Rebecca Carron, School of Nursing. Polycystic Ovary Syndrome in American Indian Women: An Exploratory Study.

The NIH mission is, in part, to discover new knowledge about the behavior of living systems and to use that knowledge to improve health. Women with polycystic ovary syndrome (PCOS) are at risk for many significant health care problems including cardiometabolic syndrome (CMS), type 2 diabetes, obesity, infertility, psychosocial stress, suicide, and decreased health-related quality of life (HRQL). No specific knowledge about the effects of PCOS in American Indian women exists. The long-term goal of the project is to improve the HRQL of AI women with PCOS. The project goal will be accomplished with the specific aims designed to fill PCOS knowledge gaps:

**Aim #1**: Determine Al ethnic specific PCOS symptoms, problems, and psychosocial stress in a sample of Al women with confirmed PCOS, and increase health care provider PCOS awareness.

**Aim #2**: Estimate PCOS population prevalence and cardiometabolic profile, including risk for diabetes, in a sample of Al women with PCOS.

**Aim #3**: Determine cultural and societal practices of Al women with PCOS for beginning development of patient-centered, culturally competent PCOS self-care interventions.

**Aim #4**: Develop a conceptual model of the Al experience of PCOS and initial development of an instrument to measure the Al experience of PCOS based on results from Aims 1-3.

The research design uses a mixed methods approach with both qualitative (interviews, group meetings, conceptual model) and quantitative (survey results, cardiometabolic profile, instrument development) methods to answer the specific aims. The evidence-based, translatable knowledge gained will significantly increase the ability of health care providers in a variety of settings to measure Al ethnic specific symptoms/problems and provide beginning evidence of effects of patient-centered, culturally competent interventions to reduce the risk for cardiometabolic diseases, psychosocial stress, and improve HRQL in Al women with PCOS.

Brian Cherrington, Department of Zoology and Physiology. The Effect of Obesity Induced Hyperinsulinemia on Lactation.

Obesity negatively affects lactation, yet a mechanistic understanding of how this occurs is lacking. This gap in knowledge is an important medical problem because breastfeeding protects both the mother and infant against obesity, diabetes, and metabolic disorders later in life. At parturition, a dramatic rise in prolactin produced by anterior pituitary gland lactotrope cells is indispensable for initiating breast milk production by mammary epithelial cells – a process termed secretory activation. In obese mothers, however, secretory activation is disrupted potentially due to the negative effects of chronic hyperinsulinemia on prolactin production by lactotrope cells. Our long-term goal is to understand the defects in the lactation mechanism in obese mothers which results in lactation problems. The overall objective of this proposal, which advances our long-term goal, is to determine if delayed secretory activation is due to obesity induced hyperinsulinemia and test if treatment with an insulin sensitizing agent can restore normal lactation. Our central hypothesis is that obesity induced hyperinsulinemia alters prolactin production by lactotrope cells delaying secretory activation. We will test our central
hypothesis by the following aims: (1) determine the molecular mechanisms through which hyperinsulinemia alters prolactin production in lactotrope GH3 cells; (2) establish if changes in lactotrope proliferation or insulin signaling activity alter prolactin production delaying secretory activation in obese mice. In aim 1, we will use GH3 cells to investigate the effects of hyperinsulinemia on activity of insulin signaling and prolactin mRNA and protein levels. In aim 2, we will use a mouse obesity model to investigate the effects of hyperinsulinemia on lactotrope cell number, insulin signaling activity, and prolactin production in vivo. Aim 2 will also test if treating obese mice with metformin restores normal prolactin production and secretory activation. The proposed research is innovative because it investigates a side effect of chronic hyperinsulinemia on lactation. The work is significant because it is an initial step in a line of research to understand the mechanisms causing lactation problems in obese mothers and test novel treatment options.

Wei Guo, Department of Animal Sciences. Role of RBM20 in the regulation of cardiac gene splicing in heart failure.

Heart failure (HF) is a serious, chronic condition that gradually deteriorates over time. The onset and development of HF are unpredictable and present individual variation possibly due to unclear etiology for HF progression. With development of HF, cardiac remodeling will occur, leading to impaired cardiac structure and function. Sarcomeric proteins are essential determinants of sarcomeric structure and function. The abnormal expression of sarcomeric proteins will result in impaired sarcomeric structure and function, enroute to impaired cardiac structure and function. Therefore, aberrant expression of sarcomeric proteins is one of the major causes for HF progression. Sarcomeric proteins such as myosin, actin and titin take up -60 to 80% of myocyte mass, so abnormal alterations of these protein will significantly affect cardiac structure and function. All of these proteins undergo isoform switch with development and under disease condition. The abnormal isoform switch of these proteins has been identified in failing heart. However, the mechanisms of isoform switch of these proteins remain elusive. Previous studies have shown that metabolic and hemodynamic switch under stress environment can cause isoform switch of these proteins, but it is unknown how these metabolic changes link to protein isoform switch. Recently, our group identified a muscle-specific splicing factor-RNA binding motif 20 (RBM20) that is a master regulator of cardiac protein isoform switches. RBM20 has been found to regulate over 30 gene splicing in cardiac muscle to date. Among these genes, titin and myosin are the major targets of RBM20, but the detailed mechanisms of how RBM20 regulates isoform switch of these proteins remain elusive. Hence, this project is proposed to use titin, the major target of RBM20 as a molecular model for investigation of the mechanisms of RBM20-mediated protein isoform switch. The increased understanding of the regulatory mechanisms regarding RBM20-mediated protein isoform switch may partially address metabolic change-induced gene expression in the heart and the progression of HF at the molecular levels.

Guanglong He, School of Pharmacy. CARD9 Signaling and Childhood Obesity-Associated Cardiac Dysfunction.

While obesity has emerged as an epidemic, strategies to curb this disease are limited. Childhood obesity and its persistence into adulthood is associated with insulin resistance and glucose intolerance with dire consequences on cardiovascular system. Therefore,
research strategies that focus on the underlying mechanisms are much needed. Obesity is accompanied with a low-grade chronic inflammation with infiltration of macrophages in target organs such as heart and vessels. As activated macrophages release pro-inflammatory cytokines with detrimental effects on the target cells/organs, our approach is to dissect the obesity-induced inflammatory response pathways and their potential impact on cardiovascular diseases. Recently, it has been reported that the pro-inflammatory protein CARD9, which is exclusively expressed in macrophages, associates with BCL10 and MALT1 as a signalosome, robustly activates NFκB signaling, and eventually eradicates the invaded pathogens. However, whether or not CARD9 plays a role in obesity induced chronic inflammation is not known. Our preliminary data indicated that CARD9 knockout reconciled obesity-induced insulin resistance and glucose intolerance. We also observed that CARD9 knockout ameliorated obesity-induced cardiac dysfunction. Therefore, we hypothesize that obesity activates CARD9 signaling in macrophages and this pro-inflammatory signaling is responsible for obesity-induced insulin resistance and myocardial dysfunction. To test this hypothesis, we will utilize an obese mouse model and the CARD9 knockout mouse strain to determine whether or not: 1) obesity induces systemic insulin resistance via activation of CARD9-BCL10-MALT1 signalosome and related TNFalpha/IL6/IL1 beta signaling in macrophages; 2) obesity suppresses myocardial mitogenesis and cardiac function via activation of CARD9- BCL10-MALT1 signalosome and related NFκB/TNFalpha signaling in macrophages in a paracrine manner. We believe that successful completion of the proposed studies will provide potential novel targets for the treatment of obesity-associated metabolic syndrome and cardiovascular abnormalities.

Anya Lyuksyutova, Department of Molecular Biology. Optogenetic control of GCS via microRNAs as treatment for liver steatosis.

Sphingolipid accumulation contributes to cardiometabolic syndrome. Inhibition of the first enzyme in sphingolipid biogenesis, glucosylceramide synthase (GCS), emerged as a powerful new approach for treating cardiometabolic syndrome and type II diabetes. Current GCS inhibitors have a long list of side effects. Inhibitory small RNAs, siRNAs and microRNA, represent an alternative approach for GCS inhibition. Recently, we showed that overexpression of microRNA-190 and microRNA-200 significantly reduces GCS mRNA levels in cell culture. However, inhibiting GCS in the whole animal is deleterious. To inhibit GCS specifically in the liver and to control the extent of GCS expression over time, we propose to use an optogenetic expression system developed in the Gomelsky lab. This system relies on light in the near infrared window (NIRW), which penetrates to the depth of several centimeters in mammalian tissues. It has been validated in mammalian cell culture. First, we will test and optimize the NIRW light expression system in mice, using hydrodynamic transfection of microRNA-190 and 200 to achieve their strong expression in the liver. Next, we will characterize effects of microRNA-190 and 200 on reducing GCS and reversing cardiometabolic symptoms in vivo. The effect of the microRNAs will be quantified by measuring changes in insulin resistance, liver lipid levels and blood free triglyceride levels over time. Precise temporal control, made possible by the NIRW light expression of the microRNAs, will allow us to fine-tune microRNA-190 and 200 expression for optimal GCS reduction. If successful, this project will provide a novel and innovative approach for cardiometabolic symptom reversal. In a broader sense, it will open up opportunities for temporal and spatial control of therapeutically important genes (i.e. cell
Amy Navratil, Department of Zoology and Physiology. Molecular Mechanisms of Luteinizing Hormone Dysregulation in PCOS.
Polycystic ovary syndrome (PCOS) is complex reproductive disorder with unclear pathophysiology. One of the hallmarks of PCOS includes elevated plasma luteinizing hormone (LH) levels that in combination with metabolic dysfunction and excess androgen lead to reproductive abnormalities. Despite recent advances, the precise mechanisms that are involved in PCOS LH hyper-secretion directly at the level of the gonadotrope are largely unknown. We propose to utilize a prenatal androgenized mouse model to test the central hypothesis that metabolic and steroid hormone dysregulation in PCOS alters gonadotrope function at both the population and cellular level to disproportionately increase LH levels. We propose three comprehensive aims to examine the hypotheses: 1. the gonadotrope network increases its size and cell-to-cell contacts in PCOS to more effectively synchronize increased pulstile LH; 2. gonadotropes will have heightened calcium activity in response to GnRH to not only enhance LH secretory events but also increase ERK activation necessary for LH synthesis; 3. histone citrullination of the LHβ gene leads to increased transcription and expression, contributing to the pathogenesis of PCOS. Taken together, this proposal seeks to advance our understanding of the relationship between PCOS, the gonadotrope, and LH secretion. We are hopeful that the experiments outlined will help in understanding the underlying mechanisms of increased LH secretion in the pituitary and provide critical insight into the pathophysiology of PCOS and impaired reproductive function.

John Oakey, Department of Chemical Engineering. Circulating Tumor Cell Capture and Release from Degradable Hydrogel Surfaces.
This proposal proposes the development of a microfluidic rare cell capture and release device that can be applied to the isolation of viable circulating tumor cells (CTCs) from clinical whole blood samples. This work is organized into two interacting components: 1) cell capture and release device platform development, 2) viable CTC isolation and recovery and genomic analysis. Central to the proposed work is the development of an immunoaffinity cell capture and release device based upon moldable photodegradable hydrogels. Antibodies lend tremendous recognition specificity and have been successfully used in surface-capture microchips to immobilize and isolate rare (1 in 109) circulating tumor cells (CTCs) from whole blood. Many devices with different geometries and surface capture motifs have already been tested to optimize for either rare population enrichment or for higher volume cell capture. Regardless of the capture target, it is of tremendous interest to subsequently release captured cells from devices in as viable a state as possible. We have found that trypsin or other enzymatic digestion approaches provide poor viable cell yields and, therefore, we have developed hydrogel-based sacrificial capture surfaces. These surfaces are fabricated by mold-polymerizing a photo-degradable hydrogel capture surface with the footprint of a microfluidic capture chamber. Capture antibodies are bound to the hydrogel by either post-polymerization conjugation or by copolymerization.
Following capture and device flushing, cells can be released from the device by a short exposure to low-intensity UV light, which degrades the gel's photocleavable crosslinks. Once fluidized, the gel's degradation products and cells may be gently eluted from the device and captured. As capture pads are molded in place, multiple capture zones, each with a unique antibody, may be created within a single device.

**Christine Porter, Department of Kinesiology. Growing Resilience Phase II: Albany County Redesign and Wind River Expansions.**

The four facets of this proposed project expand on the one-year pilot Growing Resilience program ("Phase I") that Porter led in 2013 with partners in Albany County and in Wind River Indian Reservation (WRIR). In Phase I, each team co-designed a randomized controlled trial (RCT) of the impacts of home gardens on multiple health outcomes and we trialed the design with a few families in each place. This Phase II proposed project will, in decreasing order of duration and cost:

1. Redesign and conduct a larger pilot trial of the RCT with Albany County partners, including establishing recruitment partnerships with new organizations and determining what we should select as the primary health outcome for the study design (2015-2018).
2. Expand the Native American young adult internship/mentorship program with Dr. Tarissa Spoonhunter at Central Wyoming College (CWC) for student involvement in this study (2015-2018).
3. Support the collection of health outcome data with WRIR families who participated in Phase I, and other work to sustain WRIR Growing Resilience partnerships while an R01 proposal to NIH is under review for a 2016 start (2015 only).
4. Seed-fund the nursing leadership in Native American health care action research that emerged as a priority during Phase I (2015 only).

Finally, each of the four arenas above will serve as a foundation for four different externally-funded projects.

**Baskaran Thyagarajan. TRPV1 Activation Prevents Against High Fat Diet Induced NAFLD via SiRT-1.**

High fat diet-induced obesity is associated with abnormal fat accumulation in the liver. This affects the metabolic functions of the liver leading to non-alcoholic fatty liver disease (NAFLD). The staggering statistics of increase in NAFLD in the USA necessitates the development of strategies to prevent and treat NAFLD. In our efforts to analyze the role of transient receptor potential vaniloid 1 (TRPV1) channel protein in the regulation of metabolic syndrome, we discovered that capsaicin (CAP; a TRPV1 agonist) significantly prevented high fat diet (HFD)-induced obesity and NAFLD by increasing the expression and activity of sirtuin-1 (SiRT-1) protein. SiRT-1 activates and deacetylates metabolically important cellular proteins (PPARα, PGC-1α) to trigger lipolysis and to regulate the self-digestion of lipid cells (lipophagy). Forkhead box O1 (FOXO1) is a nuclear transcription factor and is a substrate for deacetylation by SiRT-1. FOXO1 regulates lipophagy and is a potential target for treating NAFLD. We hypothesize that “HFD inhibits SiRT-1 and causes NAFLD by 1) Down-regulating TRPV1 and suppressing TRPV1/CaMKII/AMPKdependent SiRT-1 activation; 2) Disrupting SiRT-1/PPARα interaction to decrease lipolysis; and 3) Inhibiting FOXO1 deacetylation by SiRT-1 to impair lipophagy. CAP stimulates TRPV1 and
increases CaMKIIα/AMPKα-dependent SIRT-1 phosphorylation to facilitate 1). SIRT-1/PPARα interaction to promote lipolysis; 2). PGC-1α deacetylation by SIRT-1 leads to activation of UCP-1 and promotes lipophagy; and 3). Deacetylation of FOXO1 by SIRT-1 promotes lipophagy to prevent NAFLD. We propose three specific aims:

Specific Aim 1: Determine the effect of dietary CAP on SIRT-1/PPARα interaction, which stimulates lipolysis.

Specific Aim 2: Evaluate the effect TRPV1 activation on deacetylation of PGC-1α by SIRT-1 to activate UCP-1.

Specific Aim 3: Analyze the effect of SIRT-1 activation on FOXO1 deacetylation and induction of lipophagy to prevent NAFLD.

Our research will provide a new insight in the use of CAP to combat obesity and NAFLD.

Krisztina Varga, Department of Chemistry. Structure and function of TSPO, an important drug target.

The aim of this project is the structural and functional study of TSPO, an important drug and imaging target in cardiovascular disease and diabetic neuropathy and other diseases. We will elucidate the binding sites, determine its enzymatic activity, design high-affinity selective ligands, and test the effect of ligands on rat cardiac myocytes. TSPO is an 18-kDa transmembrane protein predominantly present in the outer membrane of mitochondria. TSPO has a role in regulating mitochondrial functions with implications in steroidogenesis and apoptosis. Recent studies have shown that TSPO inhibition by various ligands effectively prevented reperfusion injury after ischemia in rats. Activation of TSPO has been shown to exert neuroprotective action against diabetes induced peripheral nerve pathologies. However, we currently possess limited understanding about the interaction of TSPO with ligands. Recent evidence suggests that there are multiple ligand binding sites, and the binding pocket(s) are poorly characterized. As TSPO is widely distributed and has multiple roles, it is very important to identify the structural functional significance of TSPO ligands before their efficient application to treat specific diseases. It has been recently proposed that at least two of the bacterial TSPOs have enzymatic activity. As TSPO is widely distributed and has multiple roles, it is very important to identify the structural functional significance of ligands before their efficient application to treat specific diseases. We propose a structural functional and ligand binding study of TSPO. We have expressed and purified a well-characterized structural and functional homolog of TSPO from R. sphaeroides, and ligand binding, enzymatic studies, and NMR spectroscopy structural studies are currently underway. We are the first to show that RsTSPO has enzymatic activity (manuscript in preparation). The bacterial protein will serve as a structural and functional model to refine conditions for structure determination of the human TSPO. In order to explore the function of TSPO and test ligand binding in vivo, we will test the effect of ligands on rat myocytes. Results of this study will serve as a basis for an R01 proposal to NIH. The results of this study are expected to be transformative for drug design to combat reperfusion injury and diabetic neuropathy.