaluating the effects of aging on metabolism.
- Dr. Klein and a large team of investigators from the Department of Internal Medicine (including Drs. Abumrad, Patterson, Reeds, Fabbrini, Mittendorfer, Finck, Su, Stein, Fontana, Davila-Roman, Peterson) collaborate with Drs. Eagon (Surgery) and Colonna (Pathology and Immunology) on studies of the pathophysiology of non-alcoholic fatty liver disease.
- Drs. Cole and Hamvas (Pediatrics) and Dr. Patterson (Internal Medicine) study lung surfactant metabolism in human neonates.
- Drs. Reeds, Mittendorfer, and Yarasheski (Internal Medicine) collaborate with Dr. Cade (Program in Physical Therapy) on studies of the metabolic complications of HIV.
- Dr. Bateman (Neurology) collaborates with Drs. Yarasheski and Patterson (Internal Medicine) on studies of the metabolism of central nervous system proteins.
- Drs. Jungheim and Moley (Obstetrics and Gynecology) collaborate with Dr. Patterson (Internal Medicine) on studies of fatty acid metabolism in polycystic ovarian syndrome and diabetes.

- Dr. Klein (Internal Medicine) collaborates with Drs. Gropler and Peterson (Radiology) on studies to develop and validate PET tracers for the non-invasive kinetics of hepatic glucose and fatty acid metabolism.
- Dr. Pepino de Gruiev (Internal Medicine) is collaborating with Dr. Hershey (Psychiatry) on taste perception and reward mechanisms in obesity.

Collaborations Among Divisions Within the Department of Internal Medicine

- Drs. Reeds, Klein, and Fontana (Division of Nutritional Science); Dr. Yarasheski (Division of Endocrinology and Metabolism); Drs. Mondy, Tebas, and Powderly (Division of Infectious Diseases); and Dr. Dávila-Román (Division of Cardiology) collaborated on studies of the metabolic complications of HIV.
- Drs. Fontana and Klein, (Division of Nutritional Science), Drs. Holloszy and Weiss (Division of Applied Physiology), and Dr. Villareal (Division of Geriatrics) collaborated on studies on the metabolic effects of calorie restriction.
- Drs. Binder and Villareal (Division of Geriatrics) collaborated with Dr. Yarasheski (Division of Endocrinology and Metabolism) and Dr. Sinacore (Division of Applied Physiology) on studies of bone metabolism in aging.
- Drs. Villareal, Mittendorfer, and Klein (Division of Geriatrics); Dr. Holloszy (Division of Applied Physiology); and Dr. Yarasheski (Division of Endocrinology and Metabolism) collaborated on studies of metabolism and aging.
- Drs. Baranski and Turk (Division of Endocrinology and Metabolism and Director of the NORC Biomolecular Analysis Core) collaborate with Dr. Patterson (Division of Nutritional Science) on studies to characterize a model of high-sugar mediated insulin resistance in fruit flies.
- Dr. Cryer (Division of Endocrinology and Metabolism) collaborated with Dr. Patterson (Division of Nutritional Science) on studies of nonsteady-state regulation of glucose homeostasis.
- Dr. Klein and a large team of investigators from the Division of Nutritional Science (including Drs. Abumrad, Patterson, Reeds, Fabbrini, Mittendorfer, Finck, Su, Stein, and Fontana) collaborate with Drs. Davila-Roman, Peterson, and de las Fuentes (Cardiology Division); Dr. Eagon (Surgery); and Dr. Colonna (Pathology and Immunology) on studies of the pathophysiology of non-alcoholic fatty liver disease.
- Drs. Polonsky and Wice (Division of Endocrinology and Metabolism) collaborate with Dr. Patterson (Division of Nutritional Science) on studies of the regulation of insulin production by glucose *in vivo*.

Animal Model Research Core

Several collaborations have been facilitated by the existence of the AMR Core and investigators who otherwise may not have pursued nutrition-related research have been attracted to this work by the existence of the Core.

- Brian Finck is regularly using the Core for biochemical assays. He has developed a collaboration with Richard Gross, another Core user and an expert in mass spectrometry and lipidomics, to study the role of lipin 2. This protein is a phosphohydrolase that is regulated by

diet and may participate in lipid fluxes in several tissues. Brian is also collaborating with Mike Welch, another Core user and an expert in molecular imaging, to study metabolic substrate utilization in the rodent heart.

- John Turk is increasingly drawn to more classic nutrition-related research through his collaboration with Clay Semenkovich. These two have identified an endogenous, physiologically relevant ligand for the nuclear receptor PPAR α , prompting study of whether dietary supplementation with this ligand (a phosphatidylcholine species) can impact health. John is also collaborating with Core user Richard Gross in the study of a unique phospholipase KO mouse with a phenotype that is relevant to nutrition-related diseases.
- Nada Abumrad, an obesity scientist with a long-standing interest in animal physiology and the study of CD36, is an avid Core user and is translating her animal model observations to humans through collaborations with Samuel Klein. These have included a study of the determinants of HDL cholesterol levels in humans as well as a recent high visibility paper linking intrahepatic fat (as opposed to intraabdominal fat) to metabolic sequelae in humans.
- Jeff Milbrandt, just recently named Chair of the Department of Genetics at Washington University, is a Core user with no previous direct interests in nutrition. His work with the genetic modification of orphan nuclear receptors in mice has led to the discovery of models with obesity and diabetes phenotypes that are being characterized through the facilities of the Core. Jeff is in the process of developing relationships with Jean Schaffer and Dan Ory, also Core users, who are establishing a center at Washington University for studying the complications of diabetes.
- Jeff Gordon, who has published a series of high visibility papers with the help of the AMR Core, continues to collaborate with several NORC investigators. He is currently interacting with several groups to optimize the composition of gut flora with the goal of facilitating nutrient processing in order to treat human malnutrition. These microflora strategies are first validated in mice that are characterized by the Core.

Many other collaborations have emerged through interactions prompted by the utilization of Core services. Peter Crawford, M.D., Ph.D., a cardiologist in the process of establishing his research career, has discovered that the gut microbiota can modify myocardial function in mice (Crawford PA et al. 2009, *Proc Natl Acad Sci USA* 106:11276–81). The mechanism appears to involve ketogenesis, and Peter has initiated a collaboration with the Semenkovich laboratory to pursue relevant metabolic pathways in mice. Carlos Bernal-Mizrachi, M.D., a young endocrinologist who recently received R01 support for his work, has established a translational research program dealing with the mechanisms of action of vitamin D in obese and insulin-resistant humans (Oh J et al. 2009, *Circulation* 120:687–98). He is now using the Core to study mechanisms related to the observations he has made in humans. This has allowed him to establish collaborations with Brian Finck and Nada Abumrad (Yue P et al. 2010, *PLoS One* 5:e9906). Ken Polonsky, M.D., who is an established investigator and the Chairman of the Department of Medicine, has used the Core to study the cellular and physiological mechanisms responsible for the metabolic alterations identified in human subjects with genetic variants associated with an increased risk of type 2 diabetes; this has prompted collaborations with Colin Nichols (Villareal DT et al. 2009, *Diabetes* 58:1869–78) and Simon Fisher (Wice BM et al. 2010, *J Biol Chem* in press).

Biomolecular Analysis Research Core

Examples of nutrition- or obesity-related collaborations involving NORC investigators' use of the BMA Core are provided.

- **Free cholesterol-induced macrophage apoptosis involves group VIA PLA₂ (iPLA₂β).** Collaborative studies among NORC investigators J. Turk, C. Semenkovich, and S. Ramanadham (Division of Endocrinology and Metabolism) and Ira Tabas (Medicine Department, Columbia University) implicate iPLA₂β in ER stress-induced apoptosis. With β-cells, ER stress was induced with the SERCA inhibitor thapsigargin (Thaps), which activates iPLA₂β in many cells. Free cholesterol loading (FCL) induces macrophage (Mφ) apoptosis via cholesterol accumulation in ER membranes. This decreases fluidity and inhibits SERCA, which is analogous to Thaps action. Their iPLA₂β^{-/-} mice have altered Mφ properties, and they hypothesized that FCL-induced apoptosis might be impaired in iPLA₂β^{-/-} Mφ. They found that iPLA₂β^{-/-} Mφ are less sensitive to apoptosis induced by FCL or by Thaps and SRA ligand fucoidan, and restoring iPLA₂β expression with adenovirus increases apoptosis. WT and iPLA₂β^{-/-} Mφ incorporate arachidonic acid (AA) into glycerophosphocholine (GPC) lipids equally rapidly and exhibit identical zymosan-induced, cPLA₂α-catalyzed AA release and lysophosphatidylcholine (LPC) accumulation, as demonstrated by ESI/MS/MS in the BMA Core. WT Mφ exhibit robust AA release and LPC accumulation upon FCL, but this is attenuated in iPLA₂β^{-/-} Mφ and increases upon restoring iPLA₂β expression. Thaps + fucoidan induce mitochondrial phospholipid loss, as demonstrated by ESI/MS/MS and cytochrome c release into WT Mφ cytosol, and these events are blunted in iPLA₂β^{-/-} Mφ. Immunoblotting studies indicate that iPLA₂β associates with mitochondria in ER-stressed Mφ. AA incorporation is unimpaired in iPLA₂β-null Mφ, and their complex lipid composition is similar to WT, as demonstrated by ESI/MS/MS. Thus, iPLA₂β participates in ER stress-induced Mφ apoptosis caused by FCL. Cholesterol accumulates in Mφ during atherogenesis, and Mφ apoptosis may precipitate plaque rupture. iPLA₂β might thus represent a target for developing pharmacologic agents to retard atherogenesis and its complications.
- **Regulation of cellular cholesterol homeostasis by 27-hydroxycholesterol.** Studies conducted by NORC investigators D. Ory and J. Schaffer (Department of Internal Medicine, Cardiology Division) in collaboration with F-F. Hsu (BMA Core staff), and C. Semenkovich (Director, NORC AMR Core; Division of Endocrinology and Metabolism) indicate that the endoplasmic reticulum (ER) cholesterol content and HMG-CoA reductase (HMGR) activity respond rapidly to changes in plasma membrane cholesterol. Sterol regulatory element-binding protein (SREBP)-dependent gene expression was found not to be required for responses to changes in ER cholesterol content. Acute proteolytic HMGR inactivation triggered by cholesterol loading does require conversion of cholesterol to 27-hydroxycholesterol (27HC). Sterols were measured by GC-MS in the BMA Core. Exogenous 24,25-dihydrolanosterol induced HMGR inactivation, but depleting endogenous sterols by treatment with the squalene epoxidase inhibitor NB-598 had little effect on ER cholesterol pool or HMGR inactivation. Thus, SREBP-regulated genes are not involved in setting the ER cholesterol pool, but 27HC stimulates rapid HMGR inactivation in response to high cholesterol levels.

Niemann-Pick C1 (NPC1) deficiency leads to defective suppression of SREBP-dependent gene expression and failure to activate liver X receptor (LXR)-mediated pathways, resulting in cellular cholesterol accumulation. Effects of NPC1 deletion in BM-derived cells on atherosclerotic lesion development in *Ldlr*^{-/-} mice was examined. High-fat diet-fed chimeric *Npc1*^{-/-} mice reconstituted with *Ldlr*^{-/-}/*Npc1*^{-/-} macrophages (Mφ) exhibited accelerated atherosclerosis but lower serum cholesterol than mice reconstituted with WT Mφ, despite normal fractional cholesterol absorption (determined after [²H]7-cholesterol administration with BMA Core GC-MS measurements). Mφ from chimeric *Npc1*^{-/-} mice had impaired synthesis of the endogenous LXR ligand 27-hydroxycholesterol (27-HC), reduced LXR-regulated cholesterol transporter expression, and impaired cholesterol efflux. Oxysterol levels were determined by isotope-dilution GC-MS in the BMA Core. Lower 27-HC levels were associated with elevated oxidative stress and cholesterol oxidation products in Mφ and plasma of chimeric *Npc1*^{-/-} mice. NPC1 is thus atheroprotective through regulation of LXR-dependent cholesterol efflux and mitigation of cholesterol-induced oxidative stress in Mφ (49). This collaboration also involved the NORC AMR Core.

- **Structural identification of olfactory signaling molecules.** The olfactory system provides information about nutrient availability and social order cues to mice via pheromones (PM), but little is known about their structure(s). T. Holy (Department of Anatomy & Neurobiology) in collaboration with FF Hsu (BMA Core staff) and J. Turk (BMA Core director) identified a family of these signaling molecules through MS analyses in the BMA Core. Liquid chromatography fractions of urine were screened for ability to cause vomeronasal sensory neuron (VSN) firing using multi-electrode arrays (MEA). Active fractions contained compounds of low molecular weight, moderate hydrophobicity, low volatility, and negative electrical charge. MS and nuclear magnetic resonance (NMR) analyses of active substances revealed a family of sulfated steroids. A battery of 31 synthetic sulfated steroids triggered VSN MEA responses, but individual neurons were sensitive to small structural variations. Mice null for RPC2 sensory channels did not detect the compounds. Structural characterization involved MS analyses by BMA Core staff, including tandem quadrupole, multiple-stage linear ion trap, high-resolution MS, H-D exchange, and LC/ESI/MS/MS (16). NMR analyses provided additional information. Two sulfated steroids were identified: 4-pregnene-11 β,20,21-triol-3-one 21-sulfate (I) and 4-pregnene-11 β,21-diol-3,20-dione 21-sulfate (II), and their concentrations in urine are gender-specific and increase upon stress. Identification of this novel class of mammalian olfactory signaling molecules suggests that steroid hormones assume a new biological role upon conjugation and convey physiological information. This collaboration also involved the NORC AMR Core.
- **D-chiro-inositol and insulin action.** Studies by R. Ostlund and several external collaborators examined the relationship of D-chiro-inositol (DCI) and insulin sensitivity. Some actions of insulin are mediated by inositolphosphoglycans (IPG), and deficiency in a specific DCI-containing IPG may contribute to insulin resistance. This group has shown that women with polycystic ovary syndrome (PCOS) have increased DCI urinary clearance (uCIDCI) that correlates positively with hyperinsulinemia. To determine whether this relationship also exists in men, a recent study examined 11 brothers of women with PCOS and 21 control men. Blood and urine inositol was measured by isotope-dilution negative ion electron capture GC-MS in the BMA Core using [²H₆]racemic chiro-inositol and [²H₆]myo-inositol internal standards. In all men together, low uCIDCI correlated strongly with

hyperinsulinemia, and PCOS brothers were heavier than controls and had more severe glucose intolerance. In PCOS brothers, plasma DCI was threefold higher and the ratio of DCI urinary clearance to that of myo-inositol was threefold lower than controls. Low uCIDCI correlated positively with hyperinsulinemia in all men. Thus, hyperinsulinemia might suppress renal DCI clearance and increase DCI plasma levels and availability to compensate for insulin resistance.

- **Group VIA phospholipase A₂ and insulin secretion.** Studies by John Turk in collaboration with Dr. Louis Philipson (University of Chicago) have established a role for group VIA phospholipase A₂ (iPLA₂β) in insulin secretion that involves phospholipid hydrolysis and accumulation of free arachidonic acid, which amplifies the glucose-induced rise in β-cell cytosolic [Ca²⁺] and insulin secretion. Phospholipids were analyzed by ESI/MS/MS and free arachidonic acid by GC-MS in the BMA Core. INS-1 cells that overexpress iPLA₂β exhibit amplified insulin-secretory responses (18), and male transgenic (TG) mice in which the rat insulin promoter (RIP) drives iPLA₂β overexpression (RIP-iPLA₂β -TG mice) exhibit lower fasting blood glucose and higher plasma insulin levels than WT mice. TG mice also develop lower blood glucose levels in glucose tolerance tests, but WT and TG do not differ in insulin tolerance tests. Islets from male RIP-iPLA₂β -TG mice exhibit greater amplification of glucose-induced insulin secretion by a cAMP-elevating agent than WT. In contrast, male iPLA₂β -KO mice have impaired glucose tolerance and blunted islet insulin secretion. ESI/MS/MS analyses in the BMA Core demonstrated normal arachidonate incorporation into RIP-iPLA₂β -TG islets, and their phospholipid composition is normal. TG islets exhibit reduced Kv2.1 current and prolonged glucose-induced action potentials and cytosolic [Ca²⁺] elevations. This suggests a mechanism for iPLA₂β to amplify insulin secretion that involves inhibition of Kv2.1 current by free arachidonic acid. This collaboration also involved the NORC AMR Core.
- **Dietary phytosterols and cholesterol metabolism.** Studies by R. Ostlund in collaboration with Drs. Michael Lefevre and Marlene Most (Pennington Biomedical Research Center NORC, Baton Rouge) examine cholesterol metabolism and effects of phytosterols on its absorption. Fecal cholesterol excretion and intestinal cholesterol absorption were determined after administering [²H₇]cholesterol and [²H₄]sitostanol by measuring cholesterol and sitostanol by negative ion electron capture GC-MS in the BMA Core. Phytosterol supplementation of 2 g/d has been recommended to reduce LDL cholesterol, and effects of different phytosterol intakes were evaluated recently. A phytosterol-deficient diet was administered to 18 adults along with 0, 400, or 2000 mg phytosterols per day for 4 weeks each. Primary outcomes were fecal cholesterol excretion and intestinal cholesterol absorption measured with stable-isotope tracers and serum LDL. Increasing phytosterol intake was associated with significantly increased total fecal and biliary cholesterol excretion and reduced cholesterol absorption. Serum LDL cholesterol declined significantly with the highest phytosterol dose (2,059 mg/d). Dietary phytosterols thus favorably alter whole-body cholesterol metabolism in a dose-dependent manner.

Adipocyte Biology and Molecular Nutrition Core

Use of the ABMN Core has facilitated several collaborations among WU faculty members:

Collaborations Facilitated by Core Use

- Drs. Ory, Schaffer, and Bickel collaborated to study the role of AGPAT2 in adipocyte differentiation. The Core provided use of the OP9 adipocyte cell line and help with cell differentiation and transfection.
- Drs. Finck and Klein collaborated on fatty acid regulation of lipogenesis and the role of lipin. The ABMN Core provided access to real-time qPCR technology.
- Drs. Klein and Finck collaborated on studying adiponectin expression and plasma concentration in obese subjects before and after gastric bypass surgery. Studies used Core facilities for measuring gene expression and adiponectin levels.
- Drs. Gross and Abumrad collaborated on studying mitochondrial metabolism in mice lacking the phospholipase iPLA2 gamma. Studies used ABMN Core qRT-PCR, help in designing primers and optimizing assays for FA metabolism genes.
- Drs. Finck, Villareal, and Lambert studied expression of several genes involved in muscle protein synthesis and degradation in elderly individuals who underwent weight loss or exercise protocols. Studies used Core qRT-PCR.
- Drs. Finck and Chen examined hepatic fat metabolism in fld dystrophic mice. The studies used ABMN Core cell culture and qRT-PCR facilities.
- Drs. Permutt and Abumrad collaborated on the mechanism by which excess exogenous fatty acids induce lipotoxicity and apoptosis of mouse and human pancreatic islets. Studies used core expertise for isolating RNA, gene expression assays, and qRT-PCR equipment.
- Drs. Su and Abumrad collaborated on examining regulation of the trafficking of the fatty acid translocase CD36. Studies used Core cell culture and imaging.
- Drs. Finck, Wolins, and Gross measured expression of several genes involved in triglyceride metabolism and quantified protein levels using Core equipment. Core personnel assisted with biochemical analysis of fat metabolism.
- Drs. Klein and Finck measured expression of several metabolic and inflammatory genes in obese subjects who underwent acute caloric restriction. Core personnel helped process biopsy samples and measured gene expression.
- Drs. Finck, Gropler, and Welch examined glucose and fatty acid metabolism in Zucker rats treated with insulin-sensitizing drugs. Studies used Core equipment for measuring expression of glucose and FA transport and oxidation genes.
- Drs. Fabbrini, Klein, and Abumrad studied lipid metabolism in obese subjects as a function of intrahepatic triglyceride content. The ABMN Core processed adipose and muscle tissue biopsies, optimized and conducted qRT-PCR and protein assays, and determined membrane localization of the FA translocase CD36.
- Drs. Finck, Brunt, and Wolins examined lipid droplet proteins in fatty liver of mice with hepatic steatosis using Core cell culture, cell imaging, qRT-PCR, and protein quantification equipment. Core personnel provided antibodies and imaging expertise.
- Drs. Su and Abumrad collaborated on the role of the vesicle trafficking regulator ARF6 in adipocyte metabolism. Core personnel trained Dr. Su in lipolysis and FA uptake assays. Core cell culture, qRT-PCR, and Li-Cor equipment were used (Lui et al., 2010).
- Drs. Wolins and Abumrad collaborated on examining the role of diacylglycerol in recruiting lipid droplet proteins to the ER or nascent lipid. Studies used adipocyte cell lines from the Core and Core cell culture and cell imaging equipment.

- Drs. Abumrad, Chen, and Finck studied cholesterol flux by hepatocytes and macrophages from WT and CD36-deficient mice. Studies used cell culture, cell transfection, qRT-PCR, and Li-Cor equipment in the Core.
- Drs. Finck, Hall, Klein, and Brunt studied hepatic expression of the cell death-inducing DFFA-like effector C in obese subjects before and after weight loss. They used the Core qRT-PCR and imaging equipment.
- Drs. Klein, Fabbrini, Mittendorfer, and Finck examined whether treatment of human subjects with TUDCA, a compound that improves ER stress and insulin resistance in mice, enhances the metabolic phenotype and tissue insulin signaling. The ABMN Core processed tissue biopsies and provided qRT-PCR and protein measurements of ER stress and insulin signaling in muscle and adipose tissue (revised manuscript submitted).
- Drs. Gross, Su, and Abumrad examined mechanisms underlying resistance to diet-induced obesity of iPLA2 gamma-deficient mice. Studies by the ABMN Core included measurement of adipocyte number and size on various diets, gene expression in several tissues, adipose tissue culture for lipolysis, fatty acid oxidation and lipogenesis, and evaluation of insulin signaling in adipose and muscle tissues (manuscript in revision).
- Drs. Permutt and Abumrad collaborated on transcriptional mechanisms mediating the synergistic potentiation of islet apoptosis by a combination of fatty acids and glucose. The studies used Core help in metabolic assays and the Core qRT-PCR equipment.

New Collaborations

- Drs. Finck and Davidson examined expression of FA metabolism and ER stress-related genes in TUDCA-treated obese mice using qRT-PCR equipment (manuscript in preparation).
- Drs. Abumrad and Su continued collaboration on the intracellular organelles involved in fatty acid trafficking using Core cell culture, imaging, and facilities for biochemical assays (manuscript in preparation).
- Drs. Finck and Jay continued collaboration on myocardial FA utilization by transgenic mice with heart failure using ABMN Core cell culture, qRT-PCR, and Li-Cor (manuscript in preparation).
- Drs. Reeds and Abumrad are examining the response of FA metabolizing genes to insulin in human adipose tissue from subjects with AIDS. The Core processes adipose tissue biopsy samples using immunochemistry and mRNA analysis techniques.
- Drs. Finck, Hall, and Klein are evaluating the role of lipin in FA metabolism during acute calorie restriction using Core cell culture, qRT-PCR, and cell imaging.
- Drs. Finck and Baranski are evaluating molecular correlates of de novo lipogenesis in Drosophila as a first step in a cross-species study using Core cell culture, qRT-PCR, and Li-Cor.
- Drs. Finck and Hruz are evaluating insulin sensitivity, AMP kinase activity, and energy metabolism in mice with heart PDK4 overexpression using Core cell culture, qRT-PCR, and Li-Cor.
- Drs. Finck and Reeds initiated a collaboration to evaluate adipocyte ER stress in HIV patients treated with protease inhibitors using Core qRT-PCR and Li-Cor.

- Drs. Klein and Abumrad are sizing adipocytes in obese and lean subjects as a function of intrahepatic fat. This collaboration will use Core help in processing biopsies and the Core Beckman Coulter Multisizer 3.
- Drs. Abumrad and Mecham initiated a collaboration studying function and morphology of adipose tissue mice deficient for the extracellular matrix protein MAGP-1 that leads to unexpected adiposity. The collaboration is focused on determining the cause of the adiposity and utilizes Core expertise in adipose tissue metabolism and methodology.
- Drs Abumrad and Brunt are collaborating on studies related to the role of CD36 trafficking in NAFLD and progression to nonalcoholic steatohepatitis.
- Drs. Davidson and Abumrad initiated a new collaboration on the role of fatty acid metabolism in gallstone formation. The *in vitro* part of the work will use cell culture, imaging, and transfection equipment in the Core as well as Core expertise in nutrient flux.
- Drs. Gross, Su, and Abumrad continue to collaborate on studying the mechanisms underlying the bioenergetic dysfunction of the iPLA2gamma KO mice by examining the role of the mTOR pathway in adipose and muscle tissue metabolism. Studies will use Core equipment for gene and protein quantification and Core expertise in metabolic assays.

Leveraging of NORC To Obtain Additional Resources

Institutional Support

Washington University has demonstrated an extraordinary commitment to the NORC by providing research funds for Pilot and Feasibility projects, support for faculty recruitment, additional administrative and research space, and Biomedical Research Core Laboratory equipment.

1. **Pilot and Feasibility Funding.** During the last funding cycle, the School of Medicine provided \$60,000, approved by the Dean, Dr. Larry Shapiro, to support the NORC Pilot and Feasibility Program. Based on the success of this program, Dr. Shapiro has agreed to more than double the commitment to **\$125,000 (\$25,000/yr for 5 yrs)** during the next grant cycle.
2. **Faculty Recruitment and Support.** Dr. Kenneth Polonsky, Chairman of the Department of Medicine, provided Dr. Klein with a total of **\$2.25 million to recruit new faculty** who conduct nutrition and obesity research. These funds were used to recruit: 1) Nada Abumrad, Ph.D., Associate Director of the NORC and director of the NORC ABMN Core Laboratory; 2) Brian Finck, Ph.D., a gifted young investigator who received a NORC Pilot and Feasibility Award and is studying the molecular mechanisms responsible for fatty liver disease, and who has obtained two NIH grants as well as industry and foundation support for his research since his recruitment; and 3) Xiong Su, Ph.D., a young investigator who received a NORC Pilot and Feasibility Award and is an expert in mass spectrometry and lipidomics, and has received an NIH grant plus stimulus supplement and an American Heart Association grant since his recruitment. These investigators and Dr. Klein were the nidus for the recruitment of an additional 15 junior faculty who are NORC investigators and members of the Division

of Geriatrics and Nutritional Science. These recruitments were made possible by approval from Dr. Polonsky; grant funds from Drs. Klein, Abumrad, and Finck; and support from the Division of Geriatrics and Nutritional Science (GNS). Most of these faculty have now obtained independent funding from the NIH, medical societies, and/or foundations. The Department of Medicine contributes **approximately \$400,000/year to the Division of GNS** to provide administrative and other (e.g., computers, phone lines) support for Division faculty who are NORC members and are conducting nutrition- and obesity-related research. Dr. Polonsky has made a **new additional commitment of \$500,000** to recruit a NORC/GNS clinical investigator, which will take place during the next 5 years.

3. **NORC Space and Facilities.** The School of Medicine has been very generous in providing the NORC with space, which is a particularly precious commodity at Washington University. The Department of Internal Medicine provided the NORC and the Division of GNS with **8,500 square feet of newly renovated space** on the second floor of the West building. This space was used to accommodate the recruitment of new nutrition faculty and to support NORC activities, including administrative offices, the CSR Core, the ABMN Core, and a large conference room for NORC meetings, seminars, and lectures. The cost of these renovations (\$750,000) was covered by the Department of Medicine. The Department of Medicine has now agreed to provide an **additional 1,000 square feet of office space that is contiguous with the current main NORC area, which will be renovated at a cost of approximately \$250,000** to house NORC faculty and staff associated with the new CSR Core Lifestyle Intervention component. The Department will also provide **\$500,000 to renovate an additional 1,500 square feet of laboratory space** in the same hallway as the CSR Core and the ABMN Core to support the expansion of the research programs of Drs. Abumrad, Finck, and Su and to help in the recruitment of additional NORC/GNS faculty. These space allocations, now totaling approximately 11,000 square feet, have resulted in a major physical presence of the NORC and the Division of GNS in a central and prominent location within the medical school complex.
4. **Equipment.** During the current funding cycle (2006–2011), the Department of Medicine has helped support the equipment needs of the NORC Research Core Laboratories by providing funds for the following items: 1) total cost of an iDEXA (GE Lunar, Madison, WI), which is used jointly by the CSR Core and the Center for Applied Research Sciences Clinical Research Unit, to assess body composition in subjects who weigh up to 450 lbs (Department: \$112,000); 2) oxygraph-2k high resolution respirometer for the ABMN Core to assess the metabolic state of biopsy samples, cells, or isolated mitochondria (Department: \$13,000, NORC: \$13,000); and 3) Kodak In-Vivo Multispectral Imaging System FX for the AMR Core, which allows whole body detection of biological processes in live animals by using optical bioimaging (Department: \$45,000; Diabetes Research and Training Center: \$40,000; NORC: \$40,000).
5. **NORC Community Outreach Program.** During this funding cycle, the Barnes-Jewish Hospital Foundation, which is the foundation associated with our major teaching hospital, provided \$750,000 (\$250,000 per year for 3 years) for the Community

Outreach Program, which was developed by the NORC and the Division of Geriatrics and Nutritional Science with a gift from the Atkins Foundation Philanthropic Trust. This program provides clinical testing and intervention for underserved older adults in the Greater St. Louis metropolitan area and serves as a vehicle for conducting community-based research. An application to renew support for this program for an additional 3 years will be submitted to the Foundation in October 2010.

Philanthropic Support

Dr. Klein has successfully raised funds from charitable foundations and individuals to support and expand NORC functions.

1. **The Atkins Foundation Philanthropic Trust** provided \$1.5 million to WUSM to establish the Atkins Professor of Medicine and Obesity Research, which is held by Nada Abumrad, Associate Director of the NORC and director of the ABMN Core.
2. **The Charles Kilo Foundation**, which is a local philanthropic organization in St. Louis, gave \$1.5 million to Barnes-Jewish Hospital Foundation to establish the first Charles Kilo Professorship in Nutrition and Diabetes. Dr. Klein and the NORC are using this endowed professorship to recruit a new WU faculty member who would conduct human metabolic research.
3. **The Atkins Foundation Philanthropic Trust** provided a \$5 million endowment to the Barnes-Jewish Hospital Foundation to establish the Atkins Center of Excellence in Obesity Medicine at Washington University, which is directed by Dr. Klein and supports the mission of the NORC. This endowment is used to support: 1) education and training in clinical obesity and obesity research for medical students, medical residents, and postgraduate fellows; 2) the NORC community outreach program; 3) a postdoctoral research fellowship in obesity for an international fellow; and 4) NORC Core Laboratory support. This endowment provides approximately \$200,000 per year, depending on interest rates.
4. **David Yawitz, a local philanthropist**, has committed \$20,000 per year for 2 years to provide 50% salary support for Dr. Gemma Fraterrigo, who is a young Italian physician from the Department of Endocrinology and Metabolism, University Campus Bio-Medico in Rome, Italy. The other half of her salary is being provided by her institution. Dr. Fraterrigo will work full-time at WU with Bettina Mittendorfer and Luigi Fontana in calorie restriction studies for 2 years.