Washington University Clinical Nutrition Research Unit
Start Date: 1999
Status: Ongoing
Source of NIH Support: NIDDK
Website: chn.im.wustl.edu/cnru/cnru.htm

Organization and Goals

Since our CNRU was originally funded in 1999, it has served as a nidus for the growth and development of nutrition research at Washington University. The research infrastructure in nutrition and obesity provided by the CNRU to investigators has created an environment that supports and stimulates cost-effective and high-quality research, collaborations between investigators, community outreach, career development and training, and clinical activities in nutrition and obesity. These efforts have attracted both new and established investigators and clinicians to the field.

The importance of the CNRU and nutrition research has been recognized by the School of Medicine, which has provided significant investments, including: 1) additional space for research, clinical, and educational programs and 2) funds for space renovation, equipment, faculty recruitment, and pilot research projects. In December 2002, the Department of Medicine, in significant measure due to the success of the CNRU, restructured the Division of Geriatrics under the leadership of Samuel Klein, M.D., the appointed Division Chief. Nutrition research is the major focus of the Division, which has been renamed the "Division of Geriatrics and Nutritional Science". This new division provides unique opportunities for the CNRU, because as director of both the CNRU and the Division, Dr. Klein is able to recruit and provide a structural home for nutrition-oriented faculty, which strengthens both programs. Dr. Klein has recruited 9 new faculty members to the division, who are also full or associate members of the CNRU: 1) Nada Abumrad, Ph.D., Professor, 2) Brian Finck, Ph.D., Assistant Professor, 3) Dominic Reeds, M.D., Assistant Professor, 4) Selma Mohammed, M.D., Ph.D., Assistant Professor, 5) Richard Stein, Ph.D., Assistant Professor, 6) Luigi Fontana, M.D., Assistant Professor, 7) Monique Williams, M.D., Instructor, 8) Bernard Miller, M.D., Instructor, and 9) Kyle Moylan, M.D., Instructor. In addition, Dr. Abumrad has recruited Dr. Zaher Nahle, Ph.D. (Assistant Professor) to her research program. Among these 10 faculty, 9 are new investigators, 9 have a primary career interest in nutrition research, and 1 has a primary career interest in clinical geriatrics and nutrition education for medical students.

Most of these new faculty serve important roles within, or ancillary to, the CNRU: Dr. Abumrad is Associate Director of the CNRU; Dr. Reeds is Medical Director of the Barnes-Jewish Hospital Nutrition Support Service; Dr. Mohammed will provide sample acquisition services as part of the Clinical Science Research Core; Dr. Stein is the Behavior Therapy Director of the Weight Management Program and Director of the Family Lifestyle Intervention Program; Dr. Williams is Director of the Community Wellness Program; Dr. Miller is Medical Director of the Weight Management Program; and Dr. Moylan has been charged with improving nutrition education for our medical students and is developing a unique internet-based obesity curriculum for the medical school. These recruitments have substantially increased the critical mass of research-oriented faculty interested in nutrition and underscore the commitment of the Department of Medicine and Washington University School of Medicine to nutrition.

The programmatic themes of the CNRU are: 1) Nutrient Metabolism in Health and Disease, 2) Obesity and its Complications, and 3) Growth, Development, and Aging.

The CNRU is administratively based within the Department of Medicine because Dr. Klein's primary appointment is in Medicine, but it serves the entire School of Medicine. The CNRU 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. A progress report, which will include the findings of the External Advisory Committee, is submitted to the Dean each year in advance of an annual meeting to review CNRU activities, accomplishments, and future goals.

The CNRU interacts with other Centers and Core Laboratories at the School of Medicine to provide the most efficient and comprehensive services to CNRU investigators. The CNRU Biomedical Research Core laboratories were specifically designed 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 CNRU activities are members of the CNRU Executive Committee or the Internal Advisory Committee, which facilitates interactions between these centers, stimulates research collaborations, and avoids duplication of services.

Core Laboratories

Administrative Core: Samuel Klein, M.D., Director; David Alpers, M.D., Associate Director; Susan Sparrow, Administrative Assistant

External Advisory Group Members:

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

Robert Eckel, M.D., Charles A. Boettcher Endowed Chair in Atherosclerosis, Professor of Medicine, and of Physiology and Biophysics, Program Director, General Clinical Research Center, University of Colorado Health Sciences Center

Henry Ginsberg, M.D., Herbert Irving Professor of Medicine, Head, Division of Preventive Medicine and Nutrition, Columbia University College of Physicians and Surgeons

Arnold Strauss, M.D., James C. Overall Professor and Chair, Department of Pediatrics, James C. Overall Professor and Chair, Department of Pediatrics, Vanderbilt University

Timothy Wang, M.D., Dorothy L. and Daniel H. Silberberg Professor of Medicine Chief, Division of Digestive and Liver Diseases, College of Physicians and Surgeons, Columbia University

Biomedical Research Core Laboratories

Three core laboratories are currently supported by the CNRU: 1) Clinical Science Research Core, 2) Animal Model Research Core, and 3) Biomolecular Analysis Core. The purpose of the Cores is to provide research support and training to established investigators who are members of the CNRU, to trainee members of the CNRU, and to Pilot and Feasibility Award recipients. It is expected that contact with experienced investigators who will serve as core directors and have 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: Bruce Patterson, Ph.D., Research Associate Professor of Medicine

The purpose of the Clinical Science Research Core is to: enhance and facilitate nutrition-related clinical research; provide training for young investigators in isotope tracer methodology; and facilitate access to clinical research technology and research subjects so that hypotheses based on non-human models can be tested in humans

The services that the Clinical Science Research Core offer include: performing body composition analyses: a) dual energy x-ray absorptiometry to determine fat mass, fat-free mass, and bone mineral content, b) magnetic resonance imaging to determine intrabdominal fat mass and subcutaneous abdominal fat mass, and c) magnetic resonance spectroscopy to determine intrahepatic and intramyocellular fat content; determining energy expenditure and maximum oxygen consumption by using indirect calorimetry; and providing mass spectrometry and mathematical modeling services to determine substrate metabolism in human subjects.

Animal Model Research Core: Clay Semenkovich, M.D., Professor of Medicine and of Cell Biology and Physiology

The purpose of the Animal Model Research Core is to: promote interactions, stimulate collaborative research, and provide education and database services related to nutrition research in animal models.

The services that the Animal Model Research Core offer include: maintaining breeding colonies of genetically modified mice relevant to nutrition research; providing genotyping of mouse lines; assisting and providing training in mouse breeding and general animal husbandry; providing nutrition-related biochemical analyses of mice, including measurement of serum lipids, lipoproteins, non-esterified fatty acids, glucose, insulin, and glucose tolerance testing; providing body composition analyses; and providing quantification of atherosclerosis and histologic analyses of atherosclerotic lesions.

Biomolecular Analysis Core: John Turk, M.D., Ph.D., Professor of Medicine

The purpose of the Biomolecular Analysis Core is to: provide the powerful tools of modern mass spectrometry to CNRU investigators; provide a common set of analytical tools for a unified understanding of molecular mechanisms involved in nutrient-related physiologic and pathophysiologic processes; stimulate collaborative nutrition research involving the analytical capabilities of the core; and disseminate new mass spectrometric approaches to nutrition research developed in the core.

The services that the Biomolecular Analysis Core offer include: providing consultation and collaboration on application of mass spectrometry to nutrition research, including development of new analytical methods targeted at achieving research objectives of CNRU investigators; performing mass spectrometric analyses for structural identification and quantification of target molecules; and providing training to students and postdoctoral fellows from laboratories of

CNRU investigators in the principles of mass spectrometry and its application to nutrition research.

Pilot and Feasibility Studies

Projects Funded 2004 – 2005

Dietary Prevention of Colon Cancer. Shrikant Anant, Ph.D., Assistant Professor of Medicine, Gastroenterology. The overall objective of this proposal is to elucidate the mechanism by which curcumin, a naturally occurring agent in the Indian spice turmeric, enhances radiation-mediated apoptosis in colon cancers. Cancer is the second most common malignancy in the western world. Recent studies have demonstrated that use of non-steroidal anti-inflammatory drugs (NSAIDs) in colon cancer treatment is limited because of renal and gastrointestinal toxicity. Curcumin possesses anti-cancer properties and is not toxic at relatively high concentrations. Curcumin also does not have the side effects that are associated with NSAIDs. Thus it is important to determine the mechanisms that are regulated by curcumin. Our preliminary studies have demonstrated that curcumin enhances the radiation effects on intestinal adenomas in $APC^{min/+}$ mice, a mouse model for human colon cancers. Furthermore, the effect on normal uninvolved mucosa is relatively less compared to the adenoma. Dr. Anant has also determined that APCmin mice that were fed curcumin and given a single low-dose radiation survived twice as much as mice that were given radiation alone. In addition, in cell culture studies he has determined that curcumin inhibits AKT phosphorylation, while inducing expression of tumor suppressor proteins p53 and its downstream target gene PTEN. Since PTEN inhibits AKT activation, these data suggest that curcumin mediated apoptosis of colon cancer cells is due to the p53-mediated activation of PTEN, resulting in inactivation of AKT pathway. To confirm these results, an additional set of experiments will be performed using silencer RNAs to specifically inhibit PTEN and p53 expression. Following inhibition of PTEN and/or p53 gene expression, the effect of curcumin and irradiation on AKT phosphorylation and on cell viability will be determined. In addition the effect of curcumin on these pathways will be determined in a colon cancer cell line with a knockout of p53 gene. Finally, we propose to confirm these *in vitro* findings in an *in vivo* setting in $APC^{min/+}$ mice that have been fed curcumin in the diet and also given a single low dose irradiation. Adenomas and its surrounding uninvolved tissue will be microdissected from the mice following the treatment. RNA and proteins will be subjected to Real Time PCR and western blot analyses for p53 and PTEN.

GLUT8, Blastocyst Nutrition and Hyperinsulinemia. Mary Carayannopoulos, Ph.D., Research Instructor, Pediatrics – Laboratory Medicine. Glucose transport and metabolism are critical for mammalian blastocyst formation and further development. At this stage of development, the switch occurs from oxidative phosphorylation and the metabolism of pyruvate and lactate to glycolysis and the use of glucose as the main substrate. We have recently cloned a novel insulin regulated glucose transporter, GLUT8, which is highly expressed at this stage of development. We have shown that this transporter is localized intracellularly under basal conditions. However, upon insulin stimulation, GLUT8 redistributes within the cell with significant staining of what appears to be the plasma membrane. In addition, blocking expression of GLUT8 using anti-sense oligonucleotide probes leads to an inhibition of insulin stimulated glucose uptake into the blastocyst. The objective of this proposal is to determine the physiologic role of GLUT 8 in the developing blastocyst and determine if regulating the translocation of this transporter can rescue the blastocyst from the deleterious effects of hyperglycemia and hyperinsulinemia.

Fatty-acid Induced Central Insulin and Leptin Resistance. Simon Fisher, M.D., Ph.D., Assistant Professor, Endocrinology and Metabolism. Excess nutrition leading to an epidemic of overeating and obesity is causing major adverse consequences for human health. The homeostatic control of energy balance is regulated in the central nervous system and is responsive to signals from the periphery. The two best studied hormonal feedback systems involved in weight control are leptin and insulin. These two hormones increase when body fat stores rise and act on brain neural circuits to both suppress eating and increase the body's metabolic rate (energy expenditure). Most patients suffering with obesity have high levels of leptin and insulin but their brain neural circuits fail to respond appropriately to the anti-obesity effects of these hormones. The mechanism by which the brain gradually develops resistance to the anti-obesity actions of leptin and insulin is unknown but is thought to be due to an attenuation of the leptin- or insulin-signaling pathways in the brain. Excess consumption of lipids (e.g., fatty acids), also termed a "Western diet", has long been known to cause weight gain and obesity, but the exact mechanism is not completely understood. Nutritional studies in humans and animals have demonstrated that excess fatty acids associated with a high fat diet can cause resistance to the actions of leptin and insulin in some tissues (e.g., muscle, fat, and liver) but it is unknown whether fatty acids can impair the actions of leptin or insulin in the brain. The specific aim of this proposal is to test the hypothesis that nutrients (e.g., fatty acids) act in the brain to cause resistance to the anti-obesity hormones, leptin and insulin, leading to an energy balance favoring weight gain. By impairing the ability of insulin or leptin to exert anti-obesity effects, chronic lipid excess in the brain may set up a vicious cycle of increased food intake causing, and perpetuating, obesity.

Calorie Restriction and Markers of Aging in Humans. Luigi Fontana, M.D., Ph.D., Research Instructor, Geriatrics and Nutritional Science. Studies in a wide range of nonprimate animal species have demonstrated repeatedly that caloric restriction (CR) significantly reduces the rate of aging and increases life span. However, it is not known whether these findings are applicable to humans. Recently, Dr. Fontana and colleagues have identified a cohort of individuals committed to a voluntary CR diet in anticipation of health benefits and increased lifespan. Data obtained in these subjects have shown that long-term CR results in profound and sustained beneficial effects on the major atherosclerosis risk factors in humans and that CR provides a powerful protective effect against obesity, insulin resistance, and inflammation. These findings are consistent with data from long-term CR studies conducted in monkeys and rodents. The goals of the proposed study are to obtain information on a range of physiological and metabolic variables that deteriorate with advancing age in order to determine whether the CR subjects are aging at a slower than normal rate. The data obtained initially will be used both for a cross-sectional evaluation and to serve as baseline values for later follow-up measurements to obtain longitudinal data on the rate of aging. The initial measurements will also make it possible to measure the rate of deterioration of a range of physiological functions that usually change linearly with advancing age. A second goal is to determine whether or not CR results in some of the same changes in gene expression profiling through genechip microarrays (which measures changes in gene expression) and proteomics (which measures changes in protein abundance and state), and in the levels of various growth factors, pro-inflammatory cytokines, and hormones that have been shown to be affected by CR in lower organisms and that have been demonstrated, or hypothesized, to slow aging.

Dissecting Pathways Involved in Sensing Nutrient Status and Regulating Growth Using. Matthew Goldsmith, M.D., Instructor in Pediatrics, Pediatrics, Critical Care. Caudal fin growth

(addition of new segments of bone to nascent fin rays) in the adult zebrafish is both episodic (alternating intervals of growth and rest) and synchronous (all rays within an individual fin grow together); moreover the relationship between fin growth and body growth is isometric. Not surprisingly, fin growth terminates rapidly when adult zebrafish are fasted. Preliminary data suggest that, in part, the mechanisms regulating growth in juvenile and adult zebrafish caudal fins are distinct. In juvenile caudal fins, growth is both continuous and asynchronous. Furthermore, the relationship between fin growth and body growth in juvenile zebrafish is allometric, not isometric. Finally, growth of the juvenile caudal fin persists during unfavorable nutritional conditions (fasting). The overall goal in this proposal is to exploit the differences between juvenile and adult caudal fin growth in order to begin dissecting the mechanisms wherein the nutritional status of an organism is integrated into an overall hierarchy of growth regulatory processes.

The Dynamic Mechanism of RXR Activation. Jianyun Lu, Ph.D., Research Instructor, Gastroenterology. Retinoid X receptors (RXRs) belong to the nuclear receptor (NR) superfamily of ligand-dependent transcription factors. They bind to DNA response elements as homodimers, tetramers, or obligate heterodimer partners with a large number of other nuclear receptors. Thus RXRs serve as master regulators of multiple signaling pathways. Endogenous ligands include the vitamin A metabolite 9-*cis* retinoic acid (9cRA) and polysaturated long-chain fatty acids. The synthetic RXR agonist bexarotene is clinically used for the treatment of cancer. There is evidence that synthetic RXR antagonists may be useful in the treatment of obesity and related diseases, such as type 2 diabetes. X-ray crystallographic studies of the RXR ligand binding domain (LBD) with and without agonists have identified the C-terminal AF-2 helix as a key structural determinant for RXR oligomerization and activation. These structures, however, represent isolated "snapshots" of a dynamic process. Dr. Lu hypothesizes that (1) the RXR LBD in solution exists in an equilibrium between multiple conformations affecting discrete regions of conformational order-disorder and (2) this equilibrium is differentially shifted by RXR agonists, antagonists, and coregulatory proteins. These hypotheses will be tested by using high resolution multidimensional NMR techniques, because NMR has the unique capacity to probe dynamic processes over a range of time scales (ps to sec) at atomic resolution.

Role of Caveolin in Triacylglycerol Storage in Lipid Droplets. Douglas Lublin, M.D., Ph.D., Associate Professor, Pathology and Immunology. Fatty acids provide a key energy source, and efficient energy homeostasis is maintained by storage of the fatty acids as triacylglycerols in lipid droplets and their subsequent release by hydrolysis. These lipid droplets range from small cytoplasmic droplets to the huge lipid droplet of an adipocyte. Caveolin-1 is a 22 kDa membrane protein that forms the coat of caveolae, flask-shaped invaginations in the plasma membrane that are implicated in signal transduction, endocytosis, and cholesterol trafficking. Unexpectedly, the caveolin-1 knockout mouse had a lean body phenotype with reduced adipose mass, is resistant to diet-induced obesity, and had increased plasma triacylglycerols. Furthermore, our laboratory found that caveolin-1, normally present on the plasma membrane, can be targeted to the surface of lipid droplets under certain conditions, including supplementation of cells with fatty acids. Under this CNRU Pilot and Feasibility Grant, we have established a cell culture model using the Fischer rat thyroid (FRT) cell line, which does not express caveolin-1, and a stable FRT/caveolin-1 transfectant. FRT/caveolin-1 cells form very large lipid droplets following supplementation with oleic acid for 48 hours, whereas the FRT cells form only minute lipid droplets. Analysis of the lipids extracted from these cells using electrospray ionization/mass spectrometry demonstrated a more than four-fold increase in triacylglycerol in the caveolin-1-expressing cells. Preliminary studies using [^{3}H]oleic acid

uptake demonstrated an increased rate of uptake over the first 5 minutes in the FRT/caveolin-1 cells compared to the FRT control. This suggests that caveolin-1 expression in the cells leads to a greater rate of uptake of fatty acids, but further studies must address whether there are also changes in the rate of lipolysis. Overall, these studies support a previously unrecognized role for caveolin-1 in storage of triacylglycerols in lipid droplets.

Identifying Diet-regulated Genes That Control Obesity. Burton Wice, Ph.D., Research Assistant Professor, Endocrinology and Metabolism. Obesity is associated with many devastating diseases and has become a major health problem in the industrialized world. Thus, it is important to understand the molecular mechanisms that regulate adiposity. Enteroendocrine (EE) cells are a rare and complex sub-population of intestinal epithelial cells scattered throughout the gut. There are at least 16 different sub-types of EE cells. Glucose-dependent insulinotropic polypeptide (GIP) is produced by EE cells called K cells. Wild type, but not GIP Receptor knock out ($GIPR^{-/}$), mice develop obesity when placed on a high fat diet. Thus, a) GIP plays a critical and non-redundant role in promoting obesity and b) agents that inhibit GIP production and/or release may represent novel anti-obesity drugs. Elucidating the molecular mechanisms that regulate GIP production and secretion could therefore provide novel insights for the development of new anti-obesity drugs. Unlike hormone release from EE cells that produce chromogranin A (CGA), GIP secretion appears to be independent of K_{ATP} channels and inositol 1,4,5-trisphosphate Receptors (IP3Rs). Unfortunately, these "negative" results do not identify the molecules that do regulate GIP release *in vivo*. This project will directly test the hypothesis that DNA microarray analyses of native EE cells can identify differentially compressed genes that may regulate GIP production and secretion. Using a novel strategy to purify EE cells, two specific aims will be pursued: 1) Identify genes which are differentially expressed in K cells from lean versus obese mice. GIP production and secretion are stimulated by fat and are greatly increased in obese individuals and animals. Thus, genes that control GIP production and release should be differentially expressed in K cells from lean versus obese mice. Transgenic mice were generated that express Red Fluorescent Protein (RFP) specifically in K cells. RFP-tagged K cells from lean versus obese mice will be purified by fluorescence activated cell sorting (FACS) and subjected to DNA microarray analysis. 2) Identify genes which are differentially expressed in EE cells that produce GIP versus CGA. EE cells that produce GIP versus CGA are remarkably distinct. Thus, transgenic mice that express RFP specifically in EE cells that produce CGA will be generated. RFP-tagged EE cells that produce CGA versus GIP will be purified by FACS and compared using DNA microarray analysis. These studies will bring to the lab the abilities to: purify *native* EE cells; perform DNA microarray analysis; and utilize mouse models of obesity.

Projects Funded 2005

***In Vivo* Quantitation of Intramyocellular Lipid Content in Human Calf Muscles Using Chemical Shift Imaging.** Adil Bashir, Ph.D., Research Associate, Department of Radiology. The metabolism and composition of skeletal muscle tissue is of special interest because it is a primary site of insulin action and plays a key role in the pathogenesis of insulin resistance. A strong association has been found between levels of intramyocellular (IMCL) triglyceride accumulation in muscles and insulin resistance in obese, type 2 diabetics, and healthy subjects. Because of invasive nature of muscle biopsies only limited information about muscle IMCL lipid stores and its metabolism is currently available. Single voxel MR spectroscopy has been shown to non-invasively determine IMCL lipid concentration in calf muscles, but its applications are limited because it does not provide distribution of IMCL lipids in muscles. An alternative

method, MR spectroscopic imaging, can provide IMCL lipid distribution in muscles, but it is based on Fourier reconstruction and leads to contamination of IMCL lipid quantification in calf muscles by extramyocellular (EMCL) lipids. The goal of this project is to develop a clinically useful fast magnetic resonance spectroscopic imaging technique to quantify the distribution of IMCL lipids in human calf muscles *in vivo*, without contamination from the adipose EMCL lipid signal, and to employ this technique to quantify the IMCL fat distribution in healthy and obese people and determine its correlation with body fat content and insulin sensitivity. This technique will allow measurement of the spatial distribution of IMCL lipids and will serve as a valuable tool for longitudinal studies of changes in IMCL that occur with changes in composition or concentration of lipid pools under physiological perturbations (e.g., nutrition, disease, exercise, aging, etc.). Given the prevalence of obesity and diabetes this will provide a non-invasive tool for assessing the effect of therapeutic interventions and for guiding therapy that may be applied to population-based practice.

Fatty-acid Induced Central Insulin and Leptin Resistance (second year of funding)**.** Simon Fisher, M.D., Ph.D., Assistant Professor, Endocrinology and Metabolism. Excess nutrition, leading to an epidemic of overeating and obesity, is causing major adverse consequences for human health. The homeostatic control of energy balance is regulated in the central nervous system and is responsive to signals from the periphery. The two best studied hormonal feedback systems involved in weight control are leptin and insulin. These two hormones increase when body fat stores rise and act on brain neural circuits to both suppress eating and increase the body's metabolic rate (energy expenditure). Most patients suffering with obesity have high levels of leptin and insulin but their brain neural circuits fail to respond appropriately to the anti-obesity effects of these hormones. The mechanism by which the brain gradually develops resistance to the anti-obesity actions of leptin and insulin is unknown but is thought to be due to an attenuation of the leptin- or insulin-signaling pathways in the brain. Excess consumption of lipids (e.g., fatty acids), also termed a "Western diet", has long been known to cause weight gain and obesity, but the exact mechanism is not completely understood. Nutritional studies in humans and animals have demonstrated that excess fatty acids associated with a high fat diet can cause resistance to the actions of leptin and insulin in some tissues (e.g., muscle, fat, and liver), but it is unknown whether fatty acids can impair the actions of leptin or insulin in the brain. The specific aim of this current grant proposal is to test the hypothesis that nutrients (e.g., fatty acids) act in the brain to cause resistance to the anti-obesity hormones, leptin and insulin, leading to an energy balance favoring weight gain. By impairing the ability of insulin or leptin to exert anti-obesity effects, chronic lipid excess in the brain may set up a vicious cycle of increased food intake causing, and perpetuating, obesity.

Vitamin K, VKORC1 Polymorphisms, and Osteoporotic Fractures. Brian Gage, M.D., Ms.C., General Medical Sciences. Our long-term goal is to determine the role of vitamin K in post-menopausal women at high risk of osteoporotic fractures. Because vitamin K catalyzes the Glu to Gla conversion of protein residues in osteocalcin and other bone proteins, it is essential for optimal bone formation in humans and laboratory animals. Vitamin K epoxide-reductase (VKOR) is the key enzyme in the γ-carboxylation system and is encoded by the *VKORC1* gene. Recently, we found two common SNPs in *VKORC1* that correlate with the therapeutic dose of the vitamin K antagonist warfarin and with mRNA expression of VKOR. We hypothesize that genetic polymorphisms in *VKORC1* will correlate with bone mineral density, risk of osteoporotic fracture, and response to supplemental vitamin K_2 in post-menopausal women. We have 3 specific aims:

1. In a cross-sectional study, to quantify the association between polymorphisms in *VKORC1* and bone mineral density in 200 postmenopausal women.
2. In a case–control study, to quantify the association between polymorphisms in *VKORC1* and osteoporotic fractures.
3. In a pilot, randomized control trial, to quantify the biochemical response to oral vitamin K supplementation, stratified by *VKORC1* haplotype.

The study brings together a new multi-disciplinary team with a high probability of success. Dr. Gage is an expert on the pharmacogenetics of warfarin therapy, a vitamin K antagonist. Dr. Binder is a geriatrician and expert on hip fractures. Drs. Gage and Binder have recently collaborated on an observational study that found a 25 percent increase in the risk of osteoporotic fractures in patients prescribed warfarin. Dr. Armamento-Villareal is an endocrinologist and expert on bone metabolism. She and Dr. Napoli, a geriatrician who has great expertise in genotyping, have recently collaborated on their study that found a strong association between *CYP1A1* gene polymorphisms and estrogen metabolism and bone density. Funding of the study will bring together this promising team and allow them to begin to elucidate a new area of investigation—the role of vitamin K in bone health and osteoporotic fractures. Within 1 to 2 years of beginning the proposed pilot studies, they anticipate submitting an R01 grant to continue this promising area of investigation.

Facilitative Glucose Transport and Myocardial Function. Paul Hruz, M.D., Ph.D., Instructor, Pediatrics. The long-term objective of this project is to understand the role of facilitative glucose transport in the normal and diabetic heart. While the heart primarily relies upon fatty acid catabolism for basal energy needs, glucose provides a significant source of fuel during periods of acute stress. The relative role of GLUT4 mediated facilitative glucose transport in regulating glucose uptake remains poorly understood. Compensatory changes in glucose transporter expression in response to the chronic induction of insulin resistance have limited the ability to draw definitive conclusions from genetic and environmental models of diabetes. The studies in this proposal are intended to establish the functional role of GLUT4 mediated glucose transport in normal and diseased rodent myocardium. We hypothesize that acute and selective pharmacologic inhibition of GLUT4 intrinsic activity will lead to significant changes in cardiac contractility and susceptibility to ischemic injury. The primary goal of this pilot proposal is to establish the feasibility of using HIV protease inhibitors and related hydrophobic peptides to acutely inhibit GLUT4 activity in the heart and produce observable changes in myocardial function. First, changes in the insulin signaling pathway and glucose transporter expression will be determined following acute administration of the HIV protease inhibitor ritonavir and the GLUT4 inhibitory peptide zHFFe to healthy lean rodents under basal and hyperinsulinemic euglycemic clamp conditions. Next, the ability of these drugs to influence survival in a mouse model of dilated cardiomyopathy will be assessed. Finally, the acute effects of ritonavir on susceptibility of left ventricular myocardium to ischemic injury will be studied in healthy lean rats subjected to ligation of the left coronary artery for 30 minutes followed by reperfusion. Effects on contractilily and infarct size will be determined by echocardiography and histologic analysis respectively. Taken together, these studies will provide significant insights into the functional role of GLUT4 in meeting the energy demands of the heart. This will provide a strong rationale for pursuing means to augment glucose transport during periods of acute ischemia. This work will also provide evidence for direct effects of HIV protease inhbitors on cardiovascular morbidity in patients receiving these drugs.

The Systemic Regulation of NAD Biosynthesis and its Effect on Glucose Metabolism in Mammals. Shin-ichiro Imai, M.D., Ph.D., Assistant Professor, Molecular Biology and

Pharmacology. The Biology of NAD metabolism has recently received attention in the fields of aging and metabolism research. This is mainly because the regulation of Sir2 NAD-dependent deacetylases by NAD biosynthesis have been demonstrated to be important to connect transcription, metabolism, and aging in a variety of organisms. We previously characterized the biochemical nature of two critical enzymes, nicotinamide phosphoribosyltransferase (Nampt) and nicotinamide mononucleotide adenylyltransferase (Nmnat), in the mammalian NAD biosynthesis pathway starting from nicotinamide. We demonstrated that Nampt is the rate-limiting component in this pathway and regulates the function of the mammalian Sirt1 NAD-dependent deacetylase in mammalian cells. More recently, a Japanese group has re-identified Nampt as a "new visceral fat-derived hormone" named visfatin. Surprisingly, the authors also reported that visfatin mimics the function of insulin by binding to and activating the insulin receptor. However, the physiological relevance of visfatin is controversial because its plasma concentration is 40 to 100-fold lower than insulin. Therefore, it is possible that the major physiological function of this protein in blood is to promote NAD biosynthesis rather than binding to the insulin receptor. To begin to elucidate the function of Nampt, we made several key observations presented in this proposal. These new findings led us to a novel hypothesis that the circulating version of Nampt plays an important role in the systemic regulation of NAD biosynthesis in mammals. To address this hypothesis, we will biochemically characterize Nampt purified from mouse plasma; examine the effects of nicotinamide, nicotinamide mononucleotide, and NAD on blood glucose levels; and generate fat-specific, inducible Nampt-overexpressing transgenic mice. The study will provide a novel framework for the systemic regulation of NAD biosynthesis by adipose tissue and its physiological relevance for glucose metabolism regulation.

The Dynamic Mechanism of RXR Activation (second year of funding)**.** Jianyun Lu, Ph.D., Research Instructor, Gastroenterology. Retinoid X receptors (RXRs) belong to the nuclear receptor (NR) superfamily of ligand-dependent transcription factors. They function as either a homodimer or obligate heterodimeric partners for a large number of NRs. Endogenous ligands include the vitamin A metabolite 9-cis-retinoic acid (9cRA) and unsaturated fatty acids. Thus, RXRs serve as master regulators of multiple signaling pathways. Both RXR-specific agonists and antagonists have been synthesized. 9cRA and bexarotene, a synthetic RXR agonist, are used clinically in the treatment of cancer. RXR antagonists have been proposed as therapeutic agents for the treatment of obesity and related diseases such as type 2 diabetes. X-ray crystallographic studies of the RXR ligand-binding domain (LBD) with and without agonists have identified the C-terminal AF-2/H12 helix as a key structural determinant for RXR activation. These structures, however, represent isolated "snapshots" of RXR LBD conformations that occur in solution. The interaction of the RXR LBD with antagonists has been modeled with docking experiments based on RXR LBD-agonist crystal strucuture, but there remain little direct structural information on the RXR LBD-antagonist complex. We hypothesize that the RXR LBD in solution is in a dynamic equilibrium between multiple conformations as reflected by discrete regions of conformational order-disorder and this equilibrium is differntially shifted by RXR agonists, antagonists, and coregulatory proteins. We propose to test these hypotheses by studying the interactions of isotopically labeled recombinant RXR alpha LBD, ligands (agonists and antagonists), and coactivator proteins using high resolution multidimensional nuclear magnetic resonance (NMR) techniques. This is because NMR has the unique capacity to investigate dynamic properties of biomolecules over a range of time scales (from picoseconds to seconds) at atomic resolution. We anticipate that the results of these studies will provide further insights on the role of RXR in mediating lipid nutrient signaling and on the rational design of new RXR ligands for the treatment of cancer, diabetes, and obesity.

Brain Mapping of Hunger and Satiety in Obese Persons. Consuelo Wilkins, M.D., Assistant Professor of Medicine, Geriatrics and Nutritional Science. The purpose of the pilot proposal is to determine the feasibility of mapping the brain's response to hunger, satiety, and satiation in obese persons. Because brain activity has been associated with food stimuli and brain activity differs in the fasted and fed states, additional studies are needed to understand differences in brain activity in obese and lean persons. The neural response may provide important data to mechanisms involved in overeating and obesity. This study will use ^{15}O labeled water and Positron Emission Tomography (PET) to measure regional cerebral blood flow in persons in the fasted, fed, and fully satiated states. To our knowledge, this study has not been previously reported, so this will serve as feasibility data for designing future studies. The study will also allow for correlations between biochemical measures (including insulin and free fatty acids) and brain activity. The study will also include a comparison group of normal weight persons. The study will utilize the Clinical Nutrition Research Unit's Biomolecular Analysis Core for determinations of free fatty acids, lipids, and insulin and the Clinical Research Services Core for biostatistical analysis.

Funding Derived from Previous Pilot and Feasibility Studies

***In Vivo* Analysis of the Adipocyte S3-12 Protein.** Bickel, PE, M.D. Funding: R01 DK59577, "Flotillins & insulin-stimulated glucose transport," 2/02 – 1/07.

Nutrient Regulation of Islet Beta Cell Survival. Srinivasan, S, M.D. Funding: K08 DK067045, "Enteric neuronal survival in diabetes," 12/04 – 11/09.

Omega-3 Fatty Acids and Intestinal Stem Cell Survival Following Injury. Houchen, C, M.D. Funding: R03 for minority investigator DK065887, "Prostaglandins and gastrointestinal DNA damage," 2/04 – 1/06.

Polycystic Ovary Syndrome, Hyperinsulinemia and Embryotoxicity. Moley, KH, M.D. Funding: R01 HD40390, "GLUT8 and glucose transport in the mouse blastocyst," 2/02 – 1/07.

Myocardial Metabolism in Obesity. Peterson LR, M.D. Funding: K12 HD01459 BIRCWH: Building Interdisciplinary Research Careers In Women's Health, "Obesity's effect on Myocardial metabolism and its regulation," 1/01 – 12/06.

Bone Demineralization in HIV/AIDS Patients Treated with HAART. Tebas, P, M.D. Funding: R21 AI063995, Mitochondria and metabolic complications of HIV, 9/04 – 7/09.

Control of Intestinal Function by a Conserved Family of KCNQ-like Potassium Channels in C. Elegans. Wei, A, Ph.D. Funding: NSF grant "Control of cellular excitability by KCNQ-like potassium channels in C. elegans, 9/01 – 8/04; AHA Heartland Affiliate, 7/02 – 6/04.

Proteoglycan Binding and Atherogenicity of ApoB-48. Chen, Z, Ph.D. Funding: R01* HL73939 "ApoB-proteoglycan interaction and atherosclerosis," 7/03 – 6/07.

Retinoids in Enteric Nervous System. Heukeroth, R, M.D., Ph.D. Funding: March of Dimes 1FY0 system development "Vitamin A and enteric nervous system development," 6/02 – 5/05.

Effect of Liposuction on Insulin Sensitivity in Obese Women. Mittendorfer, B, Ph.D. Funding: R01 AR49869, "Effects of sex and adiposity on muscle protein synthesis", 2/04 – 2/09.

Regulation of Fatty Acid Utilization. Schaffer, J., M.D. Funding: R01 DK54268, "Molecular basis of long chain fatty acid transport," 7/02 – 6/07.

Metabolic Effects of Valproate and Antipsychotic Therapy. Haupt, DW, M.D. Funding: K23 MH067795, "Metabolic effects of valproate and antipsychotic therapy," 4/04 – 3/09.

Identification of Genes That Regulate Zinc Metabolism and Ras-mediating Signaling. Kornfield, K, Ph.D. Funding: R01 GM068598, "CDF-1 regulation of zinc homeostasis, 6/03 – 5/07.

Dietary Prevention of Colon Cancer. Anant, S, Ph.D. Funding: NIH R01 CA109269, "Dietary prevention of cancer", 7/05 – 6/09.

Dissecting Pathways Involved in Sensing Nutrient Status and Regulating Growth Using the Zebrafish, *Danio Rerio*. Goldsmith, M, M.D. Funding: K08 HD046656, "Growth control in zebrafish during development," 4/04 – 3/09.

Scientific Advancements/Accomplishments

The CNRU has enhanced the research activities of Washington University School of Medicine CNRU members. Moreover, each Core has not only provided key analytical services for investigators, but has also developed new experimental methods to address important questions in nutrition. Therefore, the Cores have been very responsive to investigators' needs and have developed productive working relationships with investigators to enhance their research activities and advance the nutrition field.

The presence of the CNRU provides a framework for nutrition-related research at Washington University. It serves to expand nutrition research activities, attract established investigators to the nutrition field, foster interactions between investigators—particularly basic and clinical science investigators, and encourage young investigators to pursue research careers in nutrition. The CNRU is recognized as an important and respected component of the medical center and has had a significant impact on our research base. The following are examples of recent CNRU accomplishments.

Lung Surfactant Production in Premature Infants. The Clinical Science Research Core developed a method for measuring pulmonary surfactant phospholipids production *in vivo* in infants. Aaron Hamvas, M.D., Kevin Yarasheski, Ph.D., and Bruce Patterson, Ph.D. have used this method to evaluate the mechanisms of surfactant dysfunction in infants with respiratory distress syndrome (RDS) and found that: 1) preterm infants with respiratory distress syndrome (RDS) have slower surfactant turnover and slower fractional synthetic rates (FSR) from de novo synthesis than term infants, 2) FSR and turnover rate of surfactant is similar in preterm infants ventilated with high frequency oscillatory ventilation or conventional ventilation, 3) preformed plasma fatty acids from the diet are a more important source for surfactant production phospholipid production than is de novo synthesis of fatty acids, and 4) surfactant phospholipid turnover increases markedly by 4 weeks of age.

Increase in Research Base. The CNRU research base increased by more than 56 percent, from 50 full members when the CNRU was first funded in 1999 to 78 full members in 2005. This increase occurred even though 20 (40 percent) of the original full CNRU members are no longer part of the research base because they either left Washington University for academic positions at other institutions for career advancement and are still conducting nutrition-related research (10 members), died/retired (2 members), or are no longer the PI of an extramurally-funded nutrition-related research grant (8 members). The growth in funded CNRU investigators represents adding 48 new faculty to the research base because of an increase in funded Washington University faculty who developed research interests along the themes of the CNRU and by the recruitment of new faculty with career interests in nutrition and obesity. The enhanced profile of nutrition within the medical center produced by the CNRU and the technical services and intellectual expertise of the Core laboratories, and the P/F Program have been important factors in attracting investigators from different disciplines and departments to the CNRU and the nutrition field.

Specific Accomplishments

Women's Health.

Effect of Sex and Adiposity on Fatty Acid and Lipoprotein Metabolism. Bettina Mittendorfer, Ph.D., in collaboration with Bruce Patterson, Ph.D. and Samuel Klein, M.D., found that sex and obesity have independent effects on the regulation of lipid metabolism during different physiological conditions. She made a series of novel observations, by using Clinical Science Research Core services, that underscore the complex interaction between sex and adiposity on substrate metabolism, and demonstrated for the first time that: 1) basal fatty acid and glycerol rates of appearance in plasma, indicators of whole-body lipolytic rate, is greater in women than in men; however, the relative increase in lipolysis that occurs with continued fasting is blunted in women compared with men; 2) lipolysis of adipose tissue triglycerides during moderate intensity exercise is greater in women than men, which causes a greater rate of plasma fatty acid tissue uptake and oxidation in women than men; however, total fat oxidation is the same in both groups because of a reciprocal decrease in the oxidation rate of fatty acids derived from non-plasma sources, presumably intramuscular triglycerides; 3) the lipolytic response to exercise decreases with increasing adiposity in both men and women; 4) basal hepatic VLDL-triglyceride production is greater in women than men, and it increases with increasing adiposity in men but not in women; 5) weight loss decreases the rate of VLDL-TG secretion in women with abdominal obesity, primarily by decreasing the availability of nonsystemic (i.e., splanchnic bed) fatty acids as opposed to systemic (i.e., adipose tissue) fatty acids; and 6) obesity is associated with resistance to the glucose-insulin mediated suppression of VLDL-triglyceride secretion in women, but not in men.

Obesity.

Liposuction and Coronary Heart Disease Risk. Two young investigators, Luigi Fontana, M.D., Ph.D. and Selma Mohammed, M.D., Ph.D., along with Samuel Klein, M.D., used services provided by the CSR Core to evaluate the effect of removing large amounts of subcutaneous abdominal fat by liposuction on: 1) insulin sensitivity, assessed by the hyperinsulinemic-euglycemic clamp technique, and 2) other risk factors for coronary heart disease in women with abdominal obesity. Even though ~10 kg of fat was removed from each subject, which resulted in a considerable decrease in body weight and waist circumference, liposuction did not affect insulin sensitivity in skeletal muscle, liver, or adipose tissue and had no significant effects on blood pressure; fasting plasma glucose, insulin, lipid concentrations; and concentrations of

plasma markers of inflammation (C-reactive protein, tumor necrosis factor alpha, and interleukin-6). These results provide additional insights into how weight loss improves metabolic health and suggests that induction of a negative energy balance, not simply a decrease in adipose tissue mass, is critical for achieving the metabolic benefits of weight loss.

Frailty in Older Adults. Dennis Villareal, M.D. and John Holloszy, M.D. have evaluated the metabolic and functional abnormalities associated with aging and tested novel therapeutic approaches for improving health and reducing frailty in older adults. Data from a randomized controlled trial found that replacement therapy with DHEA, which normally declines with aging, decreased visceral obesity and improved insulin action in older men and women, suggesting that DHEA replacement might be useful in preventing or treating the metabolic syndrome. In another study, Dr. Villareal found that obesity was a major cause of physical frailty in older adults and recently completed a randomized controlled trial, which demonstrated that diet-induced weight loss and activity training markedly improved physical function and quality of life in frail obese elderly men and women. This study could not have been completed without the resources of the Clinical Science Research Core and was used to generate preliminary data that resulted in Dr. Villareal's first funded R01 grant (1.6 percentile).

Gut Bacteria and Obesity. Fredrik Backhed, Ph.D. who is a postdoctoral fellow working with Jeffrey Gordon, M.D. and Clay Semenkovich, M.D., used the Animal Model Research Core to demonstrate that the development of adiposity in mice is accelerated by the presence of intestinal bacteria and that germ-free mice are protected from obesity. This remarkable observation suggests that metabolic signals originating from gut flora alter lipid metabolism and energy balance.

AIDS.

HAART on Protein and Lipid/Lipoprotein Metabolism. Two projects involving collaborations between Kevin Yarasheski, Ph.D., Dominic Reeds, M.D., Samuel Klein, M.D., Bruce Patterson, Ph.D., Clay Semenkovich, M.D., and Ellen Li, M.D., Ph.D. have used the CSR Core to evaluate affects of HAART on protein and lipid/lipoprotein metabolism. These projects bridge between clinical and basic science research. The CSR Core is used for body composition and stable isotope substrate kinetics.

Health Promotion and/or Disease Prevention.

Protein-energy Malnutrition in Children. Mark Manary, M.D. in collaboration with Kevin Yarasheski, Ph.D. has conducted a series of unique studies in Malawian children, which evaluated protein nutrition by using stable isotope tracers methods. Dr. Manary spends part of each year conducting clinical studies in Africa that involve the administration of stable isotopically labeled tracers, and using the Clinical Science Research Core provides mass spectrometry analyses of plasma samples obtained during these studies. The results from these studies have led to the development of home-based treatments with ready-to-use foods for malnourished Malawian children, which are presently undergoing clinical trials. Dr. Manary's research has shown that: 1) egg white protein results in lower rates of urea production and acute infections compared to milk protein in children with kwashiorkor, 2) the relationship between urea production and leucine oxidation (two measures of nitrogen catabolism) is altered during acute infection and that less nitrogen is lost as urea in children who are more malnourished, 3) the acute phase protein response to infection is blunted in marasmic children with infection, and

4) diets that are high in protein quantity, rather than protein quality, provide metabolic benefits (greater whole-body protein synthesis and acute-phase response) in marasmic children with acute infection.

Long-term Calorie Restriction and Cardiovascular Disease. Luigi Fontana, MD., Ph.D. (CNRU Pilot and Feasibility Awardee) and John Holloszy, M.D. made several important observations regarding the effect of long term calorie restriction on coronary heart disease (CHD) risk. They evaluated a unique group of subjects who have been practicing calorie restriction for years in the belief that long term calorie restriction will extend their life span. These subjects are members of the Caloric Restriction Optimal Nutrition Society and were flown to St. Louis from around the United States and Canada to participate in these studies. Drs. Fontana and Holloszy found that, compared with sex and aged match controls who were consuming a typical Western Diet, calorie restriction was associated with a marked reduction in CHD risk factors, including serum lipids, inflammatory markers, blood pressure, and carotid artery intima-media thickness.

Antipsychotic Medication. John Newcomer, M.D. and Dan Haupt, M.D. have continued their collaboration to study the effect of antipsychotic medication on body composition, substrate metabolism, and insulin sensitivity. Dr. Haupt is a previous CNRU pilot and Feasibility Awardee, who has a new K23 award based on this work. The Clinical Science Research Core is used for stable isotope methodology and consultation on metabolic kinetic and made it possible for these investigators to use sophisticated stable isotope tracer methods to evaluate the metabolic effects of antipsychotic medications. In fact, they are the only psychiatrists in the country doing this kind of research. Recent data from their ongoing studies demonstrate that treatment-related changes in body fat mass and distribution were significantly related to *in vivo* insulin sensitivity in adipose tissue, liver, and muscle, assessed by using the euglycemic-hyperinsulinemic clamp technique in conjunction with stable isotope tracer infusions. These data suggest that atypical antipsychotic medications cause obesity related changes in insulin action.

Fatty Acid Metabolism and Cardiovascular Disease. The Animal Model Research Core stimulated productive collaborations among several CNRU investigators, including Daniel Kelly, M.D., Jean Schaeffer, M.D., Brian Finck, Ph.D. (new investigator), John Lehman, M.D. (new investigator), Xianlin Han, Ph.D., Clay Semenkovich, M.D., Richard Gross, M.D., Ph.D., and John Holloszy, M.D., in studies that evaluated fatty acid metabolism and cardiac function, insulin resistance, and atherosclerosis. These investigators created novel mouse models of diabetic cardiomyopathy and insulin resistance by manipulating PPARy expression in heart and skeletal muscle. These models were used to demonstrate the importance of PPARy in insulin action, atherosclerosis, and diabetic cardiomyopathy and that dietary manipulation of cardiac fatty acid metabolism can regulate cardiac function.

Diet-induced Insulin Resistance and Vascular Calcification. Jian-Su Shao Shao, M.D. who is a young investigator working in Dr. Dwight Towler's laboratory, used a novel transgenic mouse model and the Animal Model Research Core services to show that the transcription factor Msx2 promotes cardiovascular calcification in the setting of diet-induced insulin resistance. This observation provides a new framework for developing new therapies to arrest or reverse vascular calcification.

Cardiac Lipotoxicity. Jean Schaffer, M.D. (Pilot and Feasibility Awardee) and Dan Ory, M.D. generated a model of lipotoxic cardiomyopathy, and showed that mice with cardiac-specific overexpression of FATP1 develop increased intracellular fat content and have impaired myocardial function and EKG abnormalities that are consistent with aberrant expression of repolarizing, voltage-gated potassium channels.

Lipid Metabolism and Alzheimer's Disease. Mass spectrometry methods were developed in the CNRU Biomolecular Analysis Core to characterize glycerophosphoethanolamine (GPE) lipids and sphingolipids (including sulfatides), which are major components of myelin and central nervous system (CNS) membranes and are essential for neuronal function. Xianlin Han, Ph.D. in collaboration with David Holtzman, M.D. and Richard Gross, M.D., Ph.D., used these methods to demonstrate that: 1) CNS plasmenylethanolamine content declines in Alzheimer's disease (AD) cerebral cortex by a magnitude that correlates with dementia severity; 2) CNS sulfatide content is modulated by apoE; 3) CNS sulfatide content falls precipitously and ceramide content increases in early AD; and 4) cerebrospinal fluid (CSF) sulfatide content falls with the onset of AD. These findings have led to testable hypotheses about interactions between dietary fat intake and CNS membranes that will provide insight into AD pathogenesis and identify potential nutrition-related targets for AD treatment. These new findings were used as pilot data for Dr. Han's first NIH RO1 grant, awarded in April 2005.

Contributions Made by Biomedical Research Core Services

Clinical Science Research (CSR) Core

Methods Development. In response to users' needs, the CSR Core implemented several new GC/MS methods this year. Bettina Mittendorfer, Ph.D. needed analysis of plasma α-ketoisocaproate (KIC) for a project that included arterio-venous leucine balance across leg muscle. A method was implemented that analyzes KIC and leucine in a single GC/MS run. Robert Gropler, M.D. has been using stable isotope tracers of lactate and pyruvate to validate the information gained from ^{11}C (PET) tracers used to examine glycolysis and intermediary metabolism in dogs. The CSR Core developed and implemented a method to measure the isotopic enrichment of ^{13}C-labeled lactate, pyruvate and alanine in a single GC/MS run. Dogs are given a ^{13}C-labeled substrate concomitant with its ^{11}C-labeled analog, and the quantity of the ^{13}C label appearing in alternate members of the lactate/pyruvate/alanine triad is quantified. The information gained from the quantification of these metabolic pathways in dogs will aid in the interpretation of PET tracers used to quantify glycolysis non-invasively in human subjects.

Facilitation of Collaborations Between Washington University Faculty, Particularly Between Basic and Clinical Investigators. Aaron Hamvas, M.D., F. Sessions Cole, M.D., and Bruce Patterson, Ph.D. have continued a collaboration to study lung surfactant production in premature infants. Metabolic tracers studies have been conducted in over 90 infants since the inception of this project. The CSR Core has provided resources for sample processing and stable isotope enrichment measurement in support of this project, and provided mentorship to five research fellows from the Department of Pediatrics for laboratory procedures. One of these fellows started a faculty position in July 2004, and has a K23 award application pending. Another research fellow has been awarded a Daland Fellowship from the American Philosophical Society (funding to begin July 2005).

Two projects involving collaborations between clinical investigators Kevin Yarasheski, Ph.D., Dominic Reeds, M.D., Samuel Klein, M.D., and Bruce Patterson, Ph.D. and basic investigators

Clay Semenkovich, M.D. and Ellen Li, M.D., Ph.D. have used the CSR Core to evaluate affects of HAART on protein and lipid/lipoprotein metabolism. These projects bridge between clinical and basic science research. The CSR Core is used for body composition and stable isotope substrate kinetics, whereas cellular analyses of human tissue are performed in basic laboratories.

Basic investigators Daniel Kelley, M.D., Brian Dieckgraefe, M.D., Ph.D. and Perry Bickel, M.D. and clinical investigators Samuel Klein, M.D., Bettina Mittendorfer, Ph.D., Kevin Korenblat, M.D., and Chris Eagon, M.D. are continuing a collaborative study evaluating the pathogenesis and pathophysiology of non-alcoholic fatty liver disease (NAFLD) in obese subjects. The CSR Core is used for subject recruitment, body composition, and stable isotope substrate kinetics, whereas cellular analyses of human tissue are performed in basic laboratories.

Two clinical investigators, Drs. Yarasheski and Patterson, have started a collaboration with a basic investigator in the Department of Neurology, Randall Bateman, M.D., to measure the rate of synthesis of amyloid-beta peptide in human subjects. Amyloid-beta is one of the proteins formed in the brain that is associated with the "neural plaques" typical of Alzheimer's disease. The project entails an infusion of $^{13}C_6$-leucine, with sampling of cerebral spinal fluid. The project also requires mathematical modeling of metabolic kinetics.

Kenneth Polonsky, M.D., John Holloszy, M.D., and Dominic Reed, M.D. in the Department of Medicine are collaborating with investigators from the Department of Biology to study the effect of ginseng extracts on the sensitivity of lipolysis and glucose production to insulin using hyperinsulinemic euglycemic clamps and substrate kinetics. The CSR Core is used for body composition and stable isotope tracer techniques. This is a novel collaboration that brings together faculty from different departments at the two campuses of Washington University: the School of Medicine and the Hilltop campus.

Mentorship and Training of Research Fellows and Junior Faculty. The CSR Core is offering a series of weekly lectures on "Methods of *In Vivo* Metabolism Research in Humans" again this year (spring 2005). This series, first offered in the 2003-2004 school year, consists of diverse background topics on stable isotope tracers and mathematical modeling of metabolic kinetics, with specialized units on glucose, lipid, and protein metabolism. The lecture series this year has been attended by approximately 20 CNRU investigators and research fellows. Dr. Patterson, CSR Core director, is responsible for organizing this series.

Within the past year, the CSR Core has provided mentoring in laboratory techniques and/or mathematical modeling of metabolic kinetics to fellows and junior faculty in the Department of Internal Medicine, W. Todd Cade, Ph.D., Bharathi Raju, M.D., Erik Kirk, Ph.D., Sharina Belani, M.D., Bernard Miller, M.D., Selma Mohammed, M.D., Ph.D., Dominic Reeds, M.D., Edward Weiss, Ph.D., and Kevin Korenblat, M.D.; Department of Neurology, Randall Bateman, M.D.; Department of Pediatrics, Kimberly Spence, M.D. and Tami Garmany, M.D.; and the Department of Psychiatry, Dan Haupt, M.D. Their studies encompass a wide range of areas involving regulation of lipolysis and lipoprotein metabolism, glucose metabolism, muscle function, and protein synthesis and turnover. The fellows' training consists of design of clinical research protocols, sample processing, miscellaneous laboratory techniques, and kinetic analysis.

Animal Model Research (AMR) Core

The AMR Core has facilitated collaborations among scientists with diverse approaches to nutrition-related problems in biology. Many have occurred between investigators of different

backgrounds who, because of the availability of easy analyses of nutrition-related variables in mice, are drawn together and share insights. Kendall Blumer, Ph.D., a basic scientist in the Department of Cell Biology and Physiology who studies G proteins, is now collaborating with Anthony Muslin, M.D., a cardiologist in the Department of Medicine, to study blood pressure regulation and heart failure in mice. Dr. Muslin is interacting with Dr. Kelly, a cardiologist pursuing the role of lipid metabolism in heart failure using mouse models. Dr. Muslin is also working with Steven Teitelbaum, M.D. in a project to define the role of the beta 3 integrin in myocardial function. Nicholas Davidson, M.D. and Deborah Rubin, M.D. are now interacting regularly to address the role of diet and weight gain in mouse models. Dr. Davidson is also collaborating with Gustav Schonfeld, M.D. to study the effects of fatty liver on systemic metabolism in a mouse model. John Turk, M.D., Ph.D. and Michael McDaniel, Ph.D. are developing novel assays of lipids that appear to affect insulin secretion in mouse models of diabetes caused by genetic manipulation of pancreatic beta cells. Dr. Semenkovich has established collaborations with Jeffrey Gordon, M.D. to define the role of intestinal bacteria in the development of diet-induced obesity, with Dr. Turk to show that dietary fat accesses unique subcellular regulatory compartments in liver to maintain homeostasis, and with Dr. Kelly to characterize the effects of tissue specific modulation of fat metabolism in mouse models. These are just a few of the examples of CNRU investigators whose productivity has been enhanced through collaborative interactions related to the AMR Core.

Biomolecular Analysis Research (BMA) Core

Users of BMA Core services are distributed among many departments of the university, including Biological Chemistry, Cell Biology and Physiology, Medicine, Molecular Biology and Pharmacology, Pathology, and Pediatrics. Users from the Medicine Department include those from the Divisions of Endocrinology, Diabetes, and Metabolism, Cardiology, Gastroenterology, Gerontology, and Lipid Research.

Multiple collaborations between investigators have been fostered by the resources available from the BMA Core: 1) John Turk, M.D., Ph.D., Dana Abendshein, Ph.D. and Richard Gross, M.D., Ph.D. are using are using ESI/MS/MS to study genetic and nutritional models of the influence of diabetes in the pathogenesis of atherosclerosis; 2) Drs. Turk and Gross are studying lipid signaling molecules involved in glucose-induced insulin secretion; 3) Dr. Semenkovich is using GC/NIEC/MS to study oxidative injury in atherosclerosis and GC/MS to study the fatty acid composition of tissues of genetically modified animals subjected to nutritional stress; 4) Drs. Manary and Yarasheski are evaluating the effect of malnutrition in children on oxidative proteins; 5) Drs. Yarasheski and Turk are continuing to develop the methods needed to quantify lipid membrane composition in diabetes and obesity by using ESI/MS/MS; 6) Richard Ostlund, M.D. is using MS techniques to study gastrointestinal absorption of cholesterol and to characterize phytosterol disposition in humans; 7) Jean Schaffer, M.D., Xianlin Han, Ph.D., Fong Hsu, Ph.D., and Daniel Kelley, M.D. are using MS to quantify lipids in genetic and nutritional induced lipotoxic cardiomyopathies; 8) Drs. Schonfeld and Patterson are continuing their studies of the effect of abnormalities in lipoprotein metabolism on hepatic steatosis; 9) Drs. Han and Holtzman are studying central nervous system complex lipid changes that occur in Alzheimer's disease; and 10) Barry Markaverich, Ph.D. and John Turk, M.D., Ph.D. have characterized linoleic acid metabolites in corn products that have endocrine-disruptive effects and accelerate the growth of breast and prostate cancer cell lines.

Community Accomplishments

Community Outreach. In 2004, the CNRU established a new initiative in community outreach, the Community Wellness Program (CWP), to improve the nutritional and overall health of selected populations in the greater St. Louis cosmopolitan area. The overall mission of the CWP is to establish innovative and effective community-based intervention strategies to prevent and treat obesity in children and their families (or primary caretakers), and to ameliorate obesity, under nutrition, and frailty in the older population (>65 years old), within the greater St. Louis community, particularly in underserved areas. This program was started with very modest administrative support from the CNRU and funds from the Division of Geriatrics and Nutritional Science. However, resources have now been raised from philanthropy that will make this program completely supported by external funding in July 2005. In addition, it is anticipated that pilot data collected from CWP activities will be used to obtain additional extramural funds to allow continued growth and expansion of the program.

Dr. Monique Williams is the director of the CWP. Dr. Williams is a young clinical investigator and geriatrician who has a career interest in developing outreach programs in financially disadvantaged older adults. She is active in the St. Louis African American community; her contacts with African American churches and subsidized elderly housing facilities enhance her ability to provide new initiatives in underserved areas of St. Louis.

The CWP has developed and implemented two outreach programs: 1) Family Lifestyle Intervention Pilot (FLIP) Program for obese adolescents and their primary caretakers, and 2) Nutrition Education, Assessment, and Treatment (NEAT) Program for elderly persons in underserved areas.

Community Education. The CNRU contributes to community education in nutrition through the BLC HealthCare system Community Education Program. Among the educational activities for the lay community are health fairs, which are held in St. Louis and throughout its surrounding area, and seminars and classes on a variety of health-related topics such as healthy hearts, diabetes, weight control, and osteoporosis.

Leveraging of CNRU to Obtain Additional Resources

We have successfully leveraged the prestige, recognition, and productivity of the CNRU to obtain considerable institutional and philanthropic support for nutrition and obesity initiatives. This support would not have been possible without the presence of our CNRU.

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

Pilot and Feasibility Funding. During the last funding cycle, Dr. William Peck, the Dean of the School of Medicine at that time, provided $40,000 to support one CNRU Pilot and Feasibility Award for two years. Dr. Larry Shapiro, the current Dean of the School of Medicine, has agreed to double the commitment and will provide support for one CNRU Pilot and Feasibility Award per year for four years ($20,000/yr; $80,000 total).

Faculty Recruitment. 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, within the Division of Geriatrics and Nutritional Science. These funds have been used to recruit Nada Abumrad, Ph.D., who joined the faculty in 2004, and who will be Associate Director of the CNRU and director of the CNRU Adipocyte Biology Core Laboratory. Dr. Abumrad has further enhanced our pool of nutrition research faculty by recruiting a young investigator (Zaher Nahle, Ph.D.) to join her research program. Brian Finck, Ph.D. was recruited by Dr. Klein and joined the Division of Geriatrics and Nutritional Sciences in August 2005. Dr. Finck is a promising young investigator who is interested in nonalcoholic fatty liver disease (NAFLD), and is working with unique murine models to study the nutritional and metabolic factors involved in the pathogenesis of NAFLD. In addition, Dr. Polonsky also approved the appointment of 7 additional new faculty members to the Division of Geriatrics and Nutritional Sciences, who are involved in nutrition research or nutrition education. Three of these faculty now have their own research support from the NIH (Dominic Reeds, M.D., K23 Award; Monique Williams, M.D., Minority Supplement Award; and Kyle Moylan, M.D., Geriatric Academic Career Award), and the other four faculty (Selma Mohammed, M.D., Ph.D.; Bernard Miller, M.D.; Luigi Fontana, M.D.; and Richard Stein, Ph.D.) are supported by Dr. Klein's research grants and clinical activities. Moreover, three of these seven new faculty are African Americans, who support the CNRU's mission to increase minority faculty participation in nutrition.

In 2001, Dr. Klein approached Charles Zorumski, M.D., chairman of the Department of Psychiatry, about recruiting Denise Wilfley, Ph.D. to Washington University to enhance our clinical and research expertise in the behavioral management of obesity. Dr. Wilfley is a productive and well-funded investigator in the behavioral management of eating disorders and obesity. Expansion into this area was not a targeted priority within the Department of Psychiatry. Therefore, Dr. Wilfley's recruitment was initiated by Dr. Klein and facilitated by the CNRU because of the increased opportunities for Dr. Wilfley's research program provided by the CNRU. Moreover, this recruitment represents an example of collaborative interactions between different departments and the Dean to support nutrition and obesity. The Department of Psychiatry provided a $500,000 package for Dr. Wilfley's recruitment; the Department of Medicine provided 2,650 square feet of space, contiguous with the Washington University Weight Management Program, for Dr. Wilfley's research program; the Dean provided $135,000 to renovate the new space; and Dr. Klein appointed Dr. Wilfley as Director of the Washington University Weight Management Program.

CNRU Space and Facilities. The Department of Medicine has been generous in providing the CNRU with space, which is a particularly precious commodity at Washington University. In 2004, the Department of Medicine provided 5,000 square feet of newly renovated space, contiguous with the existing 3,500 CNRU space on the second floor of the West building. This additional space was needed to accommodate the recruitment of new nutrition faculty and the expansion of CNRU activities. The Department also provided $600,000 to cover the cost of renovations needed to construct offices; modernize laboratories (including the CNRU's Clinical Science Research Core); develop space for the new CNRU Adipocyte Biology Core; and construct a large conference room for CNRU meetings, seminars, and lectures. The Department of Medicine also provided 1,500 square feet and $50,000 to renovate new space for the Washington University Weight Management Program, which is part of the clinical component of

the CNRU. The Weight Management Program is located on the first floor of the Storz building, across the street from the CNRU.

Equipment. The need for body composition analyses in human subjects increased markedly after our CNRU was funded in 1999, in part, because of the increase in clinical nutrition and human obesity research stimulated by the CNRU. At the same time, the dual energy x-ray absorptiometer (DEXA) dedicated for CNRU investigators for body composition analyses, which was located in the CNRU Clinical Science Core laboratory, had become outdated and could no longer be covered by a service contract. Therefore, the CNRU requested and received support ($75,000) from the Department of Medicine to acquire a new DEXA (Hologic Delphi QDR Series), purchased at a substantial discount because the order was pooled with other Washington University equipment orders. The CNRU and the General Clinical Research Center (GCRC) developed a mutually beneficial partnership, in which the new DEXA was placed within the GCRC, and the salary of a full-time DEXA technician is shared by the GCRC and the CNRU. With this arrangement, CNRU investigators are able to obtain body composition analyses for their studies without charge.

Philanthropic Support.
The CNRU has been successful in raising funds from charitable foundations and individuals to implement CNRU initiatives that are important to the mission of our CNRU but are not directly supported by the CNRU grant.

In 2003, the Robert C. Atkins Foundation committed $1.5 million to Washington University School of Medicine to establish the first Robert C. Atkins Professor of Medicine and Obesity Research. This endowed chair was used to help recruit Nada Abumrad, Ph.D., an outstanding basic scientist and adipocyte physiologist, who will become Associate Director of the CNRU in 2006. This endowment will reach its full amount in 2006 and will provide approximately $60,000/year, depending on interest rates.

In 2004, the Charles Kilo Foundation, which is a local philanthropic organization in St. Louis, committed $1.5 million to establish the first Charles Kilo Professorship in Nutrition and Diabetes. This endowed professorship is being used by Dr. Klein and the CNRU to recruit a new faculty member to Washington University who is interested in nutrition and diabetes research. The candidate for this position will be selected by members of the CNRU Internal Advisory Committee, which includes the Chairman of the Department of Medicine. This endowment will provide approximately $60,000/year, depending on interest rates.

In 2005, the David A. and Linda S. Yawitz Fund in Geriatrics and Nutritional Science was established by a generous commitment from David and Linda Yawitz of $50,000/yr for 4 years. The fund is to be used for programs aimed at reducing frailty and osteoporosis in older men and women in St. Louis by nutrition education and intervention (e.g. weight loss in obese older adults, increased energy and protein intake in cachectic older adults, and calcium and vitamin D supplementation to prevent and treat osteoporosis) and physical activity interventions in older men and women.