

## Current Research of Postdoctoral Training Faculty

The research interests of the training faculty are described in more detail in the paragraphs that follow. Postdoctoral trainees can participate in any area of ongoing research provided the project chosen is adequately funded and can be completed within a two-year period. Most faculty suggest a discrete portion of a larger project that the trainee can pursue as his/her research project.

**Steven A. Abrams, MD** ([sabrams@bcm.tmc.edu](mailto:sabrams@bcm.tmc.edu)). Dr. Abrams conducts studies of iron and zinc bioavailability in children including children in developing countries. These studies are performed to evaluate the sources of these key minerals and to identify interactions between them. Study populations of interest include children and adults of all ages, from premature infants to elderly women with osteoporosis. Studies are performed by administering stable minor isotopes of the elements of interest and following the time course of the isotopes in blood, urine, and stool. Intravenously administered isotopes are used to assess the rate of bone mineral deposition and the secretion of minerals into the gastrointestinal tract. Numerous models of calcium and magnesium kinetics are used to evaluate bone mineralization and resorption processes. The principal analytical tool utilized is a thermal ionization mass spectrometer, a state-of-the-art device capable of highly precise measurements of isotopes isolated from biological samples and one of only a few such instruments available for nutrition research. Current studies focus specifically on growing infants and children in an attempt to evaluate dietary recommendations for intake of calcium, magnesium, zinc and iron.

**Tom Baranowski, PhD** ([tbaranow@bcm.tmc.edu](mailto:tbaranow@bcm.tmc.edu)). Dr. Baranowski and his team have developed, implemented and evaluated school and community-based interventions for enabling students and their families to eat more fruit and vegetables and to be more physically active as obesity preventive and chronic disease protective lifestyle behaviors. The interventions are based on social cognitive theory and employ one or more of classroom curricula, newsletters for parents (with joint parent-child home activities), home videos, point-of-purchase education, classroom interactive multimedia educational games, summer day camps, internet-based programs for girls and their parents, troop meetings, camping and internet based activities for Boy Scouts and changes in school food services. The team collaborated on a project to use the school as a channel to change the diet of African-American parents of fourth graders and has recently been funded to design, implement and evaluate a middle school diabetes prevention program as part of a multi-site behavioral trial. Smaller projects investigate the role of goal setting and problem solving processes in children's dietary and physical activity practices, the influences on home fruit, juice and vegetable availability, and the mechanisms by which families influence children's physical activity.

**Dennis M. Bier, MD** ([dbier@bcm.tmc.edu](mailto:dbier@bcm.tmc.edu)). Dr. Bier's primary research interest is the regulation of interorgan transport of metabolic fuels. This interest encompasses the substrate and hormone regulation of glucose, lipid and protein/amino acid fuels. Under this rubric, his work has extended in two principal directions. The first entails regulation of endogenous fuel availability for metabolic functions when a subject is ill and incapable of ingesting adequate quantities of food. The second involves assessment of the metabolic fates of ingested exogenous fuels under various classical nutritional circumstances. Research employs a wide variety of stable-isotope tracer kinetic methods to quantify substrate flux, metabolism, precursor-product relationships, and irreversible oxidation of excreted end products. The physiological information thus obtained has also been used to further assess aberrations in interorgan fuel transport consequent to a wide variety of pathological conditions. Dr. Bier's work has provided data on the physiologic and pathophysiologic regulation of metabolic fuel transport of body protein, fat and carbohydrate in a wide variety of circumstances spanning a spectrum of ages from extreme prematurity to the elderly.

**Douglas G. Burrin, PhD** ([dburrin@bcm.tmc.edu](mailto:dburrin@bcm.tmc.edu)). The objective of Dr. Burrin's research is to elucidate the cellular and hormonal signals that mediate the stimulatory effects of enteral nutrition on the growth and function of the neonatal intestine. Previous studies have shown that macronutrients produce a greater trophic stimulus in the neonatal intestine than growth factors such as IGF-I ingested in breast milk. Current studies focus on establishing the optimum quantity and composition of enteral nutrients necessary to maintain normal intestinal growth and function.

Additional studies investigate the physiological significance of the gut-derived peptide, glucagon-like peptide-2 (GLP-2), and how it impacts intestinal protein and amino acid metabolism in the neonatal pig. The studies utilize isotopic tracers coupled with arteriovenous organ balance measurements to quantify the intestinal absorption and metabolism of macronutrients provided either enterally or parenterally. Using this approach, he plans to determine how the catabolic effects of dexamethasone compromise intestinal absorptive function and how provision of either minimal enteral nutrition or GLP-2 ameliorates the actions of dexamethasone. These studies also will determine how these nutritional and hormonal factors modulate rates of cellular protein turnover, proliferation and programmed cell death. How these factors affect the expression and activity of key intermediates in these cellular pathways also will be investigated.

**Nancy F. Butte, PhD** ([nbutte@bcm.tmc.edu](mailto:nbutte@bcm.tmc.edu)). The overall goal of Dr. Butte's current research is to identify environmental and genetic determinants of childhood obesity in "at risk" children. To do so, 500 obese Hispanic children and their biological parents and siblings will be identified and phenotyped. In addition, a systematic genomic scan will be performed to localize quantitative trait loci that influence quantitative variation in body composition, energy expenditure, physical activity, food intake and/or eating behavior.

Dr. Butte's previous research has played a major role in defining energy requirements of pregnant and lactating women and their infants with respect to pregnancy outcome, lactation performance as well as infant growth and development. The mechanisms underlying energy adaptations during pregnancy, lactation and infancy are now under investigation. To achieve these research goals, methodologies have been developed to measure energy expenditure and body composition in the populations of interest; these include room and portable respiration calorimeters for use in adults and infants, the doubly labeled water method for the measurement of free-living total energy expenditure and several devices for measuring body composition.

**Lawrence Chan, MD** ([lchan@bcm.tmc.edu](mailto:lchan@bcm.tmc.edu)). Dr. Chan's major research area concerns the molecular regulation of lipid homeostasis. Over the last twenty years, his laboratory has applied molecular biology techniques to the structure and regulation of various genes related to lipid homeostasis, including the genes for the major apolipoproteins and lipolytic enzymes. The effects of lipid nutrients on the expression of these genes have also been a major focus. Current projects concern the interaction of various lipid-modifying genes on plasma lipoprotein metabolism and on atherogenesis in transgenic mouse models. His trainees have created gene knockout mice that produce only apoB-100 but no apoB-48. His laboratory is now studying the apoB-100 only animals in the presence and absence of cholesteryl ester transfer protein (CETP), apoE, and LDL receptor expression. Other areas currently under exploration concern the role of adipocyte-specific gene expression on obesity and weight control and the mechanism of the abnormal lipid metabolism in diabetic animal models.

**Karen W. Cullen, DrPH, RD** ([kcullen@bcm.tmc.edu](mailto:kcullen@bcm.tmc.edu)). Dr. Cullen's research focuses on the prevention of diet-related chronic diseases through the development, implementation, and evaluation of nutrition behavior change programs for children and adolescents. Of particular interest are programs utilizing unique delivery channels to improve children's fruit and vegetable consumption. Current projects include implementing and evaluating an environmental behavior change program for middle school cafeterias and a *la carte* snack bars that includes social marketing within the cafeteria environment, developing and implementing a school-based program for the prevention of type 2 diabetes among youth, and a feasibility study of an internet-based dietary behavior change program for African-American families.

**Teresa A. Davis, PhD** ([tdavis@bcm.tmc.edu](mailto:tdavis@bcm.tmc.edu)). Dr. Davis' research focuses on identifying the mechanisms by which nutrients, hormones, and growth factors regulate protein deposition during growth and development, particularly in skeletal muscle. Early studies demonstrated that the high rate of protein deposition in the neonate is due to the elevated response of protein synthesis to nutrient intake, which is particularly profound in skeletal muscle. Using novel hormone-substrate clamps, the feeding-induced stimulation of skeletal muscle protein synthesis has been shown to involve independent regulation by both

insulin and amino acids whereas that in other tissues is mediated by either amino acids or insulin alone. Recent studies have shown that the high rate of muscle protein synthesis in the neonate is regulated by the expression and activity of components of the intracellular signaling pathway that controls translation initiation. The role of nutrient intake, insulin, growth hormone, and insulin-like growth factor I in the regulation of protein degradation, amino acid oxidation and protein synthesis during growth and development is also being explored. Other research areas currently being investigated include the regulation of protein synthesis during catabolic illness such as sepsis.

**Kenneth J. Ellis, PhD** ([kellis@bcm.tmc.edu](mailto:kellis@bcm.tmc.edu)). The objective of Dr. Ellis' research is to establish body composition references for the normal biological diversity of growth in different ethnic groups. As part of this research, mathematical models are being developed to describe the temporal and maturational changes in body composition. Contemporary reference models for the fetus, infant, child and adolescent are being developed. The measurements utilized are state-of-the-art technologies (<sup>40</sup>K counting, deuterium and bromine dilution, neutron activation analysis, dual-energy x-ray absorptiometry, bioelectrical impedance, body density assessment) that have been or are being expanded for use in children and infants.

**Marta L. Fiorotto, PhD** ([martaf@bcm.tmc.edu](mailto:martaf@bcm.tmc.edu)). The fundamental objective of Dr. Fiorotto's research is to identify the in vivo mechanism(s) that confer to the immature muscle the ability to drive protein synthesis at an extremely high rate. Ongoing studies focus on the regulation of mRNA expression, which may be a limiting factor for protein synthesis in the newly differentiated myotube, and on the role of locally produced insulin-like growth factors, which appear to exert their effect primarily on satellite cell division. As muscle maturation proceeds, ribosomal abundance becomes the limiting factor for protein synthesis; hence, the interrelationship between the regulation of satellite cell replication and ribosomal RNA transcription (the limiting step for ribosomal production) will be examined. A variety of cell and molecular biology techniques in conjunction with in vivo tracer techniques in infant animal models, including transgenic mice, the rat and the pig, are utilized to understand how transcriptional and translational events contribute to the observed outcomes.

A second research interest concerns the long-term functional consequences of impaired muscle growth in early life. One consequence of the developmentally-linked regulation of the anabolic processes in the muscle is that impairment of muscle growth during this phase of rapid growth (as occur when there is an inadequate supply of nutrients) chronically compromises muscle mass. Thus, studies to determine whether the development of sarcopenia and its sequelae, including obesity and impaired glucose tolerance, are exacerbated when muscle growth is compromised in early life are underway. These include determining if a muscle-specific, plasmid-based growth hormone-releasing hormone gene therapy that we have developed can be used to reverse or mitigate the muscle loss.

**Jennifer O. Fisher, PhD** ([jfisher@bcm.tmc.edu](mailto:jfisher@bcm.tmc.edu)). Dr. Fisher investigates the development of food preferences and the controls of food intake during infancy and early childhood. The broad goal of her research is to understand how early eating environments modify young children's eating behavior and health outcomes. Of particular interest is the parents' role in selecting foods of the family diet, in serving as models of eating behavior, and in making child feeding decisions that affect child food preferences, selection, and intake patterns. Dr. Fisher has recently demonstrated that large portion sizes promote overeating at meals among pre-school aged children. Her research has also shown that restrictive feeding practices may favor the development of childhood overweight by focusing children's attention on restricted foods and promoting intake of these foods in the absence of hunger. She is currently investigating the influence of feeding practices and family eating styles on problematic food intake regulation and overweight among Hispanic children and their siblings. Additional research evaluates the role of maternal feeding practices on food preferences, regulation of food intake, and development of overweight during infancy.

**Michael A. Grusak, PhD** ([mgrusak@bcm.tmc.edu](mailto:mgrusak@bcm.tmc.edu)). Dr. Grusak conducts both plant physiology and human nutrition research. The plant physiology effort focuses on identifying the mechanisms and regulation of short-distance membrane transport and/or long-distance translocation of organic and inorganic nutrients within and throughout plants. Of major interest is whole-plant micronutrient metal nutrition and the interrelationship of shoot and root factors that regulate expression in roots of genes relevant to metal acquisition. Emphasis also is focused on seed developmental biology, using the model legume, *Medicago truncatula*, as a tool to understand how mineral nutrients are transported to and stored within seeds. Long-term goals are to characterize the dynamics of nutrient flow within plants, thereby determining the biophysical/molecular signals that regulate source-to-sink nutrient partitioning and to use these data to enhance the nutritional quality of plant foods. Methodologies utilized range from whole-plant nutrient partitioning analysis to membrane flux analysis using radioisotopes to gene discovery and expression analysis using various molecular techniques.

The human nutrition effort involves the design and construction of controlled environment systems for growing and labeling plants with stable isotopes. Protocols have been developed to generate safe, traceable plant foods and bioproducts for human consumption. The labeled foods, in turn, are used to address questions about absorption and utilization of specific nutrients from plant food products. Current emphasis is on the bioavailability of carotenoids and phylloquinone as well as several mineral nutrients from various vegetable sources.

**Darryl L. Hadsell, PhD** ([dhadsell@bcm.tmc.edu](mailto:dhadsell@bcm.tmc.edu)). Dr. Hadsell uses state-of-the-art immunohistochemical and molecular biological techniques to analyze the relationships between signaling pathways and in-vivo developmental processes

that occur in the lactating mammary gland. Transgenic and knockout mouse models are used to determine the impact of perturbing specific signaling pathways on regulation of postpartum mammary cell turnover. Research concerning IGF-I action suggests that variation in signaling pathway use by the IGF-I receptor (Igf1r) occurs at different developmental stages. During lactation, IGF-I relies on cell survival pathways to inhibit mammary cell apoptosis but, during earlier stages of development, IGF-I action occurs through proliferative pathways. Crosses between Igf1r knockout mice and transgenic mice which overexpress downstream signaling proteins are used to analyze in vivo mechanisms of IGF-I action during early mammary gland development. The impact of overexpression of IGF-I and of the downstream Igf1r signaling proteins on lactation and mammary cell apoptosis is being analyzed both during normal lactation and during prolonged lactation. Other studies focus on determining the importance of *myc/max/mad* family proteins in the regulation of early postpartum mammary cell proliferation.

**Peter M. Haney, MD, PhD** ([phaney@bcm.tmc.edu](mailto:phaney@bcm.tmc.edu)). The long-term goal of Dr. Haney's research is to understand the molecular and cell biology of lactation. Current work focuses on glucose transport in lactating mammary gland. Glucose is the key substrate for the synthesis of lactose, the major osmotic constituent of human milk and, hence, the major determinant of the volume of milk produced. Glucose also is a key substrate for the synthesis of lipid, the major determinant of the energy content of human milk. Current studies focus on regulation of the amount, activity, and subcellular targeting of GLUT1, the only glucose transporter isoform identified in mammary gland. Established and primary mammary epithelial cell lines as well as humans and rodents are utilized in the studies. Efforts are underway to elucidate the mechanisms of previously observed alterations of glucose transporter targeting during lactation, including sequestration in Golgi-related vesicles and polarization of plasma membrane distribution, and to understand the impact of GLUT1 retargeting and of altered GLUT1 gene expression on lactose synthesis and milk production.

**Morey W. Haymond, MD** ([mhaymond@bcm.tmc.edu](mailto:mhaymond@bcm.tmc.edu)). Dr. Haymond's research focuses on the substrate and hormonal regulation of glucose homeostasis in children and adults. In collaboration with Dr. Agneta Sunehag, all of the stable isotopic techniques needed for measuring gluconeogenesis in humans have been developed. Dr. Haymond is exploring the regulation of glucose homeostasis in lactating women and has determined that lactation imposes a 30% increase in demand on the fasting lactating woman and that the human breast is capable of *de novo* synthesis of both glucose and galactose in the formation of lactose. This contribution of hexoneogenesis contributes nearly 50% of milk lactose production during fasting but, in the fed state, nearly 70 % of lactose is derived from the plasma glucose of the mother.

Dr. Haymond and collaborators also have investigated the impact of dietary fat, carbohydrate and fructose content on nutrient oxidation, insulin sensitivity and glucose homeostasis in normal children and are extending these studies to

obese children. Ongoing studies aim to determine the metabolic fate of galactose and fructose as well as their effects on hepatic glucose production and gluconeogenesis. The ultimate goal of this research is to improve care and management of children with hypoglycemia as well as those with both type 1 and type 2 diabetes.

**William C. Heird, MD** ([wheird@bcm.tmc.edu](mailto:wheird@bcm.tmc.edu)). Dr. Heird's research concerns the needs for specific nutrients during infancy. His previous research concerning fatty acid needs has helped establish that both term and preterm infants can convert the 18-carbon precursors of  $\omega$ 6 and  $\omega$ 3 fatty acids to longer chain, more unsaturated fatty acids and has helped clarify the metabolic steps involved. Current studies in this area concern the mechanisms of incorporation of these fatty acids into brain vs. other tissues, including the role of tissue-specific expression of  $\Delta$ -6 and  $\Delta$ -5 desaturases.

Current research concerning protein needs of low birth weight (LBW) infants addresses the hypothesis that there is a finite period during early infancy during which the infant can optimally use protein for growth and that failure to provide sufficient protein intake during this period contributes to the inadequate growth of these infants. This research also will define this finite period and determine if providing a higher protein intake during early infancy improves growth and neurodevelopmental outcome of these infants. Collaborators in this area of research include members of the Meyer Center for Developmental Pediatrics.

All areas of research utilize state-of-the-art stable isotope and mass spectroscopy techniques as well as standard anthropometry and nutrient balance techniques. Some also utilize measurements of energy expenditure and body composition as well as cellular and molecular biology techniques.

**Susan J. Henning, PhD** ([shenning@bcm.tmc.edu](mailto:shenning@bcm.tmc.edu)). The major goal of Dr. Henning's research is to understand the factors that control maturation of the epithelium of the small intestine. This is a clinically relevant problem, because immaturity of the intestine accounts for the high susceptibility of infants to diarrhea and for feeding intolerance in preterm infants. Previous research, using rodent models, has shown that there are two distinct regulatory pathways for intestinal maturation: *a*) a timing mechanism intrinsic to the intestinal tissue; *b*) a pathway elicited by glucocorticoid hormones which is synergistically enhanced by a second hormone, thyroxine. Current research goals are to determine whether the initiating events of each pathway occur in the epithelial cells or in the underlying mesenchymal cells and to elucidate the regulatory genes which mediate each pathway. Although prior work focused largely on sucrase-isomaltase as a marker of maturation, current efforts focus on a new marker, trehalase, a neglected member of the disaccharidase family. Its importance lies in current efforts of the food industry to use its substrate, trehalose, as an alternative sweetener. Recent research has cloned and mapped the gene for murine trehalase and has identified the human gene. Current studies are assessing the extent to which developmental and glucocorticoid –induced changes of trehalase expression reflect transcriptional activation. Future studies

will attempt to identify the transcription factors involved as a first step to dissecting the complete regulatory pathway.

An additional project is designed to identify and characterize intestinal stem cells. Although the presence of these cells has been inferred for many years, they have never been isolated. A novel sorting approach will be deployed to isolate a population of putative intestinal stem cells, together with a graft model to assess their capacity for proliferation and differentiation. If successful, such approaches should have two applications: a) new therapies for various conditions in which the bowel is damaged; b) use of the intestine as a site for gene therapy.

**Karen K. Hirschi, PhD** ([khirschi@bcm.tmc.edu](mailto:khirschi@bcm.tmc.edu)). The primary purpose of Dr. Hirschi's research is to understand, at the cellular and molecular level, the events leading to blood vessel formation. She is interested in elucidating regulators of vascular cell (endothelial and smooth muscle) recruitment, proliferation and differentiation needed for blood vessel assembly and maintenance. Mechanisms by which soluble effectors such as retinoids and TGF- $\beta$  as well as cell-cell junctional components such as gap junctions modulate vascular cell phenotype and cell cycle progression will be defined. *In vitro* co-culture systems are used to study interactions between vascular cells and their precursors and *in vivo* regulation of blood vessel assembly is studied in murine embryo culture and transgenic mouse models.

Another research focus concerns the potential of adult stem cells to contribute to neovascularization in response to tissue injury and growth. Bone marrow transplantation and localized delivery techniques are used to study mechanisms by which adult stem cells are recruited, induced to differentiate into vascular cells, and functionally integrated into existing vascular networks.

Insights gained from these cell and developmental studies are applicable to the optimization of clinically relevant treatments including autologous vascular cell and gene therapy, assembly of blood vessel grafts, and vascularization of engineered tissues.

**Kendal D. Hirschi, PhD** ([kendalh@bcm.tmc.edu](mailto:kendalh@bcm.tmc.edu)). The long-term goal of Dr. Hirschi's research is to understand the components of ion homeostasis and calcium signal transduction in plants and to assess the role of these genes in plant growth and adaptation. Using a combination of approaches, he is attempting to characterize the expression and physiological function of calcium transporters and will use these ion transporters as "bait" in a series of genetic approaches in yeast and plants to identify the molecules that interact with these transporters and, thus, regulate ion homeostasis. The ensemble of ion transporters and their regulatory molecules have been characterized and identified, thus allowing ion storage, signal transduction events, and the environmental constraints of traditional agricultural practices to be manipulated.

**Farook Jahoor, PhD** ([fjahoor@bcm.tmc.edu](mailto:fjahoor@bcm.tmc.edu)). Dr. Jahoor's research interests include nutritional requirements during growth and development and in different pathological states, interrelationships between protein and energy (glucose and

fat) metabolism in normal and pathological states, *in vivo* protein, amino acid(s), urea, glucose and lipid metabolism, and the development of stable isotope tracer methodologies to study *in vivo* substrate metabolism. Current efforts include development and use of stable isotope tracer methodologies to investigate the *in vivo* metabolism of proteins, amino acids, urea, glucose and lipids in both animals and humans. An area of primary interest is the impact of the metabolic response to the stress of infection and injury on nutritional requirements during early growth and development. Studies are being performed in both animals and humans to determine how stress alters protein and energy metabolism and the role of stress in the precipitation of severe protein-energy malnutrition. Other studies concern the effect of surgical trauma on protein and amino acid metabolism of premature neonates. Special emphasis is placed on the role of stress-induced alterations in the hormonal milieu as one of the major mediators of the deranged metabolic response to infections and injury. Stress-induced changes in the partitioning of nitrogen for synthesis of muscle proteins, acute phase proteins, and nutrient transport proteins also are being investigated.

**Heidi E. Karpen, MD** ([hkarpen@bcm.tmc.edu](mailto:hkarpen@bcm.tmc.edu)). Dr. Karpen's research focuses on mechanisms for regulation of the Sonic Hedgehog signaling pathway which is fundamental for early embryonic patterning of nearly every organ system of the developing fetus and is conserved from *Drosophila* to humans. Her lab has focused primarily on regulation of the Hedgehog receptor complex, comprised of the two large transmembrane proteins, Patched and Smoothed. Mutation analysis has revealed several domains of each of these proteins that are important for appropriate receptor complex formation, receptor trafficking and function. Current efforts are directed at studying these domains in relation to the binding of the Sonic Hedgehog ligand and signal transduction in the pathway. Work over the past two years has shown a link between Patched, cholesterol and the structural protein, caveolin-1. Recently published data confirm that Patched and caveolin-1 interact directly via a short, highly conserved amino acid motif found in each protein and that caveolin-1 is involved in the transport of Patched to lipid rafts on the membrane. Cholesterol, a key component of lipid rafts, seems to play an integral role in transport of the Hedgehog receptor to these specialized membrane microdomains and ongoing studies focus on defining its role in receptor function and signal transduction in this pathway.

**Saul J. Karpen, MD, PhD** ([skarpen@bcm.tmc.edu](mailto:skarpen@bcm.tmc.edu)). Dr. Karpen is actively pursuing two major research interests. The first concerns molecular regulation of hepatobiliary transporter genes. Over the past few years, it has become apparent that a wide variety of endogenous and exogenous substances are transported into and out of the hepatocyte via specific transporters. Thus, research has focused on understanding the nuclear factors that regulate the expression of these transport genes. A particular interest is the role of regulable factors such as nuclear receptors. The aim of this research is to enhance understanding of normal hepatobiliary physiology as well as the adaptive response to cholestasis, a condition in which elevated concentrations of

intracellular bile acids can cause and exacerbate ongoing liver damage. Recent research exploring how the hepatocyte responds to cholestasis has shown that bile acids themselves act as gene regulators.

The second research interest concerns extracellular ATP activation of hepatocyte proliferation. Extracellular ATP activates specific cell surface receptors that transduce a variety of intracellular signals, one of which appears to be events linked to hepatocyte proliferation. Research in this area focuses upon exploring signal transduction and nuclear regulatory pathways that may represent a novel fundamental mechanism for the regulation of gene expression and growth in the liver.

**Gerard Karsenty, PhD** ([karsenty@bcm.tmc.edu](mailto:karsenty@bcm.tmc.edu)). Dr. Karsenty is interested in two different areas of skeleton biology. The first concerns elucidation of the transcriptional mechanisms controlling cell differentiation in chondrocyte and osteoblast lineages. Through a molecular biology approach, a gene that controls the entire cascade of osteoblast differentiation has been identified in mice and humans. This gene, *Cbfa1*, is a mammalian homologue of a drosophila gene *runt*. It not only controls osteoblast differentiation throughout the skeleton but also controls chondrocyte differentiation in some parts of the skeleton. To date *Cbfa1* is the only factor known to control differentiation of both osteoblasts and chondrocytes, two cell types long thought to have a common progenitor. More recent efforts have shown that *Cbfa1* is required for osteoblast function, namely bone formation after birth. Thus, *Cbfa1* is both a determination factor during development and a regulator of skeleton physiology postnatally. Current efforts focus on a downstream gene of *Cbfa1* which also encodes a transcription factor. This project relies on genetic and biochemical approaches.

Despite the central role that *Cbfa1* plays during skeletogenesis, there is a five-day delay between the initiation of *Cbfa1* expression at E10 during mouse development and the appearance of the first osteoblast at E15. This could be explained by two different mechanisms; *Cbfa1* either initiates the expression of an activator of cell differentiation and/or *Cbfa1* function is inhibited by another protein. A second osteoblast-specific transcription factor has recently been identified and knock-out of this factor results in a major decrease in the number of osteoblasts per bone. Current efforts are aimed at identifying the mode of action of this factor and its relation to *Cbfa1*. A transcriptional inhibitor of *Cbfa1* called *iCbfa1* also has been identified. Its deletion corrects the loss-of-osteoblast phenotype of the *Cbfa1*-deficient mice. Thus, other experiments are aimed at identifying the mode of action of this factor.

More recently, largely by serendipity, a *Cbfa1*-dependent pathway to control osteoblast proliferation and function was uncovered by observation of a decrease in bone formation in mice and humans in which the gene encoding *Lrp5* is inactivated. *Lrp5* is a receptor for the Wnt proteins and the low bone mass phenotype of the *Lrp5*-deficient mice suggest that Wnt proteins are involved in osteogenesis. This new line of research is developing through the use of molecular biology and genetic tools.

The second major area of study focuses on the physiology of the skeleton, specifically the genes and the genetic pathways controlling bone remodeling and bone mineralization. This line of research stems from the belief that the combination of molecular biology and mouse genetics provides sophisticated tools that allow the study of physiology and pathophysiology of degenerative diseases. Using two mutant mouse strains, leptin has been determined to be a potent inhibitor of bone formation through a hypothalamic relay. The inhibitory action of leptin is so powerful that mice deficient in leptin or its receptor maintain a high bone mass even when gonadectomized (gonadal failure is the most frequent cause of bone loss or osteoporosis in mammals). Currently, a genetic approach is being used to identify the mediators of leptin in bone remodeling. Additional studies are exploring whether this novel hypothalamic regulation of physiology can be used to establish new therapeutic approaches for bone loss diseases. Finally, the role of other hormones acting through the hypothalamic regulatory loop is being explored.

Another project of molecular physiology explores the postulate that absence of mineralization in all tissues is an active physiologic function whereas bone mineralization is a passive process or a default pathway. The function of four different genes is currently being studied to establish this concept and to establish a genetic cascade to explain the absence or presence of mineralization in any tissue.

**David D. Moore, PhD** ([moore@bcm.tmc.edu](mailto:moore@bcm.tmc.edu)). The major goal of Dr. Moore's research is to understand the functions of the newer members of the nuclear hormone superfamily. Current efforts focus on three that have emerged as key regulators of metabolic pathways in the liver: CAR, FXR and SHP. He and colleagues have found that CAR functions to regulate the response of the liver to potentially toxic foreign compounds, such as drugs and environmental pollutants, collectively termed xenobiotics. Activation of CAR by specific xenobiotic stimuli results in an increased ability of the liver to metabolize and eliminate such compounds. CAR also is activated by an endogenous toxic product, bilirubin, and this activation also results in an increased rate of bilirubin metabolism and clearance. Although these CAR-dependent responses are generally protective, CAR activation also can be deleterious. For example, activation of CAR by very high doses of acetaminophen increases production of a toxic acetaminophen metabolite with severe liver toxicity and blocking CAR activity prevents the hepatotoxic effects of an acetaminophen overdose. The recent findings that chronic activation of CAR by a class of compounds called non-genotoxic carcinogens results in liver tumors suggests that this hepatocarcinogenesis is a consequence of direct effects of CAR on both hepatocyte proliferation and apoptosis; thus, current studies are exploring the molecular mechanisms for these effects.

FXR is a recently identified receptor for bile acids, which are downstream metabolites of cholesterol produced in the liver. Although they were previously thought of mainly as detergents to dissolve dietary lipids, they now appear to be important regulators of lipid homeostasis as well. Activation of FXR by high levels

of bile acids induces expression of SHP, an unusual orphan receptor that lacks a DNA binding domain. SHP acts to repress transcriptional activation by several other nuclear receptors, resulting in decreased expression of key metabolic target genes. Since one of these is the rate limiting enzyme for bile acid production, this FXR/SHP pathway accounts for the negative feedback regulation of bile acid biosynthesis. The FXR/SHP

Pathway also mediates beneficial effects of bile acids on triglyceride levels by decreasing expression of SREBP-1c, a transcription factor that promotes expression of a variety of lipogenic enzymes. FXR also regulates the expression of a number of other proteins involved in cholesterol and bile acid homeostasis. Prompted by this central regulatory function of FXR and its ability to respond to a wide range of bile acids and other ligands, we screened a number of compounds that alter cholesterol levels by unknown mechanisms for effects on FXR. This led to the identification of guggulsterone, a plant derived steroid that lowers LDL cholesterol by antagonizing FXR. The biochemical basis for the cholesterol lowering effects of guggulsterone are now being studied. Pharmacologic and mouse knockout approaches are used to define the metabolic regulatory functions of the nuclear hormone receptors.

**Paul A. Nakata, PhD** ([pnakata@bcm.tmc.edu](mailto:pnakata@bcm.tmc.edu)). Dr. Nakata's primary research interest concerns the mechanisms regulating nutrient partitioning in plants and the manipulation of these mechanisms for nutritional improvement of plant foods. His current research focuses on understanding the mechanisms regulating calcium partitioning and sequestration within plants. This research employs an integrated biochemical, cellular, molecular, and genetic approach to identify and characterize the components regulating calcium transport and storage. Such studies should lead to the rational design of strategies to enhance calcium abundance and bioavailability in plant foods.

**Buford L. Nichols, MD** ([bnichols@bcm.tmc.edu](mailto:bnichols@bcm.tmc.edu)). Dr. Nichols' research has focused upon the adaptive alterations of physiologic function in malnourished infants. In recent years, this work has been directed toward the enteric mucosal adaptations of severely malnourished infants with a specific focus on brush border hydrolases necessary for carbohydrate digestion. Extending this investigation to the molecular level, the mucosal enzyme glucoamylase cDNA as well as the entire gene have been cloned and sequenced. Structurally, the glucoamylase gene is very similar to that of sucrase, both of which are needed to digest starches. Recent studies include investigations of the transcription and translation of these brush border enzyme genes in children with chronic abdominal pain. Research conducted in children with chronic abdominal pain, utilizing tissues obtained by endoscopic biopsy, showed that 26% had low glucoamylase activities. A mouse knockout model of glucoamylase deficiency has been developed and the physiologic deficits caused by this are currently under study. Glucoamylase and sucrase are regulated during development and in response to changes in the level of starch in the diet. The present focus is on

understanding the molecular mechanisms by which these two related genes are co-regulated.

**Theresa A. Nicklas, DrPH, LN** ([tnicklas@bcm.tmc.edu](mailto:tnicklas@bcm.tmc.edu)). Dr. Nicklas's research interests encompass both the epidemiological and interventional aspects of chronic disease prevention and health promotion. Two general questions of interest include: How do eating behaviors and other lifestyles influence the development of chronic disease risk factors early in life? What are the behavioral factors influencing the development of adverse lifestyles early in life? These questions must be answered before effective interventions to prevent chronic disease risk can be developed. Specific areas of interest include: 1) environmental factors influencing the development of eating patterns early in childhood; 2) how these eating patterns relate to the onset of obesity, cardiovascular disease, cancer and type 2 diabetes; 3) effective intervention strategies for changing and maintaining healthful behaviors, particularly in children and adolescents. Dr. Nicklas's work has provided data on the eating habits and dietary intakes of children as it relates to chronic diseases over a span of 25 years. These data have provided the rationale and foundation for early intervention programs, national dietary guidelines, and public policy. In addition to epidemiologic investigations, she has been involved in the design and evaluation of nutrition intervention programs for children, adolescents, and families.

**Monique Rijkels, PhD** ([rijnel@bcm.tmc.edu](mailto:rijnel@bcm.tmc.edu)). Dr. Rijkels' primary research goal is to understand the regulation of the casein genes and the genomic domain in which these genes reside. Caseins constitute the major nutritional proteins in milk and supply basic amino acids, calcium, phosphates and bioactive peptides (*e.g.* anti-microbial and opioid). Genomic approaches are used to study the transcriptional regulation of the casein gene cluster region. This combines three lines of research: The study of the chromatin structure and remodeling in the casein gene cluster region related to tissue type and development, using Chromatin Immuno Precipitation (ChIP) assays; Computational approaches to identify evolutionarily conserved regions with potential regulatory function and the trans-acting factors that might bind them; The functional analysis of potential regulatory regions in transgenic mice.

As the casein-cluster region harbors a number of non-casein genes that potentially share functional properties as well as spatial expression patterns and evolutionary ancestry, we also study these genes.

**Jeffrey M. Rosen, PhD** ([jrosen@bcm.tmc.edu](mailto:jrosen@bcm.tmc.edu)). Dr. Rosen's research objectives are to elucidate the mechanisms regulating the normal development of the mammary gland, including the hormonal control of milk protein gene expression, and to determine how these regulatory mechanisms have deviated in breast cancer. In the mouse mammary gland, there are several critical periods of development: Ductal proliferation and branching that occur during sexual maturity; Lobuloalveolar proliferation that occurs during pregnancy; Terminal

differentiation and lactation; Involution, characterized by increased apoptosis and extensive tissue remodeling. Dr. Rosen is studying the role of systemic hormones (*i.e.*, prolactin, glucocorticoids, estrogens, progestins) and local growth factors, including members of the Wnt and Fgf families, on these processes. The role of specific transcription factors and their dominant-negative isoforms, including members of the C/EBP, Stat and NF- $\kappa$ B families, are also being examined using transgenic and knockout mouse models. Gene arrays and subtractive hybridization techniques are being employed to identify downstream targets of these transcription factors. Methods that permit the analysis of both gain and loss of specific gene function in the mammary gland also have been developed. Studies in mouse models have been translated to the analysis of alterations in the levels of these factors in breast cancer biopsies. Finally, transgenic mouse models that mimic highly aneuploid human breast cancers have been developed using a gain-of-function p53 mutant. Studies using these models to define the mechanisms responsible for genomic instability are in progress.

**Robert J. Shulman, MD** ([rshulman@bcm.tmc.edu](mailto:rshulman@bcm.tmc.edu)). Dr. Shulman's primary research interest concerns the development and adaptation of the gastrointestinal tract of the premature infant. A specific interest is the maturation of carbohydrate digestion and absorption mechanisms. Current goals are to develop strategies that will maximize the function of the immature or impaired gastrointestinal tract (*e.g.*, short bowel syndrome) and therapeutic treatments that will enhance gastrointestinal tract development and function. Studies currently being conducted in premature infants focus on: 1)The interaction between digestion and absorption of carbohydrates and other nutrients; 2)The ontogeny of carbohydrate digestion and absorption in terms of hydrolysis and/or transport; 3)The relationship between digestive and absorptive function and the mechanisms presumably regulating these functions (*e.g.*, glucose transport and transporter mRNA); 4)The factors that contribute to feeding intolerance; 5)Hormonal influences on the growth and adaptation of the gastrointestinal tract.

**C. Wayne Smith, MD, PhD** ([cwsmith@bcm.tmc.edu](mailto:cwsmith@bcm.tmc.edu)). Dr. Smith's research focuses on the mechanisms of neutrophil transendothelial migration – specifically the mechanisms of leukocyte activation and adhesion and the phenotypic changes neutrophils undergo following transmigration. Ongoing studies have demonstrated significant kinetic distinctions between LFA-1 and Mac-1 following activation by CXC chemokines, a role for PI3Kinase in the activation of LFA-1 but not Mac-1, and markedly increased synthesis of oncostatin M by transmigrated neutrophils. In addition, microarray techniques have been used to indicate significant new gene expression in neutrophils following stimulation with IL-15, a cytokine expressed by inflamed endothelial cells. Finally, studies to confirm the kinetics of expression of several of the intriguing genes identified by array analysis are underway.

**E. O'Brian Smith, PhD** ([esmith@bcm.tmc.edu](mailto:esmith@bcm.tmc.edu)). Dr. Smith provides statistical support to CNRC investigators and postdoctoral fellows. He is available to assist investigators and postdoctoral fellows in the biostatistical aspects of research including formulation of the research question, appropriate study design, sample size estimation, data collection and management, data analysis, computer applications, preparation of research proposals and manuscript preparation. He also provides a series of didactic lectures on research design, analysis and methods of data analysis as part of the required course, "Fundamentals of Clinical Research".

**Agneta Sunehag, MD, PhD** ([asunehag@bcm.tmc.edu](mailto:asunehag@bcm.tmc.edu)). Dr. Sunehag's primary research interest concerns glucose metabolism in children. A longstanding research focus has been glucose homeostasis of prematurely born infants during their first days of life. Currently, she is investigating how these immature infants utilize parenteral nutrients to produce glucose via the gluconeogenic pathway. The ultimate goal of this research is to define a mixture of parenteral nutrients that both maintains normoglycemia and promotes normal growth. A second research focus concerns the effects of dietary macronutrient content and exercise on parameters of glucose metabolism (glucose production, gluconeogenesis and insulin sensitivity) in non-obese and obese pre-pubertal children and adolescents. She also is involved in studies of glucose homeostasis in lactating mothers and the metabolism of galactose and fructose. Dr Sunehag utilizes compounds labeled with stable isotopes and gas chromatography – mass spectrometry to measure the dynamic parameters of glucose metabolism and is interested in resolving methodological issues regarding these techniques.

**Qiang Tong, PhD** ([qtong@bcm.tmc.edu](mailto:qtong@bcm.tmc.edu)). Dr. Tong's research concerns adipose tissue development, which may provide clues to the molecular mechanisms of obesity and type 2 diabetes later in life. His previous work demonstrated that both GATA transcription factors are expressed in preadipocytes and that their expression disappears in mature fat cells. GATA transcription factors suppress adipocyte differentiation through the interference of two critical regulators, PPAR and the C/EBP family of transcription factors. GATA also forms protein complexes with other proteins, some of which (e.g., C/EBP, Rb and Trap220) also are known to be involved in the process of fat cell formation, such as current research focuses on characterization of the components and dynamics of the GATA protein complexes during adipogenesis.

Another line of research concerns the function of the sirtuin family of deacetylases, the mammalian homologues of yeast Sir2 gene, which mediates the effect of caloric restriction on life span extension. Initial experiments have demonstrated that mammalian sirtuins play roles in white adipocyte differentiation and brown adipocyte adaptive thermogenesis.

**Ignatia B. Van den Veyver, MD** ([iveyver@bcm.tmc.edu](mailto:iveyver@bcm.tmc.edu)). Dr. Van den Veyver's primary research interest is the role of DNA methylation in epigenetic regulation of gene expression during development. She participated in studies

showing that Rett syndrome, an X-linked dominant neurodevelopmental disorder, is caused by mutations in *MECP2*. This gene encodes methyl-CpG-binding protein 2, which plays a role in transcriptional silencing of other genes when they are methylated at their promoters. To better understand the role of DNA methylation in development, current studies focus on the effects of varying dietary levels of methyl donor agents (folic acid, betaine) on DNA methylation and gene expression during development. These studies utilize large genomic screens in mouse models and cell culture systems. In collaboration with others, she is studying the effects of such treatments on DNA methylation and correlating these effects with phenotypic effects in Rett syndrome. This work is relevant for the understanding not only of Rett syndrome but also of other common birth defects, (e.g., neural tube defects) as well as the influence of intrauterine environment on gene expression later in life. Additional laboratory projects focus on identification and functional analysis of genes for other X-linked dominant disorders and for recurrent familial hydatidiform moles.

**Robert A. Waterland, PhD** ([waterlan@bcm.tmc.edu](mailto:waterlan@bcm.tmc.edu)). Dr. Waterland's research focuses on the basic biologic mechanisms by which the pathogenesis of several adult-onset diseases can be influenced by nutrition in early life. One probable mechanism is through early nutritional effects on epigenetics, i.e. self-perpetuating gene regulatory systems that are not dependent on DNA sequence alterations. He is particularly interested in the methylation of cytosine residues on both strands of palindromic CpG dinucleotides in genomic DNA. Mammalian one-carbon metabolism, which provides the methyl groups for biological methylation reactions, is highly dependent on dietary substrates and co-factors. The requirement to establish and maintain genomic methylation patterns during early development may therefore make it more critical to maintain appropriate levels of these diet-derived components at that time than during later life. He and colleagues have demonstrated that dietary methyl donor supplementation of viable yellow agouti ( $A^{vy}$ ) mice during early development increases CpG methylation at the *agouti* locus. The overall hypothesis is that maternal dietary methyl donor supplementation before conception and during pregnancy alters DNA methylation of specific genomic regions in the early embryo and that these alterations persist to adulthood. Because epigenetic dysregulation is implicated in a broad range of human disease, such induced epigenetic alterations may enable early nutrition to influence adult metabolism and chronic disease susceptibility.

**Leonard E. Weisman, MD** ([lweisman@bcm.tmc.edu](mailto:lweisman@bcm.tmc.edu)). Dr. Weisman's research includes both laboratory and clinical studies related to neonatal immunity, including the effects of neonatal neutrophil function and/or immunoglobulin (parenteral, enteral or transplacental) on common infectious agents (e.g., *Streptococcus agalactiae*, *Staphylococcus epidemidis*, *Ureaplasma urealyticum*, respiratory syncytial virus) and the effects of modulation of neonatal neutrophil and/or immunoglobulin function on these agents. A multi-center NIH-sponsored project focusing on the protective levels of maternal antibody against

early-onset invasive group B streptococcal disease in neonates was just completed and a single-center NIH-sponsored project focusing on determining the risk factors for late-onset invasive group B streptococcal disease in neonates is nearing completion. Dr. Weisman also directs the clinical and laboratory evaluation of an anti-*Staphylococcal* humanized monoclonal antibody for the prevention and treatment of infection.

**William W. Wong, PhD** ([wwong@bcm.tmc.edu](mailto:wwong@bcm.tmc.edu)). Dr. Wong's major research efforts focus on prevention of childhood obesity and dietary supplementation to prevent chronic diseases. He recently completed a project funded by the Texas Department of Health documenting the prevalence of and risk factors for childhood obesity in the Houston Independent School District, the largest public school district in Texas with students of diverse ethnic and socioeconomic backgrounds. A project to determine the effectiveness of an after-school physical activity program in preventing obesity among Hispanic children is planned.

He also is the project director of a USDA-funded \$4.5 million, multi-center, randomized, double-blind, placebo-controlled study to document the safety, efficacy, and optimal dosage of soy isoflavones to prevent osteoporosis in postmenopausal women. In addition, he is principal investigator of a NIH-funded project to determine the effects of soy isoflavone supplementation on nitric oxide production, blood pressure, and arterial compliance in postmenopausal women with normal and high-normal blood pressure as well as stage 1 hypertension.