University of Colorado at Denver and Health Sciences Center
Clinical Nutrition Research Unit
Start Date: 1995
Status: Ongoing
Source of NIH Support: NIDDK

Organization and Goals

The major goal of the University of Colorado at Denver and Health Sciences Center Clinical Nutrition Research Unit (Colorado CNRU) continues to be to create an environment where researchers are able to work together to conduct high quality research in order to maximally contribute to knowledge in the area of nutrition and obesity. The Colorado CNRU promotes vertical integration of research efforts that facilitate interaction and collaboration among investigators working at different levels of basic and clinical investigation, from gene to cell to organ to animal model to human to clinical to community intervention.

The specific aims of the Colorado CNRU are to:

- Enhance an existing strong and well-funded research base in areas related to nutrient utilization by providing an infrastructure to support ongoing nutrition and obesity research; providing measurements/expertise that would not otherwise be available to individual investigators; and providing measurements in a cost-effective manner.
- Promote interdisciplinary collaborative, vertically integrated research among members of the Colorado CNRU research base.
- Strengthen several NIH-funded training programs in nutrition that provide education and research training for graduate, undergraduate, and medical students, and assists promising young M.D.s and Ph.D.s in becoming independent investigators in the field of nutrition and obesity.
- Translate basic and clinical research into programs to improve health and wellness and reduce obesity in the community.
- Improve the quality of nutrition information provided to the general public.

To accomplish these specific aims, we have an Administrative Core, three Scientific Core Laboratories (Energy Balance, Metabolic, and Mass Spectrometry), and a new Clinical Component. We also have continued our pilot and feasibility (P/F) and enrichment programs. In order to maximize the resources of the CNRU and to promote interactions among members of our research base, we have identified an overall theme for the Colorado CNRU, which is nutrient utilization and function. Within the overall theme, we have three areas of research focus: obesity and its comorbidities; developmental aspects of nutrient utilization and function; and aging, nutrition, and physical activity.

Core Laboratories

Administrative Core: James O. Hill, Director; Robert H. Eckel, Co-Associate Director; Daniel Bessesen, Co-Associate Director; Andra Price, Administrator

Current External Advisory Group Members:

William C. Heird, M.D., Professor, Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX

Steven D. Clarke, Ph.D., Director of Nutritional Science, McNeil Nutritionals, LLC, a Johnson and Johnson Company

John C. Peters, Ph.D., Research and Clinical Development, The Procter & Gamble Company, Cincinnati, OH
Robert R. Wolfe, Ph.D., Professor, Department of Metabolism, University of Texas Medical School, Schriners Burn Institute, Galveston, TX
Steve Woods, Ph.D., Professor, University of Cincinnati, Psychiatry Department, Cincinnati, OH

Proposed External Advisory Group Members:
TBA. In the coming year, we will invite a new group of external advisory members to participate in the CNRU.

Energy Balance Core: Wendy Kohrt, Ph.D., Director

Mass Spectrometry Core: Uwe Christians, Ph.D., Director

Metabolic Core: Jed Friedman, M.D., Director

Clinical Component: Holly R. Wyatt, M.D., Director

Pilot and Feasibility Studies

Effects of IUGR On Ovine Fetal Myocardial Metabolism and Insulin Signaling Transduction Pathways. James Barry, M.D., Assistant Professor, Department of Pediatrics, Section of Neonatology, UCDHSC, Denver, CO. Funding: 1/2004 – 12/2004 (yr 1), 1/2005 – 12/2005 (yr 2). The goal of our studies is to uncover the metabolic and molecular mechanisms for altered cardiac glucose metabolism that occur with fetal growth restriction (IUGR). In an ovine model of IUGR, a significantly smaller placenta is produced that results in placental insufficiency and alters the fetal delivery of nutrients and oxygen. This decreased supply of substrates results in an asymmetrically growth restricted fetus with relative hypoglycemia, hypoinsulinemia, lower levels of IGF, a reduction in amino acids, and elevated lactate levels. These findings are the same essential characteristics found in human fetal growth restriction. These changes result in asymmetric fetal IUGR with maintenance of myocardial growth relative to fetal size. We hypothesize that fetal myocardial glucose metabolism adapts to the IUGR environment by developing a "thrifty phenotype," decreasing overall fetal body size and individual organ growth.

We proposed to determine the changes in myocardial glucose and lactate utilization *in vivo* in near term IUGR ovine fetuses and the associated alterations in their specific myocardial cell membrane transporter protein levels.

We have investigated the changes in myocardial glucose transporters (Glut) 1 and 4 during the latter half of the IUGR ovine gestation at 55 dGA, 90 dGA, and 135 dGA in the basal state. This was done to determine normal ontogeny as well as to define adaptations by the IUGR myocardium pertaining to glucose transport.

We found that Glut-1mRNA expression did not significantly change across gestation, and Glut-4 mRNA expression significantly increased with gestation, coinciding with anticipated maturational changes in both the control and IUGR animals. We have also found that Glut-4 plasma membrane bound protein levels were significantly increased in the near term IUGR

myocardium (135 dGA), while whole heart levels of Glut-4 were not significantly different. These results (being prepared for publication) have shifted our study focus slightly to define changes within the insulin signaling pathway which may provide a mechanism for increased Glut-4 translocation to the plasma membrane.

Our *in vivo* studies have been performed on four control animals. We have successfully performed catheter placement surgeries and completed metabolic studies utilizing the techniques of substrate labeling ([1-^{13}C] lactate and [1-^{14}C] glucose) (21) and myocardial blood flow measurements with fluorescent-labeled microspheres in determining myocardial carbohydrate metabolism. Thus far, we have found an increase in myocardial blood flow with insulin stimulation and an increase in glucose uptake and oxidation rates. We are currently working with the CNRU Core Mass Spectrometry lab in analyzing blood levels of [1-^{13}C] lactate and ^{13}CO2 so that we may be able to determine the utilization and oxidation rates of lactate (5; 6) in the IUGR ovine myocardium in basal and insulin stimulated conditions.

We determined the changes in the insulin/IGF signaling cascade within cardiac myocytes that may be affecting glycogen synthesis, protein synthesis, hypertrophy, and overall myocardial growth.

We have investigated key proteins of the insulin signal transduction pathway with Western immunoblot analysis in 90 dGA and 135 dGA IUGR and control myocardium for the insulin receptor, insulin receptor substrates (IRS-1 and IRS-2), p85α subunit of PI-3 kinase, Akt, and phosphorylated Akt.

We have found a significant increase in whole heart protein levels of the insulin receptor in the IUGR animals at 135 dGA. No significant differences in the level of other signal transduction proteins were found in the IUGR myocardium or between gestational ages 90 and 135.

We speculate that with the increase in insulin receptor levels, it is possible that this is an up-regulation in the basal state allowing for increased downstream signaling, providing for the increased translocation of Glut-4 and for the increased glycogen levels found in the IUGR myocardium. It will be important to determine insulin signal transduction in the IUGR near-term myocardium (135 dGA) after acute insulin stimulation by measuring phosphorylation levels of the insulin receptor IRS-1, p85α subunit of PI-3 kinase, and Akt, as well as to measure the IRS-1 stimulated PI-3 kinase activity. These studies may provide a mechanism for increased Glut-4 translocation or address the separate, but equally important, issue of IUGR associated myocardial insulin resistance by determining if there is a decrease in the signal transduction of insulin stimulation in the IUGR myocardium.

The findings of increased Glut-4 plasma membrane bound levels and increased levels of insulin receptor protein found in the IUGR myocardium have caused us to focus more on insulin signaling mechanisms related to Glut-4 translocation and less on further downstream pathways affecting overall myocardial growth. However, we are currently performing histological analysis to determine overall myocardial cellularity, nucleation characteristics, and myocyte size in order to better characterize the myocardial growth (18). Normally, fetal cardiomyocytes are distinctly smaller than more mature cardiac myocytes and fetal myocytes have a single nucleus per cell, with more mature myocytes commonly having multiple nuclei per cell. We also plan to investigate myosin heavy chain isoforms alpha and beta, as their expression is developmentally

related. The alpha isoform is found in adult myocytes and the beta isoform is predominantly found in fetal and early neonatal life (9). Gaining a better understanding of these growth and maturation changes will allow us to form a more focused investigation on IUGR cardiac adaptations, with specific focused interest in mTOR and downstream signaling pathways as outlined in the initial proposal.

Dr. Barry is an Assistant Professor of Pediatrics and a member of the CNRU research base. He has requested, and will likely receive, a second year of funding for his pilot project. He plans to use these studies to obtain sufficient background data and expertise to obtain independent funding from the NIH—such as a K award—or from the American Heart Association.

Insulin Control of Fat Regulation and Exercise in Teens. Kristen Nadeau, M.D., Fellow, Pediatric Endocrinology, UCDHSC, Denver, CO. Funding: 1/2004-6/2005 (extended yr 1), 7/2005-6/2006 (yr 2). Type 2 diabetes mellitus (T2DM) is increasing in epidemic proportions among adults and adolescents. Exercise is critical to the prevention and treatment of T2DM. Despite the data, adults with T2DM do not exercise sufficiently and exercise less intensely. A reduced VO_2max, diastolic dysfunction, and endothelial dysfunction have been identified in these adults, compared to equally obese and sedentary controls. These defects may limit their ability to exercise, creating a negative experience with exercise. There are no studies looking at exercise function or its components in adolescents with T2DM. Therefore, the goal of this study is to compare exercise function (bicycle Vo_2 max and Vo_2 kinetics) in children 12-19 with T2DM, compared to similarly active lean and obese teens, to independently examine the effects of obesity and inactivity. In addition, secondary outcomes will include measurement of insulin resistance (IR) (hyperinsulinemic euglycemic clamp), endothelial function (brachial artery ultrasound and plethesmography), diastolic function (echocardiogram with exercise), intramyocellular lipid content (IMCL) (nuclear magnetic resonance or NMR), and perceived exertion (Borg Scale). In addition, outcomes will be correlated with body composition; measures of inflammation (CRP, IL-6, myeloperoxidase); liver fat deposition (ALT); serum lipids (fasting lipid profile, free fatty acids); and adiponectin. By defining the exercise defects present in adolescents with T2DM, identifying noninvasive markers and examining potential non invasive causes, we hope to improve the exercise treatment approach to pediatric diabetes and help prevent exercise defects from occurring or progressing.

To date we have enrolled 32 subjects who entered into the protocol. Although the study goal number of patients is 30, several patients dropped out due to moving, inability to complete a maximal exercise test, or not qualifying for the exclusion criteria. Therefore, we still need to recruit four more subjects. The study protocol has gone very well. There have been no adverse events. Preliminary results show that exercise capacity is reduced in subjects with diabetes compared to subjects with obesity but no diabetes, and that exercise capacity in subjects with obesity but no diabetes is reduced when compared to controls. Testing for endothelial function and echocardiograms has gone well, but results are still pending. Preliminary results for the insulin clamps show that subjects with diabetes are much more insulin resistant than obese subjects, who are in turn more resistant than the control subjects. Thus, the insulin clamps appear to be working well. Other lab results from the insulin clamps are pending, as are correlation with exercise function. The muscle NMR was delayed due to the need to obtain new software and upgrade the NMR machinery. This has been addressed and NMRs are now going well—20 subjects have now had an NMR performed. Results are pending. There are not yet any publications from this research, but they will be coming soon! This activity led to the successful award of a 5-year grant (NIH K23 RR20038).

Leucine Regulation of Pancreatic B-cell Development in Fetal Growth Restriction. Paul Rozance, M.D., Assistant Professor. Funding: 1/2005-12/2005 (yr 1). This project will determine the effects of decreased nutrient supply and oxidation on pancreatic β-cell development in the growth-restricted fetus. Fetal growth restriction (FGR) is associated with the development of type 2 diabetes, a combination of insulin resistance and pancreatic islet failure. Growth-restricted fetuses develop features of pancreatic islet failure *in utero*, including decreased β-cell mass and impaired glucose-stimulated insulin secretion (GSIS). Our lab has developed a placental insufficiency model of intrauterine growth restriction (PI-IUGR) with global nutrient deficiencies in the pregnant sheep which shares these characteristics. β-cell replication is diminished, contributing to decreased β-cell mass and leading to defective GSIS. Using this model, we will investigate the role of decreased leucine signaling in diminished β-cell replication. Cellular leucine metabolism is central to this signaling. Leucine signals the initiation of protein translation via its own metabolism as well as by its ability to promote the metabolism of glutamate through allosteric activation of glutamate dehydrogenase (GDH). The signals generated by these metabolic events stimulate mTOR (mammalian target of rapamycin) in the β-cell. mTOR is a kinase central to the initiation of protein translation and maintenance of β-cell mass. Therefore, defects in the ability of leucine to stimulate mTOR may underlie the decreased β-cell mass seen in FGR. The long-term goal of this project is to demonstrate that fetal growth restriction decreases protein synthesis in the pancreatic islet by downregulating leucine stimulated mTOR activation. Determining the cause of pancreatic islet failure and decreased β-cell mass in IUGR will give a better understanding of the mechanism underlying the increased risk of type 2 diabetes in infants born small for gestational age, and may suggest nutritional therapies aimed at diabetes prevention.

We propose to determine the effects of FGR on rates of protein synthesis in isolated pancreatic islets. We hypothesize that FGR will decrease the ability of isolated islets to increase protein synthesis in response to stimulatory signals. We will stimulate isolated pancreatic islets with hormones (insulin, insulin-like Growth Factor-1) and nutrients (glucose, leucine) to determine their ability to increase protein synthesis, a downstream consequence of mTOR activation.

We also propose to determine the effects of FGR on nutrient signaling pathways that lead to reduced β-cell protein synthesis, replication, and pancreatic endocrine mass. We hypothesize that FGR, by decreasing leucine metabolism, will limit the nutrient signals which mediate β-cell protein translation and replication. We will determine glucose, leucine, and glutamine's ability to activate mTOR in isolated islets from FGR animals by measuring the phosphorylation of its target proteins, p70S6K and 4E-BP1.

In 2005, Dr. Rozance's most significant research accomplishments were in determining the cause of decreased glucose-stimulated insulin secretion in a model of intrauterine growth restriction in which fetal sheep are made selectively hypoglycemic and hypoinsulinemic by a maternal insulin infusion. Previously, our lab had demonstrated decreased *in vivo* glucose-stimulated insulin secretion. In the past year, Dr. Rozance has further characterized *in vivo* insulin secretion in this model by quantifying lysine- and leucine-stimulated insulin secretion. He showed that in normal fetal sheep both leucine and lysine given as single amino acid infusions can stimulate insulin secretion. Chronic hypoglycemia decreases lysine-stimulated insulin secretion but not leucine-stimulated insulin secretion. Dr. Rozance also isolated islets from the hypoglycemic fetuses and demonstrated defective glucose-stimulated insulin secretion. Additionally, he tested leucine-,

lysine-, and arginine-stimulated insulin secretion and found that none of these nutrients were able to stimulate insulin secretion in hypoglycemic fetal islets, but were able to do so in control fetal islets. These results have formed the basis of an abstract that has been submitted to the American Journal of Physiology – Endocrinology and Metabolism.

In addition, Dr. Rozance has further characterized the *in vitro* secretory defect in this model. He has shown that neither direct membrane depolarization nor experimentally increased extracellular calcium entry were able to restore insulin secretion. Histological studies on the isolated islets showed no difference between treatment or control islets in the islet size or percentage of insulin, glucagon, somatostatin, or pancreatic polypeptide within the isolated islets. Utilizing electron microscopy we have also shown distended endoplasmic reticulum in hypoglycemic islets and not in control islets. This finding led Dr. Rozance to investigate and characterize the ER stress response (Unfolded Protein Response) in fetal sheep. Thus far, he has characterized this response in fetal sheep fibroblasts. This required the cloning and sequencing of several previously undefined sheep genes. In the next year, he plans to characterize this pathway in isolated fetal sheep islets.

Previously, our lab has demonstrated endogenous fetal glucose production in this chronically hypoglycemic model of intrauterine growth restriction. Dr. Rozance set up several studies to determine if this glucose production was from gluconeogenesis or glycogenolysis. First, he showed that PEPCK gene expression (and Glucose-6-Phosphatase) in the liver was increased in the hypoglycemic fetuses, showing increased gluconeogenic capacity. Then he showed the hepatic glycogen content was not decreased in the hypoglycemic fetuses, indicating that the fetal glucose production was not due to glycogenolysis.

Utilizing our placental insufficiency model of fetal growth restriction, Dr. Rozance has determined that isolated islets have a defect in their capacity to oxidize glucose. This defect could help explain the decreased beta cell mass seen in this model of intrauterine growth restriction. He has been perfecting techniques to determine defects in nutrient- and hormone-stimulated protein translation and mTOR signaling. He hopes to identify a defect in these pathways in the growth-restricted fetal islets and will then test therapeutic supplements *in vitro* to correct the defect and follow this with studies to determine our ability to correct the defects *in vivo*.

Dr. Rozance's future career lies in academic medicine, including a significant amount of time spent in research as a physician-scientist. His current interests are in understanding and determining the mechanism responsible for the well described link between intrauterine growth restriction and type 2 diabetes. His efforts will focus on determining this mechanism. Dr. Rozance is obtaining the required experience and preliminary data to receive larger career development and project awards through outside funding agencies such as the NIH, American Diabetes Association, and/or the March of Dimes.

Antioxidants and Cell Signaling During Myocardial Ischemia/Reperfusion. Karyn Hamilton, Ph.D., Assistant Professor. Funding: 7/2005-6/2006 (yr 1). The long-term goal of the research in our laboratory is to identify the most effective way exercise and antioxidants can be used to preserve cardiovascular health and decrease mortality from ischemic heart disease. Progress toward our goal includes these findings: (1) Exercise results in a cardioprotective phenotype, with hearts that are more resistant to apoptosis following ischemia-reperfusion (IR), (2) The antioxidant MnSOD appears to be essential for this protection, (3) Blocking MnSOD

does not alter oxidation of cell components, and (5) Combining antioxidant supplementation with exercise does not result in additive cardioprotection. These findings have led us to hypothesize that: (1) MnSOD regulates cardioprotection via redox-sensitive signaling pathways and (2) Altering the redox environment with exogenous antioxidants will alter exercise. Dr. Hamilton is currently halfway through her first year of funding and no results are available at this time.

Identifying strategies for protecting the heart during ischemic events is an important clinical problem. While exercise has been repeatedly shown to provide such protection in cell and animal models, the mechanisms remain elusive. Evidence from our laboratory and others suggests that an exercise-induced increase in cellular antioxidant capacity is one possible mechanism by which exercise may exert protection. However, exercise is not a feasible intervention for all populations, necessitating an alternative intervention. Several of our research projects in the recent past have led us to question whether increasing cellular antioxidant capacity with dietary antioxidants will alter or enhance the mechanism(s) by which exercise provides myocardial protection during ischemia and reperfusion. However, embarking on feeding studies is not trivial from a financial or a temporal standpoint. Because the predominance of our current pilot data has been collected from models in which we manipulated endogenous antioxidants, we have been, to this point, unable to obtain recent independent funding to do experiments exploring the effects of exogenous antioxidants on ischemia-reperfusion induced cell signaling pathways. Obtaining this pilot and feasibility award will enable us to complete a variety of pilot experiments that are essential for helping delineate the type of nutritional antioxidants (i.e., phytoestrogens, flavonoids, and synthetic antioxidants), the dose, and the efficacy of feeding regimens for altering cardiomyocyte antioxidant properties. These feasibility experiments will form the foundation of an R01 application to the National Heart, Lung, and Blood Institute to continue studying nutritional antioxidants and cell signaling during myocardial ischemia and reperfusion. This activity led to the successful award of two Colorado State University College of Applied Human Sciences Research Endeavors Awards.

Effects of a HFD on Liver and Skeletal Muscle Mitochondrial Proteomes and Insulin Action in OP and OR Rats. Mathew Jackman, Ph.D., Instructor, Department of Medicine, Endocrinology; and Nicole Reisdorph, Ph.D.**,** Assistant Professor, Anesthesiology. Funding: 9/1/2005 – 8/30/2006. Despite efforts to promote healthy eating and physical activity, the prevalence of obesity and diabetes continues to increase. Similar to humans, not all rats become obese when provided an energy dense diet. Initial findings have led us to hypothesize that responses occurring within the first few days of a HFD are critical in determining the extent to which obesity will occur. Knowing which animals will become obese or remain lean before provision of a HFD is a valuable tool that can be utilized to determine underlying attributes. The primary focus of this proposal is to systematically distinguish and evaluate phenotypically inherent characteristics versus adaptive changes that occur within mitochondria from skeletal muscle to liver of OP and OR rats following a HFD feeding. We are proposing to use proteomic and metabolomics analyses to evaluate the protein and metabolite profiles of liver and muscle mitochondria. Parallel experiments evaluating changes in insulin sensitivity will also be performed. Information gained from the tissue studies will then be used to address how fundamental aspects of the inherent tissue profiles and/or HFD-induced alterations affect insulin sensitivity in OP and OR rats. Drs. Jackman and Reisdorph have just begun this project and no results are available at this time.

Dr. Jackman's goal is to become an independently funded faculty member making significant contributions to the understanding of the etiology of type 2 diabetes and to the Division of Endocrinology, Metabolism and Diabetes at UCDHSC. His plan is to achieve these goals by utilizing funds from the pilot award to establish a line of investigation that is distinct from that of his post-doctoral mentor, Dr. Bessesen. Although the applicant's career goal is to become an independent investigator, it is evident to the applicant that the greatest success will be achieved by collegial interaction with other scientists.

Dr. Reisdorph's goal is to become a leader in the field of using mass spectrometry-based proteomics in clinical diagnostics and predictive medicine. To achieve this goal, she intends to collaborate with scientists directly engaged in clinical research. In the short-term, it is Dr. Reisdorph's objective to obtain preliminary data and establish a track record, through multiple publications, which will then enable her to obtain NIH funding and become an independent, academic investigator. In addition, the goal of the proposed research is to discover and validate biomarkers with the specificity and sensitivity profiles necessary for clinical diagnosis, early detection, and prevention of disease. Dr. Reisdorph believes that achieving these research goals will enable her to reach her primary goal of becoming an independent and autonomous researcher.

This activity has led to the successful award of three NIH-funded RO1 grants and an NIH-funded P30 grant.

Scientific Accomplishments

The CNRU's scientific accomplishments enhance basic, clinical, and translational research related to the theme of nutrient utilization and function. Within this theme, we have identified the following focus areas: obesity and its comorbidities; developmental aspects of nutrient utilization and function; and aging, nutrition, and physical activity.

Basic Research

Metabolomics. Dr. Uwe Christians, Director of the Mass Spectrometer Core Laboratory, conducted research that demonstrated that metabolic profiling in urine may provide the basis for the development of toxicodynamic monitoring strategies for immunosuppressant nephrotoxicity. This study also provides the rationale for further evaluating changes of urine metabolite patterns as disease and therapeutic monitoring tools in metabolic diseases.

Fetal Metabolism. Dr. William Hay and colleagues are involved in a basic research program in fetal growth that involves the application of physiological, biochemical, and molecular methods to study maternal, placental, and fetal metabolism in the pregnant sheep model. Last year, they completed two studies that were important for contrasting different forms of reduced insulin secretion in IUGR fetuses. The first shows that chronic IUGR from placental insufficiency and reduced nutrient uptake by the fetus involves reduced pancreatic islet and beta cell development due to an arrest of beta cell replication, but not deficit in insulin exocitosis. The second shows that despite hypoglycemia being a common complication of IUGR, IUGR fetuses with hypoglycemia alone have decreased insulin exocytosis capacity, not a reduction of insulin production. These unique observations indicate that the duration, timing, magnitude, and type of fetal nutrient deprivation produce different forms of reduced insulin secretion capacity, both of which might independently contribute to the failure of pancreatic insulin secretion in later life.

Insulin Resistance in Skeletal Muscle. Drs. Jed Friedman and Boris Draznin published research showing a novel mechanism for insulin resistance in response to growth hormone excess in skeletal muscle. In a series of experiments, they showed that the main effect of growth hormone is to trigger a specific increase in a protein sub-unit that acts as a dominant negative signal. In addition, they showed in another publication that this increased p85a subunit of the phosphatidylinositol 3-kinase (PI 3-kinase) also appears to be involved in the insulin resistance of normal pregnancy. These changes revert to normal within 1 year post-partum in women with normal glucose tolerance, suggesting they are driven by pregnancy-induced factors, with placental growth hormone, and possibly Tumor Necrosis Factor (TNFa) the most likely candidate(s). Thus, this novel pathway could be involved in other forms of insulin resistance as well, suggesting it is an attractive target for anti-diabetes therapy.

AMPK as a Metabolic Switch. Dr. Greg Florant's research in the past year has focused on how mammalian energy utilization is controlled via complex inter- and intra-organ communications. AMP-activated protein kinase (AMPK) is an important metabolic cell signal that may be involved in the regulation of food intake. AMPK controls several pathways involved in energy expenditure including acetyl-CoA carboxylase (ACC), an enzyme that controls fatty acid utilization in muscle, liver, and fat. This study investigated the effects of fasting on the phosphorylation state of AMPK, and expression and phosphorylation of ACC (pACC) in tissues from golden-mantled ground squirrels (GS) (Spermophilus lateralis). This animal model was chosen because of its ability to switch from a hyperphagic state (summer) to a fasting state during hibernation. We hypothesized AMPK may be a metabolic switch, signaling the animal to stop eating and fast during hibernation. GS were sacrificed in the fed; 1, 3, or 5 day fasted; or hibernating state. Serum samples from GS were analyzed for triacylglycerols, free fatty acids (FFA), ketones, and insulin concentrations. Insulin levels decreased with fasting duration; however, hibernation and summer control values were not significantly different ($P > .05$). Triacylglycerol concentrations decreased 78 percent over a 5-day fast and significantly increased during hibernation. FFA concentrations tripled over a 5-day fast and with hibernation. Ketones increased significantly over a 5-day fast, but not with hibernation. Phosphorylation states of AMPK and ACC were determined by western blot analysis of liver and muscle tissue. AMPK and phosphorylation of ACC were expected to increase with fasting. We found that liver ACC expression dramatically decreased in hibernation and muscle pACC increased with hibernation. Furthermore, phosphorylation of AMPK was higher in animals that were hibernating. We conclude that AMPK may be a metabolic hunger switch.

Liver Glucose Metabolism and Gene Expression. Drs. Mike Pagliassotti (CSU) and Jed Friedman have collaborated on projects related to liver glucose metabolism and skeletal muscle lipid metabolism. Overall, this collaboration has been highly effective, resulting in two mentor-based K01 awards to junior faculty (Jianhua Shao from Dr. Friedman's laboratory and Michael Bizeau from Dr. Pagliassotti's laboratory) in which Drs. Pagliassotti and Friedman are co-mentors, an ADA junior faculty award to Dr. Shao (who recently relocated to University of Kentucky), and a CNRU pilot and feasibility grant to Dr. Bizeau.

Establishing an Animal Model that Demonstrates the Metabolic Changes that Occur With Weight Loss and That Facilitates Weight Regain. Dr. Paul MacLean has collaborated with Drs. James Hill, Dan Bessesen and Janine Higgins to establish an animal model to use in investigating why it is so difficult to maintain a weight loss. In particular, they aimed to identify the metabolic adjustments that occur with weight loss that promote a high rate of weight gain. In

a rodent model of obesity, they characterized the metabolic state of weight-reduced animals and followed their metabolism as they relapsed to obesity. They observed that weight reduction was accompanied by: (1) a profound energy imbalance; (2) a preference for carbohydrate as a fuel source; (3) an obesigenic endocrine, cytokine, and peripheral insulin sensitivity profile, and (4) susceptibility to adipogensis and hyperplasia. These interrelated characteristics promote a high rate of weight regain when external restriction of food provision is eliminated. Their assertion is that these metabolic adjustments likely occur with weight loss in humans and make it difficult for people to keep the weight off permanently. This collaboration used the energy balance core laboratory for measurements of energy expenditure, substrate oxidation, and body composition in rodents, and the metabolic core laboratory for assessment of insulin action and measurement of hormones such as leptin.

Clinical Research

Obesity.

Prevention of Weight Gain/Obesity. Dr. James Hill, Holly Wyatt, and collaborators have been investigating how small lifestyle changes can help prevent excessive weight gain. Using a small changes intervention in families, they were able to prevent excessive weight gain in both overweight children and their parents. Families with at least one overweight child between the ages of 8 and 12 years were recruited for the study. The intervention was based on the "America on the Move" program of producing small changes in diet and physical activity to prevent weight gain. Families in the intervention group were asked to reduce energy intake by 100 kcal per day using sugar substitutes to replace dietary sugar. Further, they were asked to use pedometers to increase walking by 2000 steps per day. The control group was asked to record diet and physical activity behaviors but not to make changes. Both groups were followed over 6 months. Significantly fewer overweight children in the intervention group increased percent BMI for age as compared to the control group. Similarly, a significantly higher proportion of overweight children in the control group increased percent BMI for age as compared to the intervention group.

Calcium and Weight Management. There is a vigorous debate about the role of calcium, and especially dairy calcium, in weight management. One way that dietary calcium may affect weight is to increase fat oxidation. Dr. Ed Melanson recently evaluated the impact of dairy calcium on fat oxidation in human subjects using a whole room indirect calorimeter. He found that dairy calcium did not affect fat oxidation when subjects were in energy balance, but when they were food-restricted and asked to exercise, dairy calcium did increase fat oxidation. This suggests that dairy calcium may increase fat oxidation during periods of negative energy balance. This work is significant in understanding reasons why calcium, and particularly dairy calcium, affects body weight regulation.

Carbohydrate Balance and Weight Gain. Dr. Robert Eckel and colleagues investigated the effects of dietary composition, insulin sensitivity, and energy balance on predicted changes in body composition. They found that the greater the positive carbohydrate balance—as assessed in a whole room indirect calorimeter, during a single day of positive energy balance—the less body fat gain over the following 4 years. As suggested in rodents, the capacity to expand the glycogen pool might reduce energy intake and protect against fat/weight gain.

Diet Composition and Obesity. Dr. James Hill studied the role of high fat diets in affecting energy intake. Several studies have shown that very high fat diets lead to increased energy intake

as compared to very low fat diets. However, the impact of dietary fat on energy intake across the range of dietary fat typically consumed has not been studied. Dr. Hill assessed energy intake in subjects who were given ad libitum access to food containing 26, 34 or 40 percent of energy from fat. Actual energy intake was assessed in the General Clinical Research Center. He found that total energy intake increased significantly with increasing dietary fat content of foods. His results provide further evidence that the fat content of the diet influences total energy intake. As the fat content of the diet increased across the range of typical western diets, subjects voluntarily consumed more total energy. Further, this occurred independent of energy density and without differences in subjective ratings of fullness, reinforcing the notion that increased dietary fat content of the diet contributes to passive overeating and obesity.

Resistance to Weight Gain. Drs. Marc Cornier and Dan Bessesen have continued to collaborate with the Department of Psychiatry in examining the effects of overfeeding on neuronal activation using fMRI technology. In the initial cohort of subjects, fMRI studies were done in the overnight fasted state after 2 days of eucaloric feeding and 2 days of 30 percent overfeeding. In the eucaloric state, visual stimuli of 'basic' foods as compared to neutral objects resulted in moderate insular, posterior cingulated, and dorsolateral prefrontal cortex activation. Interestingly, stimuli of foods of high hedonic value as compared to utilitarian foods resulted in robust bilateral activation of inferior temporal visual and posterior parietal cortex. When examining the same condition across gender, thin women had significant activation of posterior cingulate cortex as compared to thin men. Overfeeding resulted in complete attenuation of the inferior temporal visual and parietal cortex activation seen in response to the foods of high hedonic value in the eucaloric state. Less robust but still significant inactivation of the hypothalamus in response to foods of high hedonic value was seen with overfeeding, which was primarily driven by changes in thin women. Interestingly, this change in hypothalamic activation with overfeeding was correlated to premeal hunger ratings ($r = 0.56$, $p = 0.005$). In summary, visual stimuli of food items in the fasted state results in activation of insular cortex, a brain region known to be important in various processes related to feeding. Overfeeding results in inhibition of insular activity suggesting that the rewarding properties of food stimuli are diminished in thin individuals when in positive energy balance. Visual stimuli of foods of high hedonic value appear to consistently activate brain regions important in visual processing and attention, inferior temporal visual and posterior parietal cortex. Overfeeding results in complete attenuation of this robust inferior temporal activation, and also results in deactivation of the hypothalamus in response to visual stimuli of foods of high hedonic value, particularly in women, which was associated with a reduction in hunger ratings. These findings suggest an interaction between environmental cues (visual stimuli) and homeostatic regulation of food intake (hypothalamus) in the setting of positive energy balance.

Sympathetic Nervous System and Energy Expenditure. In the past year, Drs. Douglas Seals and Christopher Bell's research has focused on how β-adrenergic receptor (β-AR) modulation of resting and postprandial energy expenditure (EE) is augmented in regularly exercising compared with sedentary adults, but the underlying physiological mechanisms are unknown. Differences in thermogenic responsiveness to β-AR stimulation, perhaps secondary to reactive oxygen species (ROS) bioactivity, may be involved. To determine habitual exercise-related differences in β-AR thermogenic responsiveness and the possible influence of ROS, we measured the percent increase in EE (ΔEE percent—indirect calorimetry, ventilated hood method) above resting EE in response to non-specific β-AR stimulation (intravenous isoproterenol: 6, 12, and 24 ng kg fat-free mass^{-1} min^{-1}) in 25 sedentary (11 males; 51±4 years; body mass index 25.0±0.8 kg m^{-2}, maximal oxygen uptake 29±1 ml kg^{-1} min^{-1} (mean±SE)) and 14 habitually aerobic exercising (9

males, 46±6 years, 23.1±0.7 kg m^{-2}, 44±3 ml kg^{-1} min^{-1}) healthy adults under normal (control) conditions and during acute intravenous administration of a potent antioxidant, ascorbic acid (vitamin C; 0.04 g kg^{-1} fat-free mass). ΔEE percent was greater (*P*=0.02) in the habitual exercising (8.6±1.2, 12.9±1.2, 20.0±1.4) versus sedentary (6.3±0.7, 10.4±0.8, 16.0±1.0) adults. Ascorbic acid increased (*P*=0.01) ΔEE percent only in the sedentary adults (to 9.5±0.9, 12.4±0.7, 18.5±0.8), abolishing baseline group differences. ΔEE percent was not related to body fatness, sex, or any other baseline characteristic. Thermogenic responsiveness to β-AR stimulation is augmented in habitually exercising adults. The mechanism is ascorbic acid-dependent, suggesting that it may be linked to decreased ROS bioactivity. Our findings advance a novel mechanism by which habitual physical activity may modulate EE in humans, with potential implications for energy balance and body weight control.

Prevention of Weight Gain. Drs. Stroebele, Hill, and Wyatt analyzed results from the Colorado Statewide Survey of Walking. This survey was conducted via a telephone interview on a representative sample of 742 adults. Results revealed that the average adult in Colorado reported taking 6804 steps per day. A subsample of 57 children (ages 10-17) of the participating adult population agreed to wear a step counter for 4 consecutive days. The parents reported the child's weight, height, age, gender, and other health-related information. On average, the children reported taking 7902 steps per day. About 47 percent reported taking fewer than 7500 steps per day, and only 18 percent reported taking 10,000 or more steps per day. Results showed that 25 percent of the children were at risk for becoming overweight (using the 85^{th} percentile as a cut point for overweight). The number of steps decreased with increasing age ($p < .01$) and weight ($p < .03$) and there was a trend toward a significant positive association between parents' steps and their child's steps ($p < .06$). Also in these children, a trend toward a significant negative association between hours of watching television or playing video games and steps ($p < .06$) was found. No difference in steps per day for gender was revealed; however, girls tried significantly more often to eat fruits and vegetables each day than boys ($p< .03$). Although this sample of children is too small to be considered a representative sample of Colorado children, it provides an interesting documentation of the physical activity and its relationship not only to the child's lifestyle but also to the lifestyle of his/her parents.

Women's Health.

Menopause and Weight Regulation. Dr. Wendy Kohrt and her collaborators in the IMAGE research group made some important initial steps in 2005 toward the long-term goal of understanding the role of estrogen in the regulation of energy balance and fat distribution in women. In one study, they used gonadotropin releasing hormone antagonist therapy ($GnRH_{ant}$) to suppress estrogen and progesterone levels of premenopausal women for 1 week. Resting metabolic rate (RMR) was assessed before $GnRH_{ant}$, during different phases of the menstrual cycle, and after $GnRH_{ant}$. They found that RMR was significantly reduced by 50-75 kcal/day in response to $GnRH_{ant}$ when compared with measurements during either the early follicular or mid-luteal phases of the menstrual cycle. Although they have not yet isolated whether this is an estrogen- or progesterone-mediated effect, the findings suggest that the withdrawal of sex hormones at the time of the menopause could promote weight gain in postmenopausal women by suppressing metabolism. An R01 proposal to expand on these studies is pending (merit score 155, percentile ranking 9.1).

Children and Adolescents.

TODAY Trial. Dr. Phillip Zeitler has continued his work as Study Chair and Principal Investigator of the Colorado Clinical Center of a national multi-center 7-year trial examining

treatment alternatives in adolescent type 2 diabetes (TODAY). Among other areas of investigation, this trial examines approaches to the promotion of lifestyle change, as well as the effects of type 2 diabetes and its treatment on cardiovascular risk and psychosocial functioning among affected teenagers. In addition, Dr. Zeitler is co-investigator with Richard Hamman in the Department of Preventive Medicine on an NIH-funded study (SEARCH-CC) to examine fitness, cardiovascular, genetic, and dietary risk factors in children with type 2 diabetes, compared to those with type 1 and to normal age and gender matched controls. As an ancillary to this project, Dr. Zeitler, along with other SEARCH-CC investigators, is developing a proposal to rigorously measure insulin resistance in type 1 and type 2 patients in order to develop simple clinical predictors of resistance that can be used in future large studies. Finally, Dr Zeitler and colleagues in the Department of Medicine at the University of Colorado have developed a protocol to study exercise and cardiovascular function in obese insulin-resistant adolescents and adolescents with type 2 diabetes.

Modifying School Lunches. Dr. Nanette Stroebele has conducted research into how to improve the quality of school lunches. The objective of this study was to evaluate students' acceptability of popular school lunch items that were reduced in fat and energy density. Students from four elementary schools within the Denver Public Schools participated in this study. With the help of food manufacturers, healthier versions of three popular foods (pizza, French fries, and chicken fingers) were created. The manufacturers were asked to reduce the fat and energy density of these foods as much as possible while minimally affecting taste and appearance. The original foods and the modified versions were offered to students during lunch. The acceptability of the healthier foods was evaluated by assessing differences in meal participation and in ratings of appearance and palatability. Data from the originally offered foods were compared to the modified foods using univariate and multivariate comparisons. Meal participation did not change after introducing modified foods. Analyses of variance revealed a decrease in both appearance and palatability ratings for the modified pizza ($p \leq .01$). Ratings for French fries only decreased in taste ($p \leq .05$) but not in appearance, whereas the ratings of appearance and palatability for the modified chicken fingers did not differ from the original chicken fingers. Although some ratings for appearance and palatability decreased significantly for the modified foods, decreases were small and the healthier food versions were still rated as palatable and attractive. There is agreement on the need to provide healthier foods in schools. These results suggest that one way to do this is to work with food manufacturers to improve the nutritional value of some of the most popular foods served in schools while maintaining taste. By taking small steps to increase the nutritional value of these foods, the modified versions appear to be completely acceptable to the students. Making small changes to the most popular foods can lead to significant improvements in the overall composition of food consumed at school.

Diabetes.

Exercise and Diabetes. Dr. Patrick Sullivan analyzed results of the Medical Expenditure Panel Survey (MEPS), which is a nationally representative survey of the U.S. population. From 2000 to 2002, detailed information on sociodemographic characteristics and health conditions were collected for 68,500 adults. Normal weight was defined as BMI 18.5 to <25 kg/m^2, overweight as 25 to≤30 kg/m^2, obese (class I and II) as 30 to <40 kg/m^2, and obese (class III) as A≥40 kg/m^2. Physical activity was defined as moderate/vigorous activity ≥30 min ≥3 days per week. The likelihood of having diabetes and diabetes-related cardiovascular comorbidities increased with BMI regardless of physical activity and increased with physical inactivity regardless of BMI. Compared with normal-weight active adults, the multivariate-adjusted odds ratio (OR) for diabetes was 1.52 (95 percent CI 1.25–1.86) for normal-weight inactive adults and 1.65 (1.40–

1.96) for overweight inactive adults; the OR for diabetes and comorbid hypertension was 1.71 (1.32–2.19) for normal-weight inactive adults and 1.84 (1.47–2.32) for overweight inactive adults. These results suggest that physical inactivity and obesity seem to be strongly and independently associated with diabetes and diabetes-related comorbidities. These results support continued research investigating the independent causal nature of these factors.

HIV.

Energy Expenditure and Body Fat Distribution in HIV Patients on Protease Inhibitors. Dr. Lisa Kosmiski has combined her expertise in energy balance and her interest in HIV, collaborating with CNRU researchers Drs. Robert Eckel, James Hill, Gary Grunwald, Elizabeth Stamm, and Ann Scherzinger and with the UCHSC AIDS Clinical Trials Group for her studies. She used the whole-room calorimeter of the Energy Balance Core Laboratory to show that total energy expenditure is increased in HIV and that it seems to be the resting energy expenditure component that is most affected. In subsequent studies, she demonstrated that the percent of body fat present in the trunk of HIV-infected men with the HIV lipodystrophy syndrome was significantly greater compared to both HIV-infected and healthy controls. In addition, the percent of body fat present in the extremities was significantly lower in men with the HIV lipodystrophy syndrome compared to HIV-infected and healthy controls. HIV-infected men *without* clinical evidence of lipodystrophy also had a significantly greater percentage of total body fat in the trunk and a significantly lower percentage of body fat in the extremities compared to healthy controls despite similar body mass index, total body fat, and percent body fat. Among the HIV-infected men only, age was an independent predictor of truncal and extremity adiposity. Dr. Kosmiski's work relies heavily on the techniques available, particularly the whole-room calorimeter, through the energy balance core. She also uses the metabolic core for measurements of insulin action and hormone levels.

Educational Activities/Accomplishments

Professional/Public Nutrition Education Efforts.

Curriculum Revision. The University of Colorado at Denver and Health Sciences Center School of Medicine is undergoing a major curriculum revision. Dan Bessesen, M.D., has been very involved in this process, having served on the Curriculum Oversight Committee. This curriculum revision provides the opportunity to give medical students greater formal training in the evaluation and treatment of overweight and obese patients as well as to continue to ensure that training in other areas of nutrition receive adequate attention. In this new curriculum, the delivery of content will not be through a traditional 'departmentally based' organizational structure (Anatomy, Biochemistry, Physiology, Pharmacology, etc.), but will be organized around themes or organ systems (Cardiovascular, Renal, Pulmonary, Life Cycle, etc.). These 'blocks' will be interdepartmentally based and will attempt to help the student integrate information from a range of disciplines as they learn about a specific topic area. Each block has two co-directors: one basic science director and one clinical director. These two individuals work together to develop and organize the content that will be delivered in the block. Over the last 6 months, Dr. Bessesen has taken the position of clinical co-director of the Metabolism Block. In this block, students will learn basic biochemical pathways, but these traditional biochemical pathways will be integrated into discussions of the regulation of food intake; understanding energy balance; basic nutritional principles, including taking a dietary history; dietary intake guidelines; and the epidemiology and health complications of obesity, as well as comprehensive discussions of available treatment approaches. Carbohydrate metabolism will be taught in the context of insulin resistance and diabetes. Fat metabolism will be taught in the

context of hyperlipidemia and atherosclerosis. Micronutrients, fat, and water-soluble vitamins, and enteral and parenteral nutrition, will also be covered. Small groups, team-based learning, and problem-based learning strategies will be employed. Students will do self-monitoring exercises of their own diet and physical activity and will be taught the principles of effective behavior change counseling strategies. We hope to continue to develop content in these areas that will be delivered during the clinical years of training, including residency training in primary care fields.

The Centers for Obesity Research and Education (C.O.R.E.) consist of eight academic medical schools who have banded together to provide hands-on training to physicians and other healthcare professionals in the area of obesity. C.O.R.E. originated within the Colorado CNRU, and Colorado continues to be the national coordinating center for C.O.R.E. Last year, C.O.R.E. provided obesity training through two national meetings that reached about 500 healthcare professionals and through a series of local workshops reaching another 200 healthcare professionals.

Obesity Management is a new journal aimed at bringing practical information about obesity to healthcare providers. The editorial office for the journal is within the Colorado CNRU. Dr. James O. Hill is the Editor-in-Chief of Obesity Management and Dr. Holly Wyatt is Senior Editor.

Recruitment of Investigators: Dr. Steve Daniels has just accepted the position as Chair of the Department of Pediatrics at the University of Colorado Health Sciences Center. He will be an outstanding addition to the CNRU research base. Additionally, Dr. Ray Browning has joined the CNRU research base as a postdoctoral fellow. He is interested in the energy cost of physical activity in obesity.