ABSTRACTS

Research from The National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, and Charles R. Drew University of Medicine and Science 2007 High School Student Summer Research Program

EXERCISE TRAINING AMELIORATES CHRONIC ALCOHOL-INDUCED HYPERTENSION, ANGIOTENSIN II LEVELS AND RENAL OXIDATIVE STRESS IN RATS

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The aim of this study was to investigate the relationship of chronic ethanol-induced elevation in blood pressure (BP) with angiotensin II levels and oxidative renal injury in rats. Male Fisher rats were divided into three groups of seven animals each and treated as follows: (1) Control (5% sucrose, orally) daily for 12 weeks; (2) ethanol (4 g kg^{-1}, orally) daily for 12 weeks; and 3) exercise training on treadmill followed by ethanol daily for 12 weeks. The BP (systolic, diastolic and mean) was recorded every week. The animals were sacrificed after 12 weeks; blood and kidneys were isolated and analyzed for angiotensin II levels and oxidative stress parameters. The results show that the systolic, diastolic and mean BP was significantly elevated 12 weeks after ethanol ingestion. The increased BP was related to elevated angiotensin II levels in the plasma and kidney of the alcohol-treated group compared to control. Exercise training reduced the ethanol-induced high BP as well as angiotensin II levels. The renal NADPH oxidase activity and lipid peroxidation increased whereas antioxidant enzyme activities such as superoxide dismutase (CuZn-SOD), catalase (CAT) and glutathione peroxidase (GPX) were significantly depressed in the alcohol group compared to control. Alcohol-induced renal oxidative stress response was abrogated by exercise training. It is concluded that chronic ethanol ingestion induces hypertension, which is correlated with elevated tissue angiotensin II levels, and activation of NADPH oxidase activity causing renal oxidative injury in rats. Exercise training attenuated the chronic alcohol-induced hypertension, elevated angiotensin II levels and renal oxidative injury in rats.

IMPACT OF CARPAL TUNNEL RELEASE ON PATIENTS WITH VS WITHOUT DIABETES

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Mentors: Dr. Darryl Wayne Peterson; Dr. Shervondylnn Brown, Greater Baton Rouge Surgical Hospital; Dr. Charles J. Monlezun, Department of Experimental Statistics, Baton Rouge, Louisiana

We found a difference in the clinical relief of hand numbness and tingling (carpal tunnel syndrome) between patients with diabetes vs patients without diabetes after carpal tunnel release (CTR) surgery is performed. There was also a difference in post-operative wound healing character and pain. CTR surgery is performed in an effort to relieve numbness in the fingers and palm region. All patients who underwent CTR surgery were surveyed to determine different experiences between those who had diabetes and those who did not.

Patients with carpal tunnel syndrome were interviewed post op in order to gain a better understanding of the nature of their conditions. A survey was then mailed to all patients of a selected physician to assess the post operative results of carpal tunnel syndrome surgery. The questions in the survey were to specifically identify the feeling and sensation of the hand, wrist, and fingers after carpal tunnel release surgery. The questions were designed to isolate responses for patients with diabetes and patients without diabetes and also to measure varying conditions during different times of the day. Questions specific to patients with diabetes assessed the impact of their diabetes treatment on the carpal tunnel condition.

THE ASSOCIATION OF VITAMIN D RECEPTOR POLYMORPHISM AND THE RISK OF DIABETES IN AFRICAN AMERICAN AND HISPANIC AMERICAN FEMALES

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Diabetes mellitus is a disease characterized by high levels of blood glucose resulting from defects in insulin production, insulin action or both. According to *National Diabetic Statistics*, *2005*, 20.8 million people in the United States have diabetes. Various studies have suggested an increased prevalence of diabetes in vitamin D deficient individuals. The activity of vitamin D is mediated through vitamin D receptor (VDR) protein, a ligand-activated transcription factor. In humans, the VDR gene maps to chromosome 12q13.11 and shows various polymorphic sites such as Fok1, Bsm1, Apa1, and Taq1. We hypothesized that VDR Taq1 and Apa1 polymorphism may be associated with the risk of diabetes in African American and Latina American females.

To examine this hypothesis we analyzed blood samples from 80 female patients with diabetes and 150 normal female controls. DNA was extracted from buffy coat and genotyped by way of a polymerase chain reaction-restriction Fragment length polymorphism method (PCR-RFLP). A 745 bp PCR product was obtained after PCR with specific primers. One microgram of PCR product was subjected to digestion for 3 hours with ApaI and TaqI restriction enzymes. Taq1 was genotyped as TT (496 bp, 249 bp), Tt (496, 291, 249 and 205 bp), tt (291, 249, 205 bp), while Apa1 was genotyped as AA (745 bp), Aa (745, 531, 214 bp) and aa (531, 214 bp). We analyzed the data using SPSS software in a case control model.

We found that there was a significant association between VDR-Taq1 polymorphism and diabetes in African American and Hispanic American females, specifically in African American patients. We also found no significant association between VDR-Apa1 polymorphism and diabetes in African American and Hispanic American patients.

EVALUATION OF SHOTGUN-BASED GLOBAL PROTEOMIC PROFILING OF A *SACCHAROMYCES CEREVISIAE* STRAIN LACKING THE F-BOX PROTEIN GRR1 REVEALS A NEW ROLE FOR GRR1 IN THE REGULATION OF INOSITOL METABOLISM

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The process of ubiquitination is executed by all eukaryotic cells characterized to date. During this process protein substrates are marked for degradation via the vacuole or proteasome through their covalent attachment to the 76 amino acid protein ubiquitin. Many proteins participating in a wide variety of cellular functions are inactivated in this manner. Thus, transcription, post-transcription, translation, and post-translational processes can all be regulated by this mechanism.

One mechanism by which proteins are tagged with ubiquitin is through a multi-subunit complex termed the SCF (SKP-Cullin-F-box). Perhaps one of the most interesting attributes of this complex is that it can associate with multiple F-box's to recognize different intracellular substrates. In particular, the F-box Grr1 (glucose repression resistant) is interesting because it has been shown to coordinate the cellular response to extra-cellular nutrients (ie, glucose, ions, nitrogen) by modulating transcriptional and post-translational control mechanisms. This occurs, presumably, through Grr1 specific targeting of transcription factors and cell cycle regulators for ubiquitination and, thus, degradation.

In order to elucidate novel substrates for the SCF^{Grr1} complex, we sought to characterize the proteomic response to Grr1 deletion on a global scale using LC-LC-MS/MS and micro-arrays. Using these systems level tools it was possible to construct intricate protein networks that allowed for identification of possible novel substrates. Since quantitative proteomic analyses can be somewhat unreliable in that a protein changes can be due to differential post-translational modification or a change in the steady state level of protein we sought to verify these changes using western blots.

AWARENESS AND IMPLEMENTATION OF CLINICAL RESEARCH IN WEBB COUNTY

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The field of medicine is evolving along with new diseases and threats found daily. Research is done in order to acquire information to answer the question or validate results that can cause an impact. To arrive at a conclusion of validation, the implementation of the scientific method is utilized. The impact derived from the results must pertain not only to people as a whole, but also to the distinct community that is clearly affected by the threat. South Texas, especially the Texas-Mexico border and in particular Webb County, has unique threats not applicable elsewhere in the state. The area is known for its high rate of tuberculosis and mortality from diabetes. Shortage of researchers and physicians in the area affects the well-being of the area's population of 250,010 and the ability to generate awareness of new found risks among the population.

My research sought to examine the integration of scientific clinical research and its impact on the unique Texas border health issues. I evaluated and observed Laredo researchers and physicians who influenced research in south Texas. I will analyze the importance of the research conducted by my colleagues and how it is compatible to the unique community of border communities. During an 8-week period, I will follow these researchers to compile information on how they use scientific methods to achieve results that have value.

DEVELOPMENT OF A MOLECULAR BASED APPROACH TO DETECTING PATHOGENIC *LEPTOSPIRA* IN ENVIRONMENTAL WATER SAMPLES

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The etiologic agents of Leptospirosis, a waterborne zoonosis, are pathogenic *Leptospira* bacteria. Because *Leptospiras* are slow-growing and easily out-competed in culture by other contaminating microorganisms, traditional culture-based techniques are inefficient and often give inaccurate and underestimates of disease prevalence. For this project, we chose to do a polymerase chain reaction (PCR) based detection assay because PCR is fast, easy, and relatively economical compared to other detection techniques that have been described for *Leptospira*. In order to do this, we needed to validate our ability to isolate the DNA of pathogenic *Leptospira* from water samples and our ability to detect the DNA using PCR technology. After the initial centrifugation process, a Qiagen© kit was used for DNA isolation. PCR was then performed using species-specific primers to detect the presence of pathogenic leptospiral DNA. Subsequently, two dilution series and gradient experiments were performed to validate our DNA isolation and PCR methods. We then tested water samples from three different days obtained from Manoa Falls Stream and found all 34 initial samples to be negative. Using standard spectrophotometer techniques and gel electrophoresis, we were able to verify the DNA isolated from the stream samples were of good quality and quantity. Thus, there are likely to be no pathogenic *Leptospira* present in the initial stream samples that were tested. In conclusion, PCR appears to be a promising detection method for pathogenic *Leptospira* in water. The utility of this method for environmental samples, however, still needs to be shown.

HEALTH RISKS FOR DIABETES IN HAWAIIAN SCHOOL CHILDREN

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The prevalence of obesity in children has shown a worrisome rise in the United States in recent years. An earlier onset of obesity can lead to high blood pressure at a young age, heart disease, and even diabetes. As a member of the Health Risks for Diabetes in Hawaii School Children Study, we tried to discover some factors that could lead to an increased risk for diabetes. In this investigation of 80 children on the Big Island of Hawaii, in kindergarten and third grade, researchers recorded age, height, weight, body fat percentage, diastolic/systolic blood pressure, lying/resting heart rate and sub maximal volume of oxygen per kilogram per minute of body mass. Children were asked to sit in the Bod Pod, which measured the body fat percentage. Diastolic blood pressure was taken followed by an exercise test on a treadmill that measured the volume of oxygen per kilogram, per minute, at 2.4 mph with a 9° incline. The third grade females had the highest body fat percentage, followed by the third grade males, the kindergarten females, and finally the kindergarten males. The diastolic blood pressure results indicated the same order (higher to lower) as the body fat percentage, but the VO2 showed an interesting difference. It revealed that the younger children had to work harder on the treadmill than the older ones. The results of this study indicate that children in the third grade have a higher rate of body fat percentage and diastolic blood pressure than children in kindergarten. Yet, the VO2 demonstrated that the younger children had to put forth more effort on the treadmill than the older children.

VARIATION IN BODY MASS INDEX AND Z-SCORES AND IN CHILDREN REGISTERED IN THE PEDIATRIC INFLAMMATORY BOWEL DISEASE CONSORTIUM

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Inflammatory bowel disease (IBD) is divided into two main types: ulcerative colitis and Crohn's disease. Growth failure is a common effect of IBD in patients under the age of eighteen. Sixty to seventy percent of the children with Crohn's disease and 14% of children with ulcerative colitis experience growth failure. Growth failure can be evaluated using the Z-score in IBD range from −0.84 to 1.08 which can be compared to their scores from −0.32 to 0.05 found in children without IBD. In both ulcerative colitis and Crohn's disease, those patients who have a Z-score of less than one have osteopenia. Prevalence of osteopenia in patients with Crohns disease ranges from 32% to 38% whereas in patients with ulcerative colitis, the range is 23% to 25%. The mean deficit Z-scores were 0.44 +/− 0.08 in the spine of patients with Crohn's disease and 0.34 +/− 0.08 in those with ulcerative colitis. We hypothesized that the growth failure in Crohn's disease patients will be higher than in ulcerative colitis patients.

To check our hypothesis, we used a database of 1049 patients of different ethnicities. Using height, weight, sex, date of office visit and date of birth we were able to calculate the Z-scores, body mass index (BMI) and the prevalence rates using an online calculator. Height was compared to age and sex matched references values. Weight was recorded by a standard clinical balance. The BMI was calculated using weight over height squared. The results of our study are not yet available.

THE PREVENTIVE EFFECT OF BAMBOO EXTRACT ON OBESITY-INDUCED TYPE 2 DIABETES

Student Researcher: Dawn Vess
Mentors: Wanyu Liu; Marla J. Berry; Jun Panee, Department of Cell & Molecular Biology, John A Burns School of Medicine, University of Hawaii, Honolulu

Diabetes has become one of the leading health problems in the United States due to the impact of changes in diet and lifestyle. Native Hawaiians are over five times more likely than non-Hawaiians to develop diabetes between the ages 19–35 and two times more likely between the ages 36–64.

In this study, we investigated the preventive effect of bamboo extract on high fat diet-induced type 2 diabetes in male C57BL/6J mice. Starting from four weeks of age, seven mice were fed with a high-fat diet (45 kcal, control group), and 8 mice were fed with a high-fat diet supplemented with bamboo extract (10 g of dry mass of bamboo extract per 4057 kcal, BEX group). The diet regimes lasted for five months. By the end of the experiment, we observed the mice in the BEX group had significantly enhanced glucose tolerance and insulin sensitivity. This result indicates that bamboo extract as a dietary supplement may have a preventive effect on obesity-induced type 2 diabetes.

A ROLE FOR B CELLS IN RENAL FIBROSIS?

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Mentors: Allison Eddy, MD; Jesús López-Guisa, PhD, Children's Hospital and Regional Medical Center and University of Washington, Seattle

Chronic kidney disease (CKD) has reached epidemic proportions worldwide. Despite significant advances in scientific understanding of the cellular and molecular pathways that mediate CKD, improvements in therapeutic options for the affected patients have been very modest. The goal of the present proposal is to initiate preliminary studies to investigate the unexplored hypothesis that B cells play an important role in CKD pathogenesis. A role for B cells has not received much attention as there is little evidence that antibodies are involved. The fact that B cells also synthesize and secrete cytokines and growth factors has been largely ignored until recently. Our hypothesis is that B cells produce soluble factors that contribute to the pathogenesis of renal fibrosis and progressive kidney disease. The experimental plan will investigate two mouse CKD models, a very aggressive model induced by ureteral obstruction and a more indolent model of chronic nephrotic syndrome induced by Adriamycin. Disease severity in wild-type mice will be compared to mice with genetic B cell deficiency (aim #1) and mice with transient B cell depletion induced using anti-CD20 antibodies (aim #2). Differential expression of cytokine and growth factors known to be produced by B cells will be examined as a first approach to identifying the key fibrosis-promoting factor(s). It is anticipated that these studies will generate data that will form the basis of a new approach for treating the ever-increasing human sub-population with CKD.

IMPROVING DIAGNOSIS OF POLYCYSTIC OVARY SYNDROME (PCOS) THROUGH PROTEIN PROFILING

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Mentors: Craig Laramee, PhD, Innovative Technologies Complex, SUNY-Binghamton, New York; Olayinka Wilhelm, MD, Diabetes Education & Management Center, UHS Hospitals, Johnson City, New York

Polycystic ovary syndrome is a disorder in which abnormal hormone levels prevent matured egg cells from being released from a woman's ovaries, leading to the development of cysts of unreleased egg cells. The condition not only has a severe impact on fertility, but can also lead to weight gain (especially around the midsection) and increased risk for diabetes and glucose intolerance. Unfortunately, there is no exact diagnosis for this disorder. Quickly and definitively identifying PCOS could not only lead to a more healthful life for many women, it could save lives, especially for minorities and others already at an increased risk to develop endocrine disorders. It is our hypothesis that by identifying irregular protein levels in the blood stream of PCOS patients, we will be able to develop a more cumulative diagnosis of this syndrome. To this end, Dr. Wilhelm will extract blood samples from patients (along IRB guidelines). Blood samples will be run through surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) over a wide range of protein weights. Resulting curves will be reduced and analyzed using the R statistical computer program for potential changes in protein levels among PCOS patients.

BACTERIAL INTERFERENCE FOR PREVENTION OF URINARY TRACT INFECTION IN SPINAL CORD INJURY PATIENTS

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Background: Since recurrent catheter-associated urinary tract infection (UTI) is a significant problem in the spinal cord injury patient population, a method to prevent UTI is needed. Our goal is bacterial interference as a strategy to prevent UTI in persons with spinal cord injury (SCI) who rely on an intermittent bladder catheterization program (ICP). We used a benign bacterial strain, *Escherichia coli* 83972 (*E coli*), which demonstrates capability to safely reside in the bladder and may prevent pathogens from causing UTI. We are conducting a clinical trial to examine whether *E coli*- coated catheters can effectively prevent UTI among SCI patients practicing ICP.

Methods: Subjects with SCI for at least 12 months, 1 symptomatic UTI in the past, a neurogenic bladder, and ICP were eligible to participate. Antibiotics were used to sterilize the subject's bladder and a study catheter coated with *E coli* is inserted and left in the bladder for 3–7 days. After removal of the study catheter, urine is collected for culture for the next 28 days to examine for the presence of the benign organism. Data are collected on the rates of UTI pre and during colonization with *E coli*.

Results: Ten of 20 subjects have completed this protocol to date and 4 of the 10 (40%) became colonized for $\geq$ 14 days. In addition, two of the three patients under the new protocol where pre-inoculation antibiotics were given became colonized. The rate of UTI slightly decreased from a baseline of 1.25 UTI per patient year to 1.02 per patient year while colonized. Only 1 of the 10 patients so far got UTI.

Conclusions: The encouraging results show that *E coli*-coated catheters are an effective means to colonize the neurogenic bladder of persons practicing ICP, and use of these catheters is associated with a decreased rate of UTI. This ongoing pilot study will guide the design of a larger, placebo-controlled, randomized trial of using bacterial interference to prevent UTI among persons with SCI.

EGFP/MMP-9 NULL MICE AS A MODEL FOR GENERATING GREEN FLUORESCENT BONE MARROW DERIVED STEM CELLS LACKING MATRIX METALLOPROTEINASE

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Previous studies of bone marrow transplantation (BMT) containing enhanced Green Fluorescent Protein eGFP (+) progenitors have elucidated the presence of a quiescent bone marrow derived (BMD) stem cell population within several layers of the mouse cornea. Further research has demonstrated the ability of these BMD stem cells to differentiate in various models of ophthalmic injury and healing responses such as corneal and retinal neovascularization. Some of the differentiated BMD stem cells in the cornea are antigen presenting cells like langerhans cells, dendritic cells, macrophages, and other MHC class-2 cells, several of which are necessary during tissue remodeling and repair. Other studies have demonstrated that the corneas of mice lacking matrix metalloproteinase-9 (MMP-9, Gelatinase B, Gel B) undergo rapid and unregulated re-epithelialization after damage, in comparison to normal control animals. The development of a viable mouse line constitutively expressing eGFP while lacking MMP-9 will enable us to better visualize these BMD stem cells in the healing cornea and aid us in elucidating their role in corneal wound healing.

Although eGFP is considered to be innocuous for cells expressing it, recent studies have determined that expression of eGFP can lead to deleterious effects. The potential of eGFP interfering with the development of this mouse line is magnified when combined with the well documented developmental problems of the MMP-9 Null Mouse, and the successful generation of a viable mouse line, constitutively expressing eGFP while lacking MMP-9, is not guaranteed.

THE ROLE OF PHOSPHOLIPASE D1 IN GLUT4 TRAFFICKING

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Glucose levels in the body are regulated by a network of cellular mechanisms that maintain homeostasis during periods of high-caloric intake and starvation. Insulin regulates the uptake of dietary glucose by inducing the redistribution of GLUT4 glucose transporters from intracellular sites to the plasma membrane (PM) of adipose and muscle cells. Therefore, finding the mechanisms that control GLUT4 trafficking is key in understanding insulin regulation of glucose homeostasis.

Insulin-signaling pathway(s) to GLUT4 remain(s) largely unknown, however the PI 3-kinase pathway has emerged as a major regulator of GLUT4 trafficking. Among the PI3-kinase downstream targets is Phospholipase D 1 (PLD1), which was previously identified to regulate GLUT4 trafficking. We used siRNA mediated PLD1 knockdown in 3T3-L1 adipocytes to explore the role of PLD1 in insulin regulation of GLUT4 translocation. We hypothesized that PLD1 regulates GLUT4 exocytosis in adipocytes, whereby its absence would decrease the translocation of GLUT4 to the PM. Although PLD1 knockdowns were not confirmed, electroporation of 3T3-L1 adipocytes with PLD1 specific siRNA, UUUACUAUCAACCACCUCUUUGACC, gave promising results. As a result, insulin-induced GLUT4 translocation to the PM was greatly affected. Verifying the effect of PLD1 siRNA knockdown sequence would allow us to confirm a possible role for PLD1 in insulin-regulated GLUT4 trafficking.

COMPARISON OF CT-ANGIOGRAPHY AND TRANSESOPHAGEAL ECHOCARDIOGRAPHY FOR THE EVALUATION OF AORTIC ATHEROSCLEROSIS IN STROKE PATIENTS

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A transesophageal echocardiogram (TEE) is considered the best test for evaluating the aorta as a possible source of emboli in stroke patients. However, TEE requires a tube placed in the patient's esophagus, which causes discomfort for the patient. Recently, advances in CT technology resulted in dramatic improvement in the quality of CT angiograms (CTA), which allow the visualization of the heart, aorta and carotid arteries during the ten-second procedure. CTA is noninvasive and more comfortable for patients, but the accuracy of CTA in evaluating the aorta is presently unknown.

Our goal was to compare CTA with the TEE in the evaluation of aortic atherosclerosis in stroke patients. Two hundred fifty CTA and TEE patients, who underwent a CTA and a TEE procedure within 3 weeks from each other, were retrospectively identified. Their CTA and TEE images were reviewed independently by a neuroradiologist and a cardiologist, respectively, for the absence or presence of atheroma, possible calcifications, ulcerations and/or pedunculated/mobile appearance. The ascending, transverse, and descending aortas were also evaluated for these features. The CTA and TEE results were compared using chi-square tests. The overall agreement between CTA and TEE for the presence/absence of aortic atheroma was 67%. Agreement for the grading of the atheromas was 79%. CTA and TEE agreed in 92% in the detection of clinically relevant grades 3 and 4 aortic atheromas.

In conclusion, CTA is an adequate alternative to TEE for the assessment of aorta as a possible source of clot in acute stroke patients.

SOURCE OF SUPEROXIDE GENERATION IN RESPONSE TO HIGH GLUCOSE IN MOUSE PROXIMAL TUBULE CELLS

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Introduction: Superoxide is the major reactive oxygen species in the kidney and can be generated by mitochondria or cytoplasmic enzymes including NADPH oxidase, xanthine oxidase or by uncoupled nitric oxide synthase (NOS). Increases in glucose concentrations result in an increase in superoxide production which contributes to renal damage associated with diabetes.

Purpose: To investigate the source of superoxide generated by high glucose in proximal tubule (PT) cells.

Methods: Mouse PT cells were treated with 5 mm and 25 mm glucose concentrations, as well as apocynin, allopurinol and water in 6 well plates. Superoxide production in each well was measured by chemiluminescence.

Result: Superoxide levels were similar in cells exposed to normal or high glucose. Apocynin inhibited 65.63% of superoxide production in normal glucose and 33.6% in 25 mm. Allopurinol inhibited 85.24% of superoxide production in 5 mm glucose and 93.51%% in 25 mm glucose treated cells.

Conclusion: Both NADPH and xanthine are responsible for most, but not all of the superoxide generation in mouse PT cells. Though exposure to high glucose did not increase total superoxide production, it stimulated xanthine oxidase, making it the predominant contributor. The remaining superoxide generation, not accounted for by NADPH and xanthine oxidase, may be attributed to enzyme activity in the mitochondria or uncoupled NOS.

DNA SEQUENCING OF PHAGE E34

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Mentor: Robert Villafane, PhD, Ponce School of Medicine, Ponce, Puerto Rico

One of the earliest and best known examples of biological regulation is the pathway by which some bacterial viruses (or bacteriophages or just phages) allow the bacteria that they infect to live. This pathway is known as the lysogenic pathway. The model system for these studies is the phage e34 which infects *Salmonella newington*. In the simplest case, the lysogenic or clear genes of the phage consist of a protein that sits on DNA sites that would cause the phage to lyse the cell. This is the cI gene or protein and it is a repressor. Another protein, cII, stimulates the synthesis of the cI repressor and a third protein, cIII, inactivates a host cell protein that would otherwise inactivate the repressor.

The DNA sequence of the entire phage e34 has been determined by the Villafane laboratory in collaboration with Dr Kropinksi (Guleph, Canada) and Dr Casjens (Utah, USA). Using primers obtained from the DNA sequence, we have produced PCR fragments that should contain the three clear genes. These genes are to be cloned into a high expression vector, pET30a-LIC (Novagen) and expressed. At the same time, a few of the mutations in these genes will be sequenced.

POLYMORPHIC VARIANTS IN MC1R AS MARKER FOR RISK OF MALIGNANT MELANOMA

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Malignant melanoma is characterized as a tumor arising from melanocytes undergoing metastasis. Melanocortin-1 receptor (MC1R) gene variants are implicated in the role pigmentation plays in melanoma carcinogenesis across populations. MC1R encodes a 317 amino acid G protein-coupled receptor that controls the gene expression of melanin in the skin. The coding region of MC1R is highly polymorphic and associations of variants with pigmentation phenotypes and risk for cutaneous neoplasms have been reported. Previous studies have shown that the developments of acquired nevi are a result of a sunburn history, lower latitude of residence and high intermittent UV exposure. Nevi are recognized as a very strong risk factor for developing malignant melanoma. Current investigation suggests that differences in allele frequencies of MC1R contribute different amounts of melanin produced in the skin. Individuals with specific MC1R genotypes frequently exhibit a red hair coloring (RHC) phenotype, and these individuals also experience the greatest amount of sun sensitivity, freckling and inability to tan. We hypothesized that by looking at non-synonymous single nucleotide polymorphisms (nSNPs) within MC1R, we may identify a higher risk subgroup of people, such as RHC, who will develop malignant melanoma in the future.

In order to test our hypothesis between MC1R gene variants and melanoma risk, genetic information and phenotypic data were evaluated using the Framingham SONIC Nevi Study. This study was conducted on 416 children 10–14 years of age, in Framingham, Massachusetts. Three hundred sixty-one children provided mouthwash samples for DNA extraction and MC1R gene sequencing. We compared information on ethnic background and important phenotypic measures (hair, skin, eye color, ability to tan, and mole counts on the back) with sequence variants in the context of the different populations.

THE DEVELOPMENT AND BALANCE OF SULFATION METABOLISM IN THE PEDIATRIC LIVER

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During prenatal and childhood periods, biochemical defenses are developing, making children susceptible to toxins. The balance between sulfotransferase (SULT) and arysulfatase (AS) may help chemical detoxification or in regenerating active chemicals. SULT detoxifies chemicals by making them hydrophilic and AS cleaves sulfated chemicals back to their parent compounds. This balance affects steroid hormones like dihydroxyepiandrosterone (DHEA) as wells as drugs and chemicals. Little is known on what regulates the activity and development of SULT and AS enzymes in children. Since the liver is a major site for SULT and AS enzyme activities in humans, we have measured SULT and arylsulfatase A and B levels activities of 24 pediatric liver cytosols (ranging from 13 days to 20 years of age) using biochemical assays titrated to provide maximal enzyme activity. We then compared the maximal levels of enzyme activities with the children's ages and sexes as well as comparing the SULT and AS activities to each other. No significant differences in SULT or AS with age were observed, nor were there any differences between males and females. SULT activity was 15–150 times higher than AS and this was significant ($P<0.001$, t test). In children, the balance of sulfation favors detoxification of drugs, dietary compounds and endogenous hormones with much higher SULT than AS activties. Ultimately, further research on the development of detoxification defenses may help us understand the development of diseases such as asthma and cancer as well as adverse reactions to drugs and toxins in children.

NUTRITIONAL ANALYSIS OF SERVICE PLANNING AREA 6, LOS ANGELES COUNTY

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Healthier dietary practices are usually prescribed to individuals with impaired cardiac functioning. Whether a person can access fresh fruit and vegetables and limit fat and caloric intake depends in part at least on the local environment. Food deserts have been shown to exist in many large metropolitan areas. Depending on individual economic circumstances, limitations in geographic access to healthy nutrition can be severe, particularly when considering concentrations of fast food restaurants and the corresponding lack of full service grocery stores that are common in inner-city areas. We hypothesized that certain areas within inner-cities of Los Angeles County suffer from limited geographic accessibility to sources of nutritious food (ie, full chain grocery stores) and have greater geographic accessibility to less nutritious food outlets, particularly fast food restaurants. If this is true it could be a contributing factor to impaired cardiac functioning.

To test the hypothesis, we examined geographic access to food outlets by type for health districts in Los Angeles County. All businesses that sell food in the county are required to be licensed by the Department of Health Services. We obtained all food vending licenses for the year 2002. Using the business addresses we geo-coded with ArcView GIS software to locate businesses geographically. We then classified the food outlets according to their North American Industrial Classification System (NAICS) index. These classifications were used to derive measures of geographic access to certain types of health-inhibiting or health-promoting foods (ie full chain grocery stores vs fast food restaurants). We then conducted a statistical analysis comparing inner-city areas with the other areas in the county to determine whether areas are nutritionally underserved.

MUTATIONS IN THE MYOSTATIN GENE

Student Researcher: Tameka Watson, King/Drew Magnet High School of Medicine and Science
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Mutations in the myostatin gene are associated with hypermuscularity which means that myostatin inhibits skeletal muscle growth. Myostatin has been identified as GDF8 (growth and differentiation factor 8) and is a muscle specific gene expressed only in the skeletal muscle. Overexpression of the myostatin gene in transgenic mice is associated with smaller muscle mass, better muscle performance, and is thought to cause a change in the skeletal muscle plasticity. We hypothesized that in adult skeletal muscle, myostatin has an effect on muscle performance by changing the muscle fiber type composition (transition).

To test the hypothesis the laboratory generated two genetically modified animals in order to learn the role of the myostatin on skeletal muscle performance. One of the animals was the myostatin transgenic mouse while the other was the myostatin knock out mouse. The myostatin transgenic mouse had a transgene that overexpressed the myostatin (Mst) protein under the regulation of a muscle specific creatine kinase promoter. The myostatin knock out mouse did not have the functional Mst protein. As control, we used wild type mice (C57B1/6) for our study.

ESCHERICHIA COLI THIOREDOXIN TRANSFORMATION AND PURIFICATION

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Research has shown that Parkinson's disease is linked to oxidative stress. Oxidative stress is noted by an imbalance between the production of oxygen in a biological system and the detoxification process of the system, where damages are also repaired. Thioredoxin (Trx) is a protein that comes in two different forms, Trx and Trx2. In this study we worked with Trx, which has 108 amino acids. Trx helps with the reduction of oxygen, and therefore may be able to help diseases affected by oxidation stress, such as Parkinson's disease and Alzheimer's disease. In order to test the beneficial factors of the Trx protein and learn more about how it can help and/or prevent these diseases, our lab needed to make and analyze the protein.

In order to prepare for the analysis using nuclear magnetic resonance (NMR), we used BL21(DE3) *Escherichia coli* (*E coli*) cells to make the Trx protein. Since there was a shortage of Trx DNA in the lab, we also used Novablue competent *E coli* cells to make more DNA, which will be used to make the protein further along in the process. The BL21(DE3) *E coli* cells were grown in *E coli* bacteria using agar plates with LB Broth and the ampicilin antibiotic. After growing the *E coli*, we sonicated the bacterial cells to lyse them and extracted the Trx. To purify the protein, we ran columns using the Trx extraction buffer (20 mm KH_2PO_4, 3 mm EDTA), and concentrated the protein and continued to run columns. By running acrylamide gels with protein samples using electrophoresis, we could see whether the Trx was purified. After purifying the protein, it was important to repeat the process to make sure our lab knew how to successfully purify the protein. We split the protein in two and recombined them to create a tighter quaternary structure needed to work with the protein in the laboratory. We then analyzed the thioredoxin protein using NMR.

CRUCIFEROUS INDOLE-BASED COMPOUNDS REGULATE CHOLESTEROL METABOLISM

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Increased blood low-density lipoprotein (LDL) levels, also known as bad cholesterol, are marked by increased secretion of apolipoprotein B (apoB), the protein moiety found on the surface of a LDL. Elevated levels of LDL-apoB can lead to increased risk of developing cardiovascular disease. Pharmaceutical drugs such as statins have proven to down-regulate LDL-apoB levels. Little is known, however, about nutritional factors, particularly indole derivatives from cruciferous vegetables such as broccoli and cauliflower, and their potential effect in lowering LDL-apoB levels. Our proposal was to screen various concentrations of two indole-based compounds, indole-3-carbinol (I-3-C) and 3,3' diindolylmethane (DIM), in an *in vitro* study using human hepatoma (HepG2) cells for their ability to inhibit apoB secretion following a 24-hour treatment. We hypothesized that I-3-C and DIM would inhibit LDL-apoB secretion dose dependently. The secretion of apoB and albumin was determined using an immunoassay called enzyme-linked immunoabsorbant assay (ELISA) with anti-bodies specific to each protein. Human serum albumin levels in the tissue culture media were also measured by ELISA to determine whether the effects observed were specific for apoB or were a result of no specific protein production effects. Trypan blue assays were performed to detect any cytotoxicity. Finally we attempted to extrapolate our *in vitro* findings using an *in vivo* hamster model with I-3-C. For the *in vivo* study, we performed an ELISA using an antibody specific to hamster apoB plasma from animals fed with a high fructose diet to induce hyperlipidemia, treated with or without I-3-C for 28 days.

In our *in vitro* study both indolic compounds were effective in lowering apoB secretion. Our I-3-C results indicated that I-3-C inhibited apoB secretion dose dependently. DIM showed to be the more potent than I-3-C, however, another secreted protein (albumin) was also inhibited. This indicated that the effects of DIM were not specific to apoB. In our *in vivo* study we tested blood triglyceride and apoB levels. This study showed that triglycerides levels decreased and ApoB levels remained the same when compared to control animal group. This finding in our *in vivo* study is still under investigation to explain why triglyceride levels decreased while apoB remained the same. These studies may lead to better understanding of how indole-based compounds can be beneficial in down regulating LDL-apoB levels.