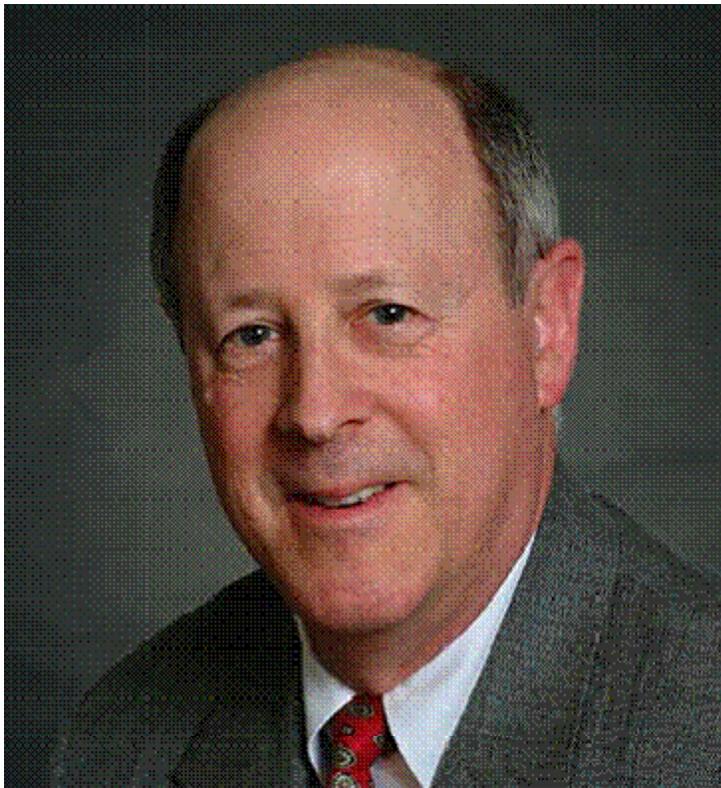


***DR. KENNETH A. SUAREZ  
MIDWESTERN UNIVERSITY  
RESEARCH DAY***

*Arizona College of Osteopathic Medicine  
College of Dental Medicine  
College of Health Sciences  
College of Pharmacy Glendale  
College of Optometry*



***Wednesday, May 1, 2013  
3:00-6:00 p.m.  
Gymnasium***



**Midwestern University's Annual Research Day is named in memory of Dr. Kenneth A. Suarez, whose leadership guided the Office of Research and Sponsored Programs for 25 years from 1982-2007. Dr. Suarez was instrumental in conceptualizing and implementing a number of MWU research-related policies and procedures, including Research Day.**

Kenneth A. Suarez, Ph.D, Professor of Pharmacology and Associate Vice President, Office of Research and Sponsored Programs received his Pharmacy Degree in 1967 and his Ph.D. in Pharmacology from the University of Rhode Island in 1972 before joining the faculty in the Department of Pharmacology at the Chicago College of Osteopathic Medicine. Dr. Suarez spent his early research career studying modifiers of microsomal electron transport on carbon tetrachloride hepatotoxicity and published most of his work in major toxicology journals. He wrote numerous review articles in Pharmacology and served as a book reviewer for the JAOA; he was a member of a number of professional societies both related to pharmacology and research administration. Ken served on many appointed, elected and *Ad Hoc* Committees during his tenure at Midwestern University. He received two Animal Facility Improvement grants and applied for and received funding for student research programs on campus from various pharmaceutical companies.

In 1982, as an Associate Professor, Dr. Suarez was also appointed to the position of Assistant Director of Research Affairs and rose to the rank of Professor of Pharmacology and was promoted to Associate Vice President of the Office of Research and Sponsored Programs. As Associate VP he was the Institutional Official responsible for ensuring the day to day requirements of the MWU research community were met. Dr. Suarez initiated a number of programs on campus to support student participation in research and The MWU Research Day Program was instituted by him to encourage student interest in academic medicine.

Dr. Suarez was a retired Lt. Col., in the USAR and served in the Medical Service Corp with the 427<sup>th</sup> Medical Laboratory until retiring from the USAR in 1996. He served in the USAR from 1970 until his retirement in 1996. He was married To Eileen and had one daughter, Christina. In his spare time he enjoyed bike riding and antiquing.

# 2013 Dr. Kenneth A. Suarez Student Research Day

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# AZCOM

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## **FUR Titration Assay shows *in vivo* *Klebsiella oxytoca* Fur activity**

Vinay Acharya, Allison Lambert and Jose Hernandez

*AZCOM, Midwestern University, Glendale, Arizona*

Almost all organisms have adapted ways to attain, store, utilize, and regulate the vital nutrient iron, which is used in processes ranging from DNA replication to oxygen transport and energy generation. Bacteria rely on Ferric Uptake Regulator (Fur), an iron-dependent DNA binding protein, to control the expression of more than 90 genes including the genes for iron acquisition, siderophore synthesis, oxidative stress, energy metabolism, and virulence factors. Iron metabolism has been widely suggested as a promising antimicrobial target, and the long-term goal of this project is to target the Fur protein due to its essential nature for cells viability. In this project, we tested the *in vivo* activity of the *Klebsiella oxytoca* Fur protein. First, by transforming  $\Delta fur$  *E. coli* strain 1780 with a plasmid containing *K. oxytoca fur*, we were able to recreate the actions of the Fur (+) *E. coli* strain JB1698 *in vivo*.  $\beta$ -galactosidase tests in *E. coli* 1780 and JB1698 using a Fur-controlled *lacZ* reporter gene show iron-responsive regulation only when the *fur* gene is present. In addition, we applied the Fur Titration Assay (FURTA) to test the degree of affinity of Fur for various gene promoters, which were cloned in a plasmid, and transformed into an *E. coli* strain H1717 (*fur*+). Twelve different plasmids containing various promoters of interest were prepared to transform *E. coli* H1717. *krrF* and *fepA* gene promoters were found to have the strongest affinity for the Fur protein *in vivo*. These results were independent of the vector system used for cloning purposes. Finally, an enriched *K. oxytoca* genomic library was prepared. Genomic FURTA experiments using this library identified four new intergenic regions potentially under Fur regulation. The results provide a proof of concept that FURTA experiments may be considered a useful strategy for testing *in vivo* *K. oxytoca* Fur activity.

*Vinay Acharya was supported by the Midwestern University Summer Fellowship Program. Jose Hernandez was supported by Midwestern University intramural funds.*

# **Arachidonic Acid-Induced Apoptosis in Human Renal Proximal Tubular Epithelial Cells**

Larry D. Alexander

*AZCOM, Midwestern University, Glendale, Arizona*

Arachidonic acid can induced several injury effects on renal tubular cells. The present study was aimed to investigate the effects of arachidonic acid on cellular apoptosis in human proximal tubular epithelial cells (HK-2) and its signaling mechanisms. Treatment with various doses of arachidonic acid resulted in dose-dependent decreases of cell viability and increases of reactive oxygen species (ROS). Arachidonic acid induced the phosphorylation of p38 MAPK, extracellular signal-regulated kinase (ERK1/2), and c-Jun N-terminal kinase (JNK), the nuclear translocation of NF- $\kappa$ B, and I $\kappa$ B-degradation. Cell death detection assay revealed arachidonic acid induced apoptotic cell death of HK-2 cells. Arachidonic acid-induced apoptosis was significantly attenuated by inhibition of the NF- $\kappa$ B and mitogen-activated protein kinase (MAPK) signaling pathways. Moreover, the antioxidant N-acetyl-L-cysteine (NAC) significantly inhibited arachidonic acid-induced apoptosis and NF- $\kappa$ B and MAPK activation. Taken together, these results indicate that arachidonic acid induced apoptosis mainly by stimulating ROS production and through NF- $\kappa$ B and MAPK signaling pathways in human renal proximal tubular cells.

*Larry D. Alexander was supported by Midwestern University intramural funds.*

## **Initial Migration Route Leading to the Settlement of Easter Island**

Kelsey K. Allen and Heather F. Smith

*College of Biomedical Sciences, Midwestern University, Glendale, Arizona*

*Arizona College of Osteopathic Medicine, Midwestern University, Glendale, Arizona*

The initial human settlement of Easter Island has been researched through archeology, osteology, linguistics, anthropology, and genetics, but the results thus far have been inconclusive. However, measurements of secondary dentition have yet to be examined as a genotypic proxy. Two initial migration models that have been proposed and disputed, which are a single-wave model and a two-wave model. The single wave model proposes that Polynesians travelled directly to Easter Island. The two-wave model proposes that Polynesians travelled to South America, then later continued on to Easter Island. In this study, the phenotypic data from the dentition, specifically the mesiodistal and buccolingual diameters of each tooth, are used to estimate genetic similarity between native Easter Islanders and South American and East Polynesian populations. Similarities in dental morphology assist in reconstructing patterns of gene flow and the population origin of Easter Islanders. Data were collected from skeletal remains of eight populations: Easter Island, Chatham Island, New Zealand, Marquesas Island, Solomon Island, Chile, Peru, and Venezuela. Distance matrices were utilized to calculate Euclidean distances between populations, and the native Easter Islanders were found to be most similar to South Americans when geographic regions were considered, and most similar to Peruvians when all populations were considered. Discriminant function analysis utilizing cross-validation provided high correct classification rates of Easter Islanders but high cross-validation rates of Easter Islanders as Peruvians, with lower classification rates of Easter Islanders as Solomon Islanders and Marquesas Islanders. When only regions were considered, Easter Islanders were classified and cross-validated as Polynesian 60% of the time and South America 40% of the time.

These results indicate that there is significant genetic admixture between Easter Island and Polynesia, as well as South America. This supports previous research, which has described evidence of a two-wave migration model from Polynesia to Easter Island.

*Kelsey K. Allen was supported by the College of Biomedical Sciences at Midwestern University. Heather F. Smith was supported by Midwestern University faculty start-up funds.*

# Investigating the Middle Eocene Micromammal Community at WU-26: A High-Resolution Stratigraphically Controlled Fossil Locality

Carl A. Astbury and K.E. Beth Townsend

*AZCOM, Midwestern University, Glendale, Arizona*

Micromammals (very small mammals: e.g., shrew, mice, some rats) have long been absent from discussions of middle Eocene mammalian communities due to their poor representation in historical collections. Since 2006, we have been screen-washing the locality WU-26 found in the Uinta Formation, Uinta Basin, Utah, for small mammals and have recovered over 400 individual specimens. WU-26 is a stratigraphically controlled single locality very high in the Uinta Formation and we predict that it should yield a late Uintan, or Ui3 fauna. With few exceptions, micromammals from the Uinta Formation have only been recognized or placed in faunal lists without any definitive morphological study of any specimens. Of the small mammals lipotyphlans (moles and shrews) and marsupials (opposums) have only been recognized, but have never been formally described from the Uinta Formation. Here we present the first assemblage of Uinta Formation micromammals that have undergone comparative study and resulted in a definitive faunal list for this single locality. The assemblage includes two species of the small opossum Herpetotherium, the lipotyphlans Centetodon and cf. Scenopagus, the smallest morph of the omomyid primate Omomys carteri, and the first rabbit in North America, Mytonolagus petersoni. A number of small rodents have also been recovered: Pareumys, Pauromys, Microparamys, and Janimus. We have determined that the ranges for the sespedectid, Scenopagus, and the geolabidid, Centodon, have been extended through to the late Uintan, basing the late Uintan designation on the presence of the rodent Janimus.

*Carl A. Astbury was supported by the Midwestern University Summer Fellowship Program. K.E. Beth Townsend was supported by Midwestern University intramural funds.*

# Natural Redistribution of End-protection Proteins in Ageing Cells as Telomeres Shorten

Michelle E. Baribault\*, Mark J. Swanson<sup>#</sup>, and Nancy S. Bae<sup>%</sup>

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Telomeres are a specialized structure of tandemly repeated, G-rich sequences that protect the gene-encoding region of the eukaryotic linear chromosome. These structures are oriented toward the ends of chromosomes and are composed of a double-stranded region followed by a single-stranded overhang of telomeric repeats. However, due to the linear nature of eukaryotic chromosomes, this inadvertently puts the ends at risk for being recognized as damaged or broken DNA. To escape inappropriate cellular repair mechanisms, telomeres are bound by a six-member protein complex known as the Shelterin complex. These proteins maintain and protect the integrity of the genome. Overtime, replication will cause telomere shortening, leading to ageing of the cell. Our hypothesis is that as telomeres become progressively shorter, the number of protein binding sites would decrease. This suggests that the components of the Shelterin complex lose their ability to assemble at telomeres, exposing the ends and potentially leading to the activation of DNA damage response pathways. In an attempt to answer the question of whether there is a relationship between telomeres and the Shelterin complex through the natural progression of ageing, we utilized a human primary fibroblast cell line. Human Dermal Fibroblasts from neonatal foreskin (HDFn) were grown in culture, and the levels of a number of Shelterin complex components were measured using immunoblotting techniques. The protein levels of TRF2, RAP1, POT1 and TIN2 were compared between young cells and aged cells. Accompanied by quantitation analysis, each protein component mentioned above was found at higher levels in young cells when compared to aged cells. This data was complemented by the telomere length analysis utilizing Southern blotting techniques. Younger cells had longer telomeres and thus more binding sites for Shelterin components to assemble. Our findings highlight the importance of studying the natural progression of ageing and indicate for the first time that a correlation exists between telomere length and Shelterin protection.

## **Deciphering how general anesthetics work: the role of ion channels**

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For more than 160 years, volatile anesthetics (VAs) have been used, yet the action of VAs upon the central nervous system is unknown. Recent evidence suggests that VA effects occur through multiple targets as opposed to a single common mechanism. Previous studies have indicated that ion channels may be VA targets. Our previous study was the first comprehensive analysis of ion channels in the response to isoflurane. We tested 359 ion channel genes within *Drosophila melanogaster* in an RNA interference (RNAi) screen to determine potential candidates for VA action. In the previous study, a small halothane screen was performed for data verification on a few ion channels; however, this revealed differences in their responses to the two VAs. As isoflurane and halothane have overall similar chemical structures, similar responses were expected. This study explores the effects of halothane on ion channels and compares them with the isoflurane data. RNAi constructs were used for gene-specific silencing of ion channels throughout the genome. An inebriometer was used to quantitatively study the effects of halothane on the flies. The data were analyzed and compared with the isoflurane data. Of the 340 genes tested, almost 39% exhibited resistance which is very similar to the 40% resistant to isoflurane. Of all the genes that showed resistance to VAs, 69 were resistant to halothane only, 78 had resistance to isoflurane only, and 64 showed resistance to both VAs. In this shared resistance category, we found that 11 genes were either resistant or strongly resistant to both VAs. A more detailed analysis will be presented at the meeting. Analysis of the data revealed that there are significant differences between the actions of isoflurane and halothane on ion channels. These differences indicate that there are many potential pathways for VA action, even among those with similar chemical structures.

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## Identifying the cellular mechanisms responsible for genistein's stimulatory action on jejunal chloride secretion

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We have previously shown that daily subcutaneous injections with the phytoestrogen genistein (600 mg genistein/kg body weight/day, 600G) significantly increases basal intestinal chloride (Cl<sup>-</sup>) secretion (Isc, a measure of transepithelial secretion,  $\mu\text{A}/\text{cm}^2$ ) by  $70 \mu\text{A}/\text{cm}^2$  (n=15) in intact C57BL/6J female mice after 1-2 weeks of treatment, compared to controls (DMSO vehicle injected). Orcheicectomy (OVX), and thus removal of endogenous estrogen, had no effect on the 600G-mediated increase in basal Isc, i.e. both OVX and intact females treated with 600G had significantly increased basal Isc (by 1.6-fold) compared to controls. Given the estrogen-like characteristics of genistein (i.e. it is structurally similar), we compared the effects of daily estradiol (E2) injections (10 or 20 mg E2/kg body weight/day, 10E2, 20E2) on basal Isc in intact and OVX mice. In intact mice, neither 10E2 nor 20E2 had an effect on Isc, however, in OVX mice, 10E2 significantly increased basal Isc (i.e. it mimicked 600G). The goal of this study was to characterize the potential intracellular signaling pathways responsible for mediating the genistein-stimulated (or 10E2-stimulated) increases in basal Isc. We measured total protein expression in isolated segments of jejunum using western blot from the following six groups of mice: intact 0G, intact 600G, intact 10E2, OVX 0G, OVX 600G, OVX 10E2. The proteins of interest were: Akt, pAkt, pTen, pGSK3B, pCRaf, and PDK1. All blots were normalized to GAPDH levels (n=6-7 mice/group). Total Akt was significantly increased 3.5-fold in OVX 10E2 compared to intact counterparts. In intact mice, pAkt levels were significantly increased 2.3-fold by 600G compared to controls. Interestingly, OVX controls had 3.2-fold significantly greater levels of pAkt compared to intact counterparts. These data suggest that the presence of endogenous sex steroids alter the intracellular pathways (i.e. intact and OVX mice are different). Moreover, these data also suggest that genistein and E2 do not necessarily follow the same intracellular signaling pathways. *Ashesh Bhakta was supported by the MWU DO Summer Fellowship Program. Layla Al-Nakkash was supported by the Soy Health Research Fund.*

## **The Treatment of TBI: A Review of the Evidence**

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Traumatic Brain Injury (TBI) affects 1.4 million Americans per year, including many of those we consider our heroes: athletes and soldiers. Nearly 23% of soldiers coming back from a Brigade Combat Team had a TBI confirmed history, and 65% of NFL players report at least one concussion in their career. Most of those with moderate or severe TBI are incapacitated to some degree; mild TBI is often followed by mental disorders such as depression, substance abuse and anxiety disorders, and chronic pain syndromes as well as other physical, cognitive and behavioral problems.

TBI is diagnosed using the Glasgow Coma Scale, which tests for three patient responses: eye-opening, verbal response, and motor response. The GCS measures TBI on a 15-point scale, using the categories of mild TBI (13 points or higher), moderate (9-12), and severe (below 12). The most common method for diagnosing TBI is the non-contrast computerized tomography scan.

Articles evaluating the treatment of TBI were identified using Boolean search terms in Google Scholar. Of the more than 520,000 articles discovered, 85 met the review criteria and were selected for the study. The most commonly studied treatments for acute TBI are hypothermia, hypertonic saline, hyperventilation, and decompressive craniectomy. The most commonly studied treatments for chronic TBI are Ach-ase Inhibitors, Methylphenidate, and Amantidine. We recommend that physicians use hypertonic saline treatment as a substitute for Mannitol, and that hypothermia, decompressive craniectomy, and Dexanabinal are other promising treatments. Osteopathic medical treatments have only been studied as components of multitherapeutic treatments, and only in studies of lower quality. Osteopathic treatments may show promise of properly researched.

## **A Preliminary Study of the Effects of Acute Oxytocin Administration on Cardiovascular Function in Conscious Unrestrained Rats using Telemetry**

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Oxytocin (OT) is the most abundant hormone in the body and is traditionally associated with female reproductive. However, we have demonstrated that heart and vasculature are key sites of OT synthesis, exerting robust effects on the cardio-renal axis, including diuresis, bradycardia and reductions in blood pressure. In this study, we determined the effects of acute OT administration on hemodynamic parameters in conscious unrestrained rats exhibiting hypertension. Male spontaneous hypertensive rats (SHR) were used, with Sprague-Dawley rats (SD) serving as controls. Rats were anesthetised with isoflurane and then implanted with TA12PA-C40 radiotelemeters in the abdominal aorta for the direct measurement of blood pressure (BP) and heart rate (HR). After a 10-day recovery period, OT, given intravenously at the concentrations of 0.1, 0.2, 0.4 mg/kg were recorded over a 90-min period. Baseline recordings revealed that HR was significantly lower in SHR rats compared to SD rats. However, all measurements of arterial blood pressure and cardiac work were significantly elevated in SHR rats compared to SD rats. Infusion with all concentrations of OT increased BP in both SD and SHR rats. In SD rats, BP remained elevated with all concentrations used, whereas in SHR, 0.2 and 0.4 mg/kg restored BP back to control (saline) levels. There were no effects of OT on HR in SD rats at the concentration of 0.1 mg/kg, but at higher concentrations, HR was reduced and periodically kept below control heart rates. In SHR, similar effects of OT on HR were seen with the exception at the concentration 0.1 mg/kg, in which a brief increase in HR compared to the control infusion was observed. Our results indicate that OT produces a rapid and favourable drop in BP in the SHR only, whereas a bradycardia is seen in both SD and SHR rats.

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## **Where Did My Aspie Go? A Case Study and Exploration of Changing Diagnostic Criteria**

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The American Psychiatric Association is scheduled to introduce the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) in May 2013. There will be changes made to several disorders including ADHD, Bipolar Disorder, Depression, OCD and Asperger's to list a few. One of the more notable changes will be the incorporation of Asperger's into the Autism Spectrum Disorders. The diagnostic criteria for Autism Spectrum Disorder have been adjusted in the hope that future diagnoses will be more specific and reliable. Using the case of a 15-year-old male with a long history of Asperger's, we explored the potential ramifications of this new classification system. These include the challenges of determining diagnoses based upon spectrum classification, the impact on social and educational systems for management and the potential stigma related to patients with the change in diagnosis. It is imperative that physicians who treat mental health issues become familiar with the proposed changes.

## **The Effects of Resveratrol on Bone and Growth Plate Cartilage in Leptin-Deficient Mice**

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In this study, we tested the effects of resveratrol treatment on bone and cartilage tissue in wild-type and ob/ob mice. Ob/ob mice are leptin-deficient and serve as an animal model for obesity and diabetes and exhibit reduced bone mass relative to controls. Resveratrol is a phytoestrogen that has been shown to maintain bone mass by inhibiting osteoclastogenesis and stimulating osteoblast activity. Our findings indicate ob/ob mice have reduced bone strength relative to controls. However, ob/ob mice treated with resveratrol exhibited significantly greater cortical area and resistance to torsion than untreated ob/ob mice ( $p < 0.05$ ). A similar pattern was observed with resveratrol treatment of wild-type control mice. In both wild-type and ob/ob mice, resveratrol treatment reduced the thickness of the calcified layer of the proximal tibia growth plate ( $p < 0.05$ ), but the overall thickness of the growth plate remained unchanged. Our results suggest resveratrol treatment affects bone and cartilage tissue in the mouse model for obesity and diabetes.

*Joseph Cooley was supported by the Midwestern University Summer Fellowship Program.*

## **Malignant melanoma and Spitz nevus incidence in the pediatric population**

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The Rochester Epidemiology Project, a complex array of medical record data as well as medical and surgical indexing systems, provides accurate incidence data for diseases diagnosed in Olmsted County and was used to calculate incidence data for pediatric melanoma and Spitz nevus. All cases of melanoma and Spitz nevus in persons <18 years of age were identified from 1950-2004 through the Rochester Epidemiology Project database. A chart review of over 228 charts was performed to identify clinical characteristics and diagnoses. Review of pathology was performed by a single dermatopathologist to confirm diagnoses and further delineate pathologic characteristics. Cases found not to be consistent with melanoma or Spitz nevus were excluded from the study. Variables considered included diagnosis, age, sex, treatment, recurrence, and follow-up time. During 1950-2004, 7 cases of melanoma, 55 cases of Spitz nevus, and 1 case of atypical Spitzoid tumor were identified. The overall incidence of melanoma was 0.49 per 100,000 persons [95% CI, 0.1-0.9] and of Spitz nevus was 3.63 [95% CI, 2.7-4.6]. The incidence of Spitz nevus increased significantly from 1990 to 2004 while the incidence of melanoma remained stable throughout the studied timeframe.

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## **When Screening Mammograms Mislead: Delayed Diagnosis of Triple Negative Breast Cancer in a 39-year-old Female**

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The leading cause of death in Hispanic women is breast cancer, even though its incidence and death rate are lower than in non-Hispanic white women. Hispanic women are also more likely to have a higher stage of breast cancer at the time of diagnosis and less likely to be diagnosed through screening mammograms. Furthermore, Hispanic women are more likely to develop triple negative breast cancer, which is typically associated with younger age at diagnosis, being an interval cancer, BRCA gene mutation, and a poorer prognosis. The American College of Obstetrics and Gynecology currently recommends annual screening mammograms beginning at age 40 for all women and enhanced screening in women with high risk of breast cancer. Once a palpable breast mass is discovered, the American College of Radiology recommends diagnostic mammography as the initial evaluation, followed by a breast ultrasound. This is a case of rapidly aggressive, triple negative breast cancer in a 39-year-old Hispanic female with strong family history of breast cancer. The patient noticed a palpable mass in her left breast and presented to a clinic less than two months after a screening mammogram with benign findings. Because of this recent benign mammogram and previous ultrasound and biopsy showing multiple fibroadenomas of the breast, further diagnostic imaging was deferred. Seven months after the screening mammogram and five months after the patient's initial complaint of a breast lump, diagnostic mammography revealed findings that were highly suggestive of malignancy. Subsequent biopsy was consistent with infiltrating ductal carcinoma, and MRI showed diffuse skeletal involvement. This case highlights the need for a high index of suspicion and prompt workup for breast cancer in a Hispanic woman with a palpable breast mass and positive family history, despite a recently benign mammogram.

## **The Neurotoxic Effects of Bisphenol A in Neonatal rat pups and Adult in Sprague-Dawley rats.**

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Bisphenol A [BPA; 2,2-bis- (4-hydroxyphenyl) propane] is an environmental estrogen, which disrupts the endocrine system and has been shown to exert toxic effects on mammalian cells and tissue. BPA is used in countless products such as water bottles, coating from the inside of cans, food containers and dental composites. In the present study, we investigated the neurotoxicity of BPA and the underlying mechanisms of action in 1) the brains of adult male Sprague-Dawley rats through direct exposure and 2) the developing brain of male pups exposed to BPA via their mothers. Adult male rats and pregnant female rats were administered BPA orally for 20 and 34 consecutive days, respectively. Protein levels of cytochrome c, caspase 3 and cleaved caspase 3 were measured using western blotting in striatal and pituitary tissue. Oxidative stress was assessed by examining reduced glutathione (GSH) and reactive oxygen species (ROS) generation in the presence of catalase and superoxide dismutase. Cell apoptosis was evaluated by TUNEL staining using fluorescent microscopy. Our findings suggest that pituitary tissue may display more damage from BPA exposure than striatal tissue. Moreover, the enhanced toxicity in male pups who were exposed to BPA through maternal exposure may suggest a more neurotoxic effect of BPA compared to adult exposure, thus providing a potential lead for the different effects that low-BPA dose can have on the developing and adult neuronal system in human body.

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## **Not Just Constipation: A Case of Wilms' Tumor in an Eight-Year Old Girl**

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Wilms' tumors are the most common renal tumors found in children, with about 500 cases occurring in North America each year. Wilms' tumors are usually found in children under the age of five presenting asymptotically with a palpable abdominal mass. This case illustrates an 8-year-old Hispanic female who presents to the emergency department with complaints of worsening abdominal pain, sudden onset of fever, and constipation that has been unresponsive to conservative outpatient treatment. Physical examination reveals tenderness to palpation in the right upper and lower quadrants warranting imaging studies. A chest x-ray reveals an elevated right hemidiaphragm, and an abdominal CT reveals a large right-sided tumor involving the right kidney, right lobe of the liver, and a portion of the diaphragm. A diffusely anaplastic stage III Wilms' tumor is found. This case emphasizes the importance of a complete physical exam and plain film imaging in evaluating childhood abdominal complaints.

## Comparative morphology of owl and hawk extraocular muscles

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Every vertebrate has six extraocular muscles that attach to the optic globe for the purposes of eye movement. Birds have two additional muscles attached to superior and inferior nictitating membranes, transparent membranes that can be drawn over the eye for protection. Owls and hawks are both relatively large-bodied, predatory groups of birds. Both groups use visual cues to hunt and as such exhibit large relative and absolute eye sizes. Hawks appear to have extraocular muscle function similar to other vertebrates in that they utilize eye movements to observe visual space. However, owls do not utilize extraocular motion and instead have evolved elaborate neck movements to observe visual space. While owls do not appear to move their eyes at all, they do retain reduced extraocular muscles in the same configuration as all other vertebrates. To date owl extraocular muscles have not been described. In this study, we describe owl extraocular muscles by comparing them to hawks. We dissected and observed extraocular muscles of eight birds of varying body size, including two barn owls (*Tyto alba*), two Great Horned Owls (*Bubo virginians*), two Red-tailed Hawks (*Buteo jamaicensis*), and two Harris Hawks (*Parabuteo unicinctus*). The extraocular muscles were removed and weighed to obtain relative sizes, and then prepared for histology and individual muscle fibers were counted. We discovered that hawks consistently have larger extraocular muscles than do owls, even when accounting for eye size and body size differences. However, the two additional muscles attached to the nictitating membranes were also reduced in the Barn Owl, but not in the Great Horned Owl.

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*Wade Grow, Lawrence Suchocki, and Margaret Hall were supported by Midwestern University intramural funds*

## **The effect of inhibition of the renin-angiotensin system on the histopathology of spinal compression injury**

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**Objectives:** Inhibition of the renin-angiotensin system (RAS) has been shown to be neuroprotective in a variety of neurological disorders, including experimental autoimmune encephalomyelitis, Parkinson's disease, and stroke. One proposed mechanism for the protective effects of RAS inhibition is modulation of the inflammatory response. Because post-injury inflammation has been implicated in degenerative cascades initiated by mechanical trauma to the spinal cord, we sought to determine whether a similar protective effect of RAS inhibition would occur following spinal compression injury.

**Design:** Spinal compression injury in female Sprague-Dawley rats was induced at the T8 vertebral level. Beginning one day post-injury (dpi) and continuing for the duration of the study, rats received daily intraperitoneal injections of either captopril (angiotensin converting enzyme inhibitor), losartan (selective antagonist of the angiotensin II, type 1 receptor), or vehicle. Rats were sacrificed at 7, 14, or 28 dpi and the spinal cords collected for immunohistochemical staining of specific inflammatory cell populations.

**Results:** The overall magnitude of the macrophage response to injury was evaluated by OX-42 immunohistochemistry (IHC). The ED-1 antibody labels actively phagocytic macrophages and has been used as an index of post-injury macrophage activation. There was no significant effect of captopril or losartan treatment on OX-42 or ED-1 immunoreactivity at the injury epicenter. The T-cell response to injury was evaluated by OX-19 IHC. Captopril and losartan appeared to have divergent effects, with captopril treatment significantly increasing the number of T-cells infiltrating the injury site and losartan decreasing it.

**Conclusions:** Our data demonstrate that modulation of the RAS by post-injury administration of either captopril or losartan had no effect on the overall macrophage response to injury at the compression site. With regard to T-cells, captopril enhanced infiltration of the injured spinal cord while losartan diminished it. Previous reports have suggested that RAS inhibition produces neuroprotection by modifying the functional phenotype of CNS macrophages (e.g., shift from a pro-inflammatory M1 profile to an anti-inflammatory M2 profile). The antibodies we used to evaluate the macrophage response to injury did not allow us to determine whether such a phenotype shift occurred in response to our treatment. In summary, our data show that inhibition of RAS selectively modifies cellular aspects of the inflammatory response to spinal compression injury. Further research is necessary to further define the therapeutic potential of RAS inhibition.

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## **Dental Dimensions, Polar and Nonpolar Teeth, and Genetic Relationships**

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Teeth are organized as a series of recurring and closely related components that undergo changes in structure and position with evolution. Butler's field theory states that when teeth grow, they differentiate into morphogenetic fields, which ultimately determine how teeth in each field will develop structurally. Each morphogenetic field has one more genetically-stable tooth, which is referred to as the polar tooth. Previous research has suggested that dental dimensions reflect genetic relationships among human populations, and it is known that there is a valuable genetic component to tooth dimensions, but a direct comparison of dental morphology and molecular distances among modern human populations had not been conducted. In this study, mesiodistal and buccolingual measurements were recorded with dental calipers from skulls of museum samples from 8 different modern human populations, and quantified to determine if these dimensions reliably reflect molecular distances. It was also investigated whether the dental dimensions of polar or nonpolar teeth, males or females, and mandibular or maxillary teeth reflected genetic distances more reliably. Despite what past studies have suggested, no significant correlations were found between any of the dental dimensions studied here and genetic distances. Since there was no apparent correlation in the samples studied, future studies should utilize dental morphology with caution when attempting to infer genetic relatedness from dental dimension data and/or utilize them in combination with other types of biological data.

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*Heather F. Smith was supported by Midwestern University faculty start-up funds.*

## **Case Study: Treatment of Hydrocephalus with Osteopathic Manipulative Medicine**

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**Context:** To present a case in which Osteopathic Manipulative Medicine (OMM) was used to treat an infant with hydrocephalus.

**Patient and Method:** A 5-month-old female presented with her parents for osteopathic evaluation and treatment based on her diagnosis of hydrocephalus. She underwent resection of a cerebrospinal fluid-filled encephalocele and placement of a ventriculoperitoneal shunt at birth. She has been slow in reaching her neurodevelopmental milestones and attaining fine motor skills. The infant's parents also note that she has difficulty turning her head to the left and tenderness to palpation of her neck. A full osteopathic evaluation was performed and somatic dysfunction found in several body regions including the cranium. Significant findings included scaphocephaly, elevated cranial rhythmic impulse rate, inferior vertical and right lateral cranial strains, compressed left occipital condyle, and hypertonic left sternocleidomastoid muscle. These somatic dysfunctions were treated with cranial osteopathy and other gentle OMM techniques. She has been treated for a total of five treatments at two-week intervals. The patient concurrently underwent physical therapy, occupational therapy and speech therapy as prescribed by her neurosurgeon.

**Results:** After the first treatment, the infant's parents noted improvement in her ability to turn her head to the left and a decrease in her neck tenderness. In addition, she was able to reach in front of her body and above her head, something she was unable to do before. After her third OMM treatment, she was able to lift her head off the ground from the prone position for the first time. Her parents and her therapists have noticed slow improvement in her fine motor skills.

**Conclusion:** This infant with hydrocephalus experienced significant improvement during the course of her OMM treatments. The osteopathic literature describes OMM as a safe complementary treatment option for patients with hydrocephalus once acute hydrocephalus has been ruled out. Further studies need to be conducted to determine the effect that OMM has on the hydrocephalic brain and cerebrospinal fluid flow.

# Glial Fibrillary Acidic Protein Interacts with a Human Telomeric Protein That Stabilizes Genome Integrity

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As human cells age and senesce, their chromosomes shorten at their ends, or telomeres, due to a problem with replicating linear DNA. Cells prevent the loss of critical information by having a buffer at the end of the chromosome, which consists of repeats of a short sequence. This sequence is bound by a set of proteins in a complex that also protects the chromosomes from DNA repair mechanisms that would join them end-to-end as if they were double-stranded breaks. One of the proteins in this complex, RAP1 (repressor activator protein 1), plays a critical role in preventing chromosomal end-fusion. In the absence of RAP1, the ends of human chromosomes fuse together, resulting in typical cancerous phenotypes. This raises the question as what other proteins might interact with RAP1.

A yeast two-hybrid screen was conducted to identify proteins that interact with RAP1. Using RAP1 as bait and a human fetal brain cDNA library as the prey, GFAP (glial fibrillary acidic protein) was identified as a positive candidate. The direct interaction was verified *in vitro* using tagged versions of GFAP and RAP1 expressed in bacteria. Further domain mapping of the interaction was conducted using four well-defined domains of RAP1. The interaction was mapped between the conserved myb domain of RAP1 and carboxy-terminus of GFAP and between the coiled domain of RAP1 and carboxy-terminus of GFAP.

In order to identify the cellular location of GFAP, a cell line that expresses high levels of endogenous GFAP was used. Cellular fractionation and immunofluorescence imaging were carried out concurrently. Results from cellular fractionation indicated that GFAP is located in both the nucleus and the cytoplasm. Immunofluorescence imaging showed the localization of GFAP primarily in the cytoplasm, with a small amount at the nuclear periphery.

Mutation and misregulation of GFAP are involved in several neurodegenerative diseases such as Alexander's disease (AxD). Based on our data, it is possible that part of the effect of AD is through RAP1 and telomere dysfunction, *i.e.*, through premature aging.

## **The effects of TNF- $\alpha$ and Ceramide on Insulin Receptor signaling and its localization to Caveolae in C2C12 Myocytes**

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Tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and ceramide are associated with the etiology of insulin resistance. Since skeletal muscle is a key metabolic tissue, defects in insulin signaling within skeletal muscle are central to the pathogenesis of diabetes. There is evidence for the involvement of caveolae membrane rafts and caveolin proteins in the modulation of insulin signaling and glucose uptake. Thus, we propose to elucidate the signaling mechanisms by which TNF- $\alpha$  and ceramide induce insulin resistance in C2C12 myocytes and the role of caveolins in the organization of this pathway within the membrane. We hypothesized that exposure of C2C12 myocytes to TNF- $\alpha$  and ceramide will result in altered insulin signaling, decreased glucose uptake, and translocation of the insulin signaling cascade away from caveolae. C2C12 myocytes were treated with TNF- $\alpha$ , ceramide, and insulin, individually and in combination. Treated cells were subjected to Western blot analysis for insulin receptor- $\beta$  (IR- $\beta$ ), phosphorylated IR (p-IR), Protein Kinase B (PKB/Akt), phosphorylated Akt (p-Akt), caveolin-3 (Cav-3), glucose transporter-4 (Glut-4), and actin (as loading control). Total protein expression of IR, Akt, Cav-3, and Glut-4 was unaltered with treatment. IR phosphorylation was decreased in cells treated with TNF- $\alpha$ . However, Akt phosphorylation was significantly increased with TNF- $\alpha$ , particularly after 48 hours exposure. This data suggests that the effect of TNF- $\alpha$  on insulin signaling is a result of a cross-talk mechanism. Analysis of total glucose uptake after TNF- $\alpha$  treatment is currently underway. This data will further the identification of novel signaling mechanisms leading to insulin resistance as a prerequisite to improved diagnosis and therapeutic treatments for diabetes.

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# Th17 Pathway Genes are Altered by Spinal Cord Injury

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**Abstract** Spinal cord injury (SCI) elicits a neuroinflammatory response involving cells of both innate and adaptive immunity. A new subtype of T-helper cells, Th17, plays a role in several neuroinflammatory pathologies including multiple sclerosis and stroke. However, the role of Th17 cells in neuroinflammation secondary to spinal cord injury has not been evaluated.

**Findings** Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) arrays were used to evaluate postinjury expression of 84 genes relating to the Th17 pathway. At 7 days post injury (dpi) mRNAs of 18 genes were increased and 27 were decreased compared to controls. Of these, 4 genes remained up- and 15 downregulated at 28 dpi. Although the genes for transforming growth factor (TGF) $\beta$ -1 and interleukin (IL)-6, cytokines required for differentiation of Th17 cells, were both upregulated at 7 dpi this was not associated with increased IL-17. Indeed, *Rorc*, IL-17b, and IL-17f mRNAs were all decreased while IL-17a and IL-17c mRNAs were unaffected by injury. This was perhaps because IL-23a was also decreased, which is required for the survival and proliferation of Th17 cells. Similar effects were observed at 28 dpi, although generally lower in magnitude.

**Conclusion** Although Th17 cells and the IL-17 family of cytokines mediate cross talk between adaptive and innate immunity in many pathologic conditions such as multiple sclerosis, our data do not support a role for this T-cell subset in the chronic inflammatory response to SCI.

*T. Bucky Jones and Carleton B. Jones were supported by funds from MWU Arizona College of Osteopathic Medicine and MWU College of Health Sciences.*

## **Beta-Arrestin in Alzheimer's Disease**

Luke Killpack, Douglas Jones, PhD, Pamela E Potter, PhD

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Muscarinic receptors become from G-proteins in Alzheimer's disease (AD). We have demonstrated uncoupling in brain samples from patients with AD, as well as those with  $\beta$ -amyloid deposition and neuritic plaques who are not diagnosed with dementia.<sup>1</sup> Thus, uncoupling of the receptors may be one of the steps involved in the pathogenesis of AD. In To further determine the mechanism underlying this phenomenon, we examined signal transduction in four groups: patients diagnosed with AD and age-matched controls with many plaques (MP), sparse plaques (SP), or no plaques (NP). Plaque levels were correlated with loss of cholinergic neurons as assessed by choline acetyltransferase activity. Levels of signal transduction markers were measured using Western blot. Levels of  $\beta$ -amyloid were measured using ELISA.

We found previously that the Gq/11 protein was shifted from the cytosol to the membrane fraction and that levels of G-protein coupled receptor kinase GRK-2 were significantly decreased as plaque levels increased, which correlated with the increase in  $\beta$ -amyloid and loss of ChAT activity. We then measured levels of  $\beta$ -arrestin, a protein involved in receptor recycling, in the four groups. There was a trend towards decreased  $\beta$ -arrestin as plaque level increased. It is possible that alterations in GRK, coupled with decreased beta-arrestin, could impair muscarinic receptor recycling. Loss of recycling could lead to down-regulation or uncoupling of the receptors. That this occurs prior to the onset of dementia, coupled with studies showing that loss of cholinergic input can increase deposition of beta-amyloid<sup>2</sup>, suggest that the cholinergic receptor deficit should be addressed as early as possible when attempting to prevent or treat Alzheimer's disease.

1. Potter, PE *et al*, Acta Neuropathol. 122:49-60, 2011
2. Beach, T *et al*, Neurosci. Lett. 283, 9-12, 2000

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# **The Dilated Rarity of Complications With Herpes Zoster**

## **Case Study**

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Herpes zoster, commonly known as Shingles, is a disease caused by the reactivation of varicella-zoster virus, a human herpes virus. Only 10-20% of herpes zoster cases have subsequent ocular involvement, commonly known as herpes zoster ophthalmicus (HZO), which is a result of viral invasion of the ophthalmic branch of the trigeminal nerve.

We present a unique case of herpes zoster ophthalmicus in an elderly patient, which rapidly progressed to oculomotor nerve palsy, a rare sequela of HZO. Recognition of HZO as a complication of herpes zoster is imperative, due to the potential for cranial nerve involvement and irreversible visual deficits that ensue if the diagnosis is missed. Symptoms of oculomotor palsy and other rare complications are easily diagnosed with a simple ophthalmic exam at the bedside and can start a chain of therapeutic measures, which can save vision in the effected eye. This case serves as a reminder of the importance of vaccination against herpes zoster. Primary healthcare providers should encourage their elderly patients to boost cell-mediated immunity with the vaccination, which has been shown to be safe and effective in preventing herpes zoster infections.

Because of the possible visual consequences, HZO requires a heightened awareness, diligent daily examination for signs of ocular complications, and strict adherence to preventive vaccination protocols to increase successful outcomes for these patients.

# **Ontogeny of Locomotion and Head Movement of the African Spurred Tortoise (*Geochelone sulcata*)**

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The aim of this research is to describe the changes of head and neck kinematics as they relate to the growth of the African spurred tortoise (*Geochelone sulcata*). The basics of tortoise locomotion have been described previously. These studies, however, have not investigated locomotor changes in head kinematics due to body size. Infra-red motion capture was used to obtain detailed kinematics of the head, limbs, and carapace on six live tortoises ranging in body mass from 10.8 to 66.2 kg. The results of the study showed that while there is no correlation between body size and stride duration ( $p = 0.198$ ) or duty factor ( $p = 0.109$ ), there is a significant positive correlation between size of tortoise and stride length ( $p = 0.016$ ). To assess head stability, we compared the average magnitude of the maximum positive and negative differences between the rotational acceleration of the head and body in the sagittal plane. Head instability, regardless of body size, increases as stride average linear velocity increases (all  $p < 0.004$ ). This rate of head instability increase with stride velocity was less marked in the larger tortoises compared to smaller tortoises ( $p = 0.017$ ). Thus, larger tortoises maintain a more constant head stability. Our data do not differentiate increases in muscle mass, larger moment of inertia because of increased head mass, or other possible control factors as the source of this increase in head stability.

*Ben LeCheminant supported by the Midwestern summer fellowship*

## **Analysis of *Escherichia coli*'s Adaptive Response to ATP Depletion**

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**Background.** The goal of synthetic biology is to create novel biological molecules and answer basic, fundamental questions in order to connect the science of synthetic biology to the world of natural biology. A synthetic protein, DX, was created through selective evolution with the specific function of binding ATP by protein-ligand sequestration. Previous characterization of the effect of DX on the physiology of *Escherichia coli* revealed phenotypic adaptations due to the perceived depletion of ATP in this living system. Following DX expression, *E. coli* cells were filamented, formed novel lipid bodies (endoliposomes), and entered into a viable-but-non-culturable state (VBNC). The current study investigates *E. coli*'s adaptive response to DX to gain a better understanding of the mechanisms used to survive ATP depletion. With that, the stringent response, composed of the *relA* and *spoT* genes, was analyzed as (1) it is a general stress response in *E. coli*, (2) generates the alarmone, ppGpp, which redirects *E. coli*'s transcriptome potentiating VBNC and lipid biosynthesis and (3) requires ATP as a substrate for (p)ppGpp production. **Methods.** *E. coli* strains MG1655 (WT), *relA::kan* and *relA::kan spoT::cam* were transformed with pBAD18::*DX* or pBAD18::*UBQ*. Cells were grown under inducing conditions for 8 h ("expression phase"), washed, and then allowed to recover for 24 h in the absence of inducer ("recovery phase"). At various time points, cells were analyzed for growth, viability, filamentation and endoliposome development. **Results.** WT cells expressing DX experienced reduced growth, extensive filamentation and endoliposome development during both growth phases. In contrast, the *relA* and *relA spoT* strains (which produce moderate or no (p)ppGpp, respectively) showed normal growth patterns, normal shaped bacilli, and delayed endoliposome organization. **Conclusion.** The stringent response plays a role in *E. coli*'s adaptive response to perceived ATP depletion, potentially redirecting the transcriptome and influencing growth rate, cell shape and lipid biosynthesis.

*Danielle Lee was supported by Midwestern University Biomedical Sciences*

*Shaleen B. Korch was supported by Midwestern University intramural funds.*

## **Basal Secretion is reduced in the *ob/ob* mouse jejunum compared to lean controls**

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The *ob/ob* leptin-deficient mouse is hyperglycemic and obese, however, the role of intestinal monosaccharide uptake and intestinal secretion is unclear. We used *ob/ob* and lean mice aged ~14 weeks. The *ob/ob* mice had a significantly increased weight ( $49.3 \pm 1.6$  g,  $n=5$ ) compared to the lean mice ( $25.1 \pm 0.5$  g,  $n=5$ ). Plasma glucose levels were almost 2-fold greater in the *ob/ob* mice ( $242 \pm 26$  mg/dL,  $n=5$ ,  $P < 0.05$ ), compared to lean mice ( $124 \pm 8$  mg/dL,  $n=5$ ). The goal of this study was to characterize jejunal function in this clinically relevant mouse model. We measured transepithelial short circuit current (Isc), across freshly isolated segments of jejunum from *ob/ob* and lean mice. Basal Isc was significantly decreased, by almost 2-fold, in the *ob/ob* mice ( $50.5 \pm 9.5$   $\mu\text{A}/\text{cm}^2$ ,  $n=5$ ) compared to lean mice ( $95.2 \pm 20.3$   $\mu\text{A}/\text{cm}^2$ ,  $n=4$ ,  $P < 0.05$ ). The Isc in response to the adenylate cyclase activator, forskolin (10  $\mu\text{M}$ , bilateral) was similar in both groups of mice, indicating no change in the cAMP-dependent Isc. The Isc in response to bumetanide (100  $\mu\text{M}$ , basolateral) to inhibit the  $\text{Na}^+/\text{K}^+/\text{2Cl}^-$  co-transporter, and to acetazolamide (100  $\mu\text{M}$ , bilateral) to block the  $\text{HCO}_3^-$  secretory component was similar for *ob/ob* and lean mice, indicating analogous  $\text{Cl}^-$  and  $\text{HCO}_3^-$  secretory components for both groups. A comparison of jejunum morphology suggested that crypt depth was similar for both groups, and thus structural changes do not appear to contribute towards the reduced Isc of the *ob/ob* mice. Villi length was significantly increased (by ~100  $\mu\text{m}$ ) in the *ob/ob* mice, suggesting a greater surface area for absorptive function. Expression of the transporter protein, Glut5 (normalized to GAPDH), was significantly increased in *ob/ob* mice  $0.74 \pm 0.06$  ( $n=14$ ,  $P < 0.05$ ), compared to lean counterparts ( $0.37 \pm 0.06$ ,  $n=11$ ). These data suggest that basal jejunal Isc in *ob/ob* mice is ~1/2 that of lean mice, and may reflect slower transit times in the gastrointestinal tract in the *ob/ob* mice, which may contribute towards increased nutrient absorption (specifically increased fructose uptake via Glut5), increased weight gain and the associated diabetic phenotype. Current studies aim to determine whether the contribution of key epithelial transporters is reduced in the *ob/ob* mice, which may thus account for the reduced basal Isc.

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## **P-cadherin Overexpression Modulates Insulin-Like Growth Factor Signaling in Oral Squamous Carcinoma Cells**

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Altered expression of cadherin cell-adhesion proteins is a frequent occurrence in epithelial tumor development. P-cadherin, a component of normal epithelia, has been ascribed both causative and preventive roles in tumor development, contingent upon the tissue of origin. In oral epithelia, histological studies demonstrate an upregulation of P-cadherin during dysplasia, and a subsequent loss of P-cadherin in later stage oral squamous carcinomas. A possible mechanism by which P-cadherin may modulate tumor development is via alterations in ligand-mediated signaling of receptor tyrosine kinases. To better understand the role that P-cadherin plays in tumor development, retroviral transduction was used to deplete or overexpress P-cadherin in two oral squamous carcinoma cell lines. Six growth factors were independently utilized to screen for P-cadherin-dependent alterations in signaling, with the greatest effects occurring in response to insulin-like growth factor (IGF) and fibroblast growth factor (FGF). The increased level of P-cadherin in IGF-treated cells greatly altered the kinetics of mitogen-activated protein kinase (MAPK) signaling, but had little direct effect on the activation of the phosphoinositide 3-kinase (PI3K) pathway. P-cadherin overexpression increased steady-state levels of Snai1 protein, with further increases noted upon IGF administration. Inhibition of either MAPK or PI3K signaling pathways in P-cadherin overexpressing cells resulted in decreased Snai1 expression. Ser-9 phosphorylation of GSK-3 $\beta$  was unchanged after IGF treatment, suggesting P-cadherin contributes to Snai1 stability independent of GSK3 $\beta$  activity. P-cadherin over-expressing cells treated with IGF showed a time-dependent decrease in P-cadherin levels. This decrease was accompanied by a decrease in immunofluorescence staining of P-cadherin at cell borders. P-cadherin internalization could be inhibited by treatment with dynasore, a dynamin inhibitor. Increases in motility were found in P-cadherin overexpressing cells compared to control. Our data suggests that the increase in P-cadherin seen in early oral cancer may promote tumor aggressiveness by altering cell behavior, and increasing EMT- related signaling.

## **Achilles tendon estrogen receptor signaling after ovariectomy and genistein treatment in rats**

Karl Martineau, Katie Corbell, Tom L. Broderick, Layla Al-Nakkash, and Chad C. Carroll

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We have previously demonstrated that ovariectomy (OVX) results in a large decline in rat Achilles tendon collagen content. The loss of collagen in these rats was prevented by administration of genistein, a naturally occurring isoflavone phytoestrogen. The mechanism(s) contributing to the changes, however, remain to be determined. The objective of this study was to evaluate the effect of ovariectomy, genistein, and exercise on Achilles tendon estrogen receptor content and signaling. Rats were separated into eight groups: intact or OVX, treadmill exercised or sedentary, genistein-treated (300 mg/kg/day) or vehicle. After 6 weeks, Achilles tendons were evaluated for estrogen receptor (ER)- $\alpha$  and - $\beta$ , total and phosphorylated extracellular signal-regulated kinase1/2 (ERK1/2), and c-Raf via Western blotting. ER- $\alpha$  levels were 31% lower ( $p < 0.05$ ) in OVX animals but not influenced by genistein or exercise ( $p > 0.05$ ). ER- $\beta$  levels were not influenced by any condition ( $p > 0.05$ ). Phosphorylation of c-Raf was lower in untreated OVX animals ( $p < 0.05$ ) but c-Raf phosphorylation in OVX animals given genistein was similar to intact animals. ERK1/2 phosphorylation was enhanced ( $p < 0.05$ ) in OVX animals due to an increase in total ERK1/2. ERK1/2 in genistein treated OVX animals was similar to untreated controls animals. The decline in tendon collagen associated with OVX may be due to a reduction in ER- $\alpha$ . The beneficial effect of genistein on tendon collagen in OVX animals may be due to alterations in ER signaling rather than an increase in ER content.

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## **Tissue specific effects of 8-weeks of treadmill exercise on collagen and cross-linking in male Wistar rats**

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The purpose of this study was to determine if there are differences in how the extracellular matrix of skeletal muscle and tendon adapt to chronic exercise. Male Wistar rats (8-week-old) were divided into sedentary (S, n=15) or exercised (E, n=9) groups. Exercised animals ran on a treadmill 5 days•week<sup>-1</sup> for 8 weeks with progression to 60 minutes per day, 20 m•min<sup>-1</sup>, and 8° incline. The Achilles tendon, gastrocnemius, and soleus were assayed for hydroxyproline by HPLC to estimate collagen content. Hydroxylysyl pyridinoline (HP) was also determined via HPLC. Gastrocnemius (p<0.05, S: 55±8 vs. E: 108±15 µg collagen•mg dry weight) and soleus (p<0.05, S: 52±7 vs. E: 104±27 µg collagen•mg dry weight) but not tendon (p>0.05, S: 1308±89 vs. E: 1364±52 µg collagen•mg dry weight) collagen content increased with exercise. In contrast, HP content was 37% greater (p<0.05) in the Achilles tendon (S: 167±17 vs. E: 243±44 mmol HP/mol collagen) but lower (p<0.05) in the gastrocnemius (S: 481±129 vs. E: 194±41 mmol HP/mol collagen) and soleus (S: 840±155 vs. E: 113±31 mmol HP/mol collagen) of trained animals. HP content was also lower in the gastrocnemius when compared to the soleus (p<0.05). Our findings suggest that exercise training-induced changes in the extracellular matrix of tendon and skeletal muscle are not synchronized. Although collagen increases in skeletal muscle with treadmill exercise, it does not appear that the addition of cross-links is sufficient to maintain a normal ratio of cross-links to collagen fibrils.

*MWU Kenneth A. Suarez Research Fellowship to K. Martineau and MWU Intramural Funds to C. Carroll.*

## **The Effect of the Cold Shock Gene *cspA* on Biofilm Formation by *Staphylococcus aureus* Varies in MRSA and MSSA Strains**

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*Staphylococcus aureus* is commonly isolated in prosthesis infections, due to its capacity to form biofilms. These diseases are recalcitrant to both antimicrobials and the host immune response. The exact mechanism of biofilm formation has yet to be fully elucidated. Recent research examining the fabrication of biofilm by methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) strains of *S. aureus* has revealed that there can be a dramatic variation between MRSA and MSSA strains harboring an identical genomic mutation. Previous work has shown that the cold shock protein CspA regulates the expression of virulence factors in *S. aureus*, decreased pigment production as well as a reduction in alternative sigma factor, *sigB*, mRNA. In this study we compare the effect of a deletion in the cold shock gene *cspA* on biofilm in two strains, *S. aureus* COL (MRSA) and *S. aureus* Newman (MSSA). The wild type strains of COL and Newman produced observable biofilms when grown in biofilm broth. For the MSSA strain, the *cspA* mutant showed a decrease in biofilm formation. However in the MRSA strain, the *cspA* mutation increased biofilm production. Since NaCl has been shown to induce biofilm formation in *S. aureus*, we chose to delete this component of the biofilm broth to determine if this effect was consistent in these strains of MRSA and MSSA. Although the relationships within wild type and mutant strains of COL and Newman remained constant, the production of biofilm was significantly decreased in the absence of NaCl. Based on the evidence in these experiments, we propose that the cold shock gene *cspA* of *S. aureus* not only plays an integral role in biofilm production that is affected by NaCl but under these conditions the presence of *cspA* enhances production of biofilm by *S. aureus* Newman (MSSA) while decreasing production in *S. aureus* COL (MRSA).

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## **Mitochondrially-Associated Amyloid Precursor Protein in a Mouse Model of Alzheimer's Disease**

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Studies have implicated the trafficking of amyloid precursor protein (APP) to the mitochondria of cells as a cause of dysfunctional bioenergetics in Alzheimer's disease (AD). The focus of this project was to localize and identify APP and its cleavage products in the mitochondria of brain tissue from the 3xTG transgenic mouse model of AD. Specifically, we investigated whether APP localized to mitochondria in the subiculum and CA1 regions of the hippocampus, where the heaviest amyloid plaque load is seen in these mice. Fluorescent immunohistochemistry was performed targeting the N-terminus of APP and subunits of the outer mitochondrial membrane transport system. A Zeiss Apotome LED-based fluorescent microscope was used to visualize the fluorophores and assess the potential co-localization of APP and the mitochondrial marker. Cell fractionation into mitochondrial and cytosolic components was optimized and performed, and Western blotting was completed on these fractions to demonstrate the mitochondrially-localized APP species in this model. Definitive evidence of co-localization was not found via immunohistochemistry; however, multiple bands indicating putative APP cleavage products could be identified in the mitochondrial fraction from the mouse brain via Western blotting. We continue to work to identify these bands, and to further optimize the immunohistochemistry to uncover the brain localization of mitochondrially-associated APP. We were unable to localize APP to CA1 or subicular mitochondria of this AD mouse model, but our Western blotting demonstrated that APP and several cleavage products likely exist in the mitochondrial fraction of the mouse brain. Further work will optimize the immunohistochemistry and additional studies will investigate the intracellular trafficking route of APP. While studies have indicated that mitochondrially-associated APP can cause energetic dysfunction in AD, the signals that control the trafficking of APP outside of the cell secretory pathway are unknown.

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# Compressive Injury to the Thoracic Spinal Cord Increases Angiotensin Receptors Type I (AT1R) and Type 2 (AT2R) Expression in the Rat

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The locally-derived Renin-Angiotensin System (RAS) in the central nervous system (CNS) has been shown to play a role in various signaling cascades independent of the well-studied systemic regulation of blood pressure and volume throughout the peripheral circulation. RAS enzymes, peptides, and receptors have been found to modulate immune responses locally within the CNS and inhibition of this system has been shown to be neuroprotective in a variety of CNS conditions (e.g., stroke). However, the manifestation of injury in the spinal cord has been shown to be distinct from that in the brain. Thus, evaluation of the effectors of this process as it occurs in the spinal cord is critical to our understanding of how this system participates in the events of secondary injury. The purpose of this project was twofold: we sought to characterize the expression of Angiotensin-II (Ang-II) receptors subtypes 1 and 2 (AT1R, AT2R) in the intact spinal cord and to evaluate how compression of the spinal cord affected the expression of these receptors. Double-label immunofluorescence techniques were applied to determine the cellular expression of AT1R and AT2R, in the intact spinal cord and the effect of compressive injury on these RAS components. The uninjured tissue strongly expressed AT1R in neurons, while moderate expression of AT2R was observed in neurons and was associated with endothelial cells. We found that AT1R and AT2R expression was increased in the injured spinal cord (n = 3) as compared with uninjured controls (n = 2). Injured tissue showed increased expression of AT1R and AT2R in cells within lesioned tissue at the compression site as well as throughout the rostrocaudal gray matter. Expression of AT1R and AT2R was predominantly localized to regions that contained a high density of phagocytic macrophages in lesioned tissue centered about the dorsal funiculi, proximal to the central canal.

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## **Discovering the potential role of novel protein C14ORF43 in modulating RAP1 function**

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The preservation and protection of DNA is essential for all living organisms. Humans, like other eukaryotes, possess linear chromosomes. As a consequence of this linear structure, the process of DNA replication results in the natural ends of chromosomes, telomeres, being shortened with each round. The chromosomal ends are vulnerable to degradation and can be targeted by cellular repair mechanisms, especially nonhomologous end-joining (NHEJ). Central to the protection of telomeric regions of chromosomes from double-strand break (DSB) repair is a complex of six proteins known as Shelterin. This complex is able to recognize and bind to the tandem telomeric repeat sequence TTAGGG. The protein RAP1, a component of the Shelterin complex, is the most highly conserved telomere protein. It has been shown to provide protection to telomeres against DSB repair and improper fusion by NHEJ. Recently, it has been discovered that RAP1 has a function independent of telomeres as an IKK adaptor protein involved in NF- $\kappa$ B gene expression. This suggests the existence of multiple functions of RAP1 as well as other possible interacting partners. Affinity purification using telomeric sequences followed by mass-spectrometry identified C14ORF43 as a potential interacting protein of RAP1. C14ORF43 contains two conserved motifs, an ELM2 domain and a SANT domain. ELM2 is a DNA binding domain and SANT domains are commonly found in proteins that play a role in chromatin remodeling. Our aim is to characterize the protein C14ORF43 and its potential role in telomeric protection. After cloning and expressing C14ORF43 in bacterial cells, we found that C14ORF43 and RAP1 interacted. Cellular fractionation experiments using HeLa cells showed C14ORF43 is localized to both cytoplasm and nuclear fractions, with the majority being in the nucleus. Immunofluorescence imaging of HeLa cells confirmed localization of C14ORF43 to be both in the nucleus and cytoplasm. Additional studies on the interaction of RAP1 and C14ORF43 and the importance of this interaction in HeLa cells are currently underway.

## **Cyclic Stretch Induces IL-8 Secretion in Human Proximal Tubular Epithelial Cells Through cPLA<sub>2</sub>, MAPK, and NF-κB Signaling Pathways**

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This study aimed to investigate the effect of cyclic mechanical stretch on interleukin-8 (IL-8) production in human proximal tubular epithelial cells (HK-2) and the underlying intracellular regulatory signaling mechanisms. The concentration of IL-8 was analyzed by ELISA, intracellular phosphorylated p38 MAPK, c-Jun NH<sub>2</sub>-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK1/2) was assessed by Western blot analysis, while NF-κB was analyzed by an ELISA-based NF-κ-binding assay. Exposure to cyclic mechanical stretch (20%) resulted in a significant increase in IL-8 secretion. In addition, we showed that cyclic stretch induced the phosphorylation of p38 MAPK, ERK1/2, and JNK in HK-2 cells. Cyclic stretch also increased NF-κB-binding activities and inhibition of NF-κB prevented stretch-induced IL-8 production. Moreover, we showed that selective inhibitors of PLA<sub>2</sub> (Mepacrine), cPLA<sub>2</sub> (MAFP and AACOCF<sub>3</sub>), ERK1/2 (PD98059), JNK (SP600125), and p38 MAPK could suppress the effect of cyclic stretch on IL-8 production, which strongly suggests that these pathways play important roles in the stretch-induced IL-8 production in renal tubular cells. Moreover, this study demonstrates the pro-inflammatory effects of cyclic mechanical stretch in the renal tubular cells.

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## **Advancing a Functional Assay for Mitochondrial Cytochrome c Oxidase: Diagnostic Potential in Alzheimer's Disease**

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Mitochondrial deficits, specifically in cytochrome c oxidase (CO; electron transport chain Complex IV) activity, occur in both brain and blood platelets of Alzheimer's disease (AD) patients. However, existing modes for measuring CO activity are time-consuming and highly variable, limiting their diagnostic potential. We have created a CO activity assay that is reliable and could be widely performed via an easily-accessible tissue. Platelets, isolated by differential centrifugation, were maintained in buffered suspension and anti-coagulated with EDTA and prostaglandin. A histochemical method based on the redox turnover of the CO substrate, cytochrome c, was applied. The redox-active marker for the reaction was diaminobenzidine (DAB). Buffer composition, pH, and ionic strength were optimized to increase enzyme-mediated DAB polymerization rates. Enzyme specificity was verified with co-incubation with potassium cyanide. Densitometric imaging was used for measurement of reaction product. Earlier work indicated that enzyme turnover was far below the level required for clinical differentiation (AD vs control). Subsequent optimization has increased the enzymatic rate by a factor of ~3, decreasing the required incubation time from 60 min to 20 min, while maintaining signal-to-noise as well as overall simplicity and low cost. Notably, the revised assay does not require the addition of exogenous enzyme substrate, but is able to utilize endogenous mitochondrial cytochrome c, greatly reducing cost and complexity. Hypothetically, very-low-ionic strength buffering removes the rate-limiting association-dissociation step of the enzyme-substrate interaction, speeding reaction velocity, and our results are consistent with that hypothesis. The assay yielded high inter-rater reliability, repeatability, and a typical dose-response curve under cyanide inhibition. Given its ease of performance, low equipment demands, and low cost, this new assay could fill a significant need.

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## Genistein diet increases intestinal secretion in female R117H cystic fibrosis mice but not in males

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Cystic fibrosis results from the reduction in function of the CFTR (cystic fibrosis transmembrane conductance regulatory protein) chloride channel. R117H, which has partial CFTR function is the third most common mutation found clinically. Genistein, a naturally occurring phytoestrogen, is known to stimulate CFTR function *in vitro*. We have previously shown that female wild-type mice (Wt, C57BL/6J) fed a genistein-rich diet (600 mg/kg food, 600Gd) for 4-weeks have elevated basal levels of jejunal chloride (Cl) secretion (i.e. transepithelial short circuit current, I<sub>sc</sub>) compared to genistein-free fed mice (0Gd), with no effect seen in males. Both male and female Wt mice injected daily with genistein (600 mg genistein/kg body weight, 600Gi) for 1-week have elevated basal levels of I<sub>sc</sub> compared to controls (0Gi). We aimed to determine whether route of administration of genistein mediated differential effects between male and female R117H CF mice. In female R117H 600Gd female mice, there was a subgroup that responded significantly to the 600Gd regimen (53.14±7.92, n=6), and a subgroup of non-responders to 600Gd (12.05±6.59, n=4), compared to 0Gd controls (29.3±6.5 μA/cm<sup>2</sup>, n=7). In the male R117H mice, there was no effect of 600Gd. In R117H mice injected with 600Gi for 1-week, basal I<sub>sc</sub> was unchanged in both male and female mice compared to 0Gi controls. We measured the effects of I<sub>sc</sub> in response to the following: the adenylate cyclase activator, forskolin (10 μM, bilateral) the Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> co-transporter, bumetanide (100 μM, basolateral) to indicate the Cl<sup>-</sup> secretory component, and acetazolamide (100 μM, bilateral) to indicate the HCO<sub>3</sub><sup>-</sup> secretory component. Jejunal morphology analyses (i.e. villi length, number of goblet cells/villi, crypt depth, number of goblet cells/crypt) for R117H mice revealed no significant difference between the genistein treated groups and the control groups. Serum levels of genistein from all groups are being measured and analyzed. This data suggests that there is a sex-dependent effect between the R117H mice fed 600Gd for 4-weeks. The female 600Gd mice showed an overall increase in basal secretion compared to their male counterparts. This promising data indicates that in female R117H mice, partial CFTR function may be sufficient for genistein's action to take effect. Sarah Polito, Ashesh Bhakta and Jonathan Kang were supported by Midwestern University Summer DO Fellowships. Layla Al-Nakkash was supported by the Soy Health Research Fund. R117H mice were generously provided from Dr. M. L. Drumm (Case Western Reserve University).

# **The Effects of Environmental Estrogens on Acetylcholine Receptor Clustering**

Sarah M. Renteria and Wade A. Grow

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Estrogens are endogenous hormones that have numerous effects due to the high number of target cells in a number of different locations throughout the body. Most of these target cells are found in breast and reproductive tissue. However, endogenous and environmental estrogens have been shown to bind to estrogen receptors found in skeletal muscle and alter gene transcription. Many studies have linked the loss of estrogen during menopause to a loss in muscle strength and mass that may lead to sarcopenia. Our lab investigates myogenesis and neuromuscular formation using the C2C12 myotube cell culture model. C2C12 myoblasts proliferate in growth medium, and then fuse into myotubes when switched to low-serum differentiation medium (DM) for 72 hours. AChRs in the myotubes are stimulated by the addition of agrin for the last 16 hours in DM. Our hypothesis was that each of the four environmental estrogens we chose (methoxychlor, DDT, estrone, & 17 $\beta$ -estradiol) would have an effect on AChR clustering in C2C12 myotubes.

*Sarah M. Renteria was supported by the Midwestern University Biomedical Sciences Program.  
Wade A. Grow as supported by Midwestern University intramural funds.*

## Utilizing Exercise as Medicine to Improve the Health of America's Truckers

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**Background:** Long-haul commercial truck drivers have a variety of health problems, some of which are due to their work environments. Paradoxically, truckers often have sedentary lifestyles, leading to increased rates of obesity, hypertension, and sleep disorders. Improvements to truckers' health also have the potential to improve public safety on the highways.

**Objective:** A self-administered, facilitated survey was conducted to determine trucker sentiment toward increasing exercise while on the road.

**Hypothesis:** Truckers will exercise at on-site truck stop gyms if suitable facilities are available.

**Methods:** We conducted a 14-question survey at truck stops in Arizona, New Mexico, California, Kansas, and Oklahoma. Truckers were approached by investigators, who obtained consent and either facilitated completion of the survey or provided instruction for self-completion. The 14 questions documented age, sex, height, weight, waist size, smoking habits, hypertension, diabetes mellitus, high cholesterol, sleep apnea, seeing a doctor regularly, exercising regularly, exercising more if given the opportunity, and, lastly, if participants were willing to use a truck stop gym for a nominal fee. Each answer was self-reported.

**Results:** A total of 100 truckers answered the survey. 42% reported regular exercise, and 70% said they would exercise more if they had the opportunity. Interestingly, this rate was increased by nearly one-third to 93% who indicated they would exercise regularly at on-site facilities if they were made available for a nominal fee. Additionally, our study supports other research showing higher rates of smoking in truckers than in the general population (49% vs 18%). Diabetes mellitus is higher in truckers as well (13% vs 8.7%). Responding truckers had poor access to primary care compared with the general population (42% vs 80%). Surprisingly, only 8% of truckers reported sleep apnea, contrary to the 28% reported by the Federal Motor Carrier Safety Administration.

**Conclusion:** It is well known that exercise can lower all-cause morbidities. We found in this survey that 93% of the truckers would work out at an on-site truck stop facility if available and would do so for a nominal fee. This is a 23% higher rate than if given only the opportunity (eg, had more time) to work out. These findings reveal a potential for improvement in truckers' health and public safety and warrant further study.

# The Effects of Bisphenol A on the Dopamine System in a Rat Pituitary Cell Line

Gina Robinson and Douglas Jones

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Bisphenol a (BPA: 4,4-[propane-2, 2-diyl]diphenol), an organic compound used in the manufacturing of plastics, is an environmental estrogen that mimics estrogenic activity in the body and may disrupt the brain's dopaminergic system. Using a rat pituitary cell line (GH3), we investigated the effects of BPA on pre- and post-synaptic dopaminergic neurotransmission. Pre-synaptically, the cellular levels and function of the dopamine transporter (DAT) were examined using western immunoblot analysis and a neurotransmitter transporter uptake assay kit, respectively. BPA increased cellular DAT levels in a time- and concentration-dependent manner, suggesting that BPA may alter DA uptake. Therefore, DAT function was examined using a fluorescent dopamine (DA) analog. BPA increased DA uptake in a concentration-dependent manner. If DAT levels and DA uptake are affected, it follows that synaptic DA levels and subsequent post-synaptic DA signaling should also be altered. DA receptor signaling was examined by measuring the phosphorylation of element regulating kinase (ERK) using a *fixed-cell* enzyme-linked immunosorbent assay kit (ELISA). BPA caused the phosphorylation of ERK in a time- and concentration-dependent manner. To investigate the role of the DA D1 receptor in BPA-induced pERK activation, GH3 cells were pre-treated with a DA D1 receptor antagonist (R(+)-SCH- 23390) followed by exposure to BPA. ERK activation was inhibited in the presence of the D1 antagonist suggesting that BPA's effect of pERK is in part, mediated by DA D1 receptors. Finally, prolactin (PRL) release was examined using western immunoblot analysis. BPA altered the release of PRL in a concentration-dependent manner. Taken together, our results demonstrate that BPA alters pre- and post-synaptic dopaminergic neurotransmission in pituitary cells, which may ultimately affect PRL release.

*Gina Robinson is supported by the Midwestern University Biomedical Science Program.*

*Douglas Jones is supported by Midwestern University intramural funds.*

## **Voluntary Exercise Training Impairs the Expression of Cardiac Natriuretic Peptides in the Ob/ob Mouse**

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Regular exercise is generally recommended for the treatment of obesity and type 2 diabetes. Exercise reduces body weight, improves glycemic control and cardiovascular (CV) function. This study was designed to determine the impact of voluntary wheel running on the metabolic state and cardiac oxytocin (OT)-natriuretic peptide (NP) system in the ob/ob mouse, a model of insulin resistance coupled with severe obesity. Five-week-old male ob/ob mice and non-obese heterozygote control littermates were assigned to either a sedentary or running group. Voluntary running was performed using a wheel system and recorded for a period of 8 weeks. Daily running activity, expressed in kilometers, was significantly greater in non-obese mice compared to obese mice yet body weight and CV plasma markers were not altered by running in non-obese mice. In obese mice, however, decreased running activity was associated with improved body weight, but exacerbated hyperglycemia and plasma triglyceride levels. Cardiac OT receptor and B-type NP gene expression was decreased in obese mice, whereas no difference in the expression of A- and C-type NP was observed between non-obese and obese mice. Gene expression of NPs was unchanged after running in non-obese mice. In obese mice, however, voluntary running was associated with a downregulation in the expression of all three NPs. Our results show that decreased voluntary exercise running in the obese diabetic mouse is associated with hyperglycemia and reduced expression of cardioprotective peptides.

*Leslie Tamura was a recipient of a MWU Summer Research Fellowship. The work was funded by the MWU Office of Research (TLB), the Diabetes Action and Research Education Foundation (TLB) and by the Canadian Institute for Health Research (MJ, JG).*

## **Using micro-CT to investigate middle and inner ear structures in five species of carnivores with known hearing abilities**

Gregory Taylor and Mark N. Coleman

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Little is known about the functional morphology of the auditory system in carnivores. In this study we used high-resolution microCT to investigate several functionally relevant structures of the middle and inner ears in five species of carnivores with known hearing characteristics (dogs, cats, weasels, ferrets and raccoons). The measurements taken include the areas of the tympanic membrane and stapedia footplate, the lever arm lengths of the malleus and incus, the volume of the middle ear cavity, and the length of the outer spiral of the cochlea. Three-dimensional digital models of these structures were constructed and measured using 3D Slicer open source software.

All of the auditory structures investigated scale with negative allometry against body mass similar to patterns observed in a comparative primate dataset measured with similar techniques. To evaluate the relationships between auditory morphology and hearing sensitivity, the carnivore data were analyzed independently and combined with the primate dataset. Most of the structures demonstrated highly significant correlations with measures of low-frequency sensitivity but no significant relationships with measures of high-frequency sensitivity were detected. In particular, the length of the cochlea and the area of the stapedia footplate showed the highest correlations. The implications for these findings are further discussed.

*Greg Taylor was supported by the Midwestern University Summer Fellowship Program. Mark N. Coleman was supported by Midwestern University intramural funds.*

## Regulation of Toxin:Antitoxin Modules in *Mycobacterium tuberculosis*

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**Background.** *Mycobacterium tuberculosis*, the etiologic agent of tuberculosis, persists as one of the leading causes of death from a single infectious agent worldwide. As an intracellular pathogen that resides within human macrophages and dendritic cells, *Mtb* encounters challenging environmental conditions during host aerosolization, phagocytosis, active growth, latency, and reactivation. Bacteria exposed to a plethora of environments possess molecular responses that regulate the degradation of defective or unnecessary proteins and mRNA molecules. One well-described quality control mechanism of *Mtb* involves toxin-antitoxin (TA) modules, protein pairs where one protein, the toxin, is toxic to or inhibits the growth of the bacterial cell through targeted mRNA degradation, and the second protein, the antitoxin, binds to and neutralizes the toxin's inhibitory effects. The *relBE* TA modules has been implicated to have a role in *Mtb*'s latency program, but the regulation of *relBE* has yet to be defined and is the goal of this study. *Mtb* possesses 12 serine-threonine protein kinases (PknA-L), stress-responsive proteins which modulate gene expression and protein activity through phosphorylation. **Methods.** Using the M-PFC (Mycobacterial Protein Fragment Complementation) technique, we examined whether PknK and RelB or RelE interact *in vivo* through protein-protein interactions. Briefly, when two mycobacterial interacting proteins are independently fused with domains of murine dihydrofolate reductase (mDHFR, F1-2 or F3), functional reconstitution of the two mDHFR domains occurs, allowing for the selection of mycobacterial resistance against trimethoprim (TRIM). The following protein pairs were cotransformed into *M. smegmatis*: RelB<sub>[F1,2]-C</sub>/PknK<sub>[F3]-C</sub>, RelB<sub>[F1,2]-N</sub>/PknK<sub>[F3]-N</sub>, RelE<sub>[F1,2]-C</sub>/PknK<sub>[F3]-N</sub>, and RelE<sub>[F1,2]-N</sub>/PknK<sub>[F3]-C</sub>. Transformants were plated on 7H11-Kan-Hyg plates and incubated at 37°C for 3 days. Transformants were restreaked onto 7H11-Kan-Hyg and 7H11-Kan-Hyg-TRIM and incubated at 37°C for 5 days. **Results.** *M. smegmatis* RelB<sub>[F1,2]-C</sub>/PknK<sub>[F3]-C</sub> and RelE<sub>[F1,2]-N</sub>/PknK<sub>[F3]-C</sub> grew on TRIM-agar plates, indicating that PknK does interact with RelB and RelE. Future studies will analyze direct phosphorylation of RelB and RelE by PknK.

*Jared Taylor was supported by the Midwestern University Summer Fellowship Program.*

*Shaleen B. Korch was supported by Midwestern University intramural funds.*

## **Piriformis Syndrome: Perhaps more than just the Sciatic Nerve**

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### *Background:*

Piriformis Syndrome is defined as “a peripheral neuritis of the Sciatic Nerve caused by an abnormal condition of the Piriformis muscle” termed somatic dysfunction in the Osteopathic profession. Specific Aim: We hypothesize that other anatomical structures contribute to this clinical syndrome, in particular the Posterior Femoral Cutaneous Nerve (PFCN). We propose to show this based upon anatomic dissection, neurologic mapping, and clinical presentation.

### *Materials/Methods:*

Anatomic dissection of Caucasian female cadaver performed in Advanced Dissection course at Midwestern University, Arizona College of Osteopathic Medicine, Glendale, Arizona from March-April 2012. Dissection included the deep gluteal region, posterior lower extremity, and plantar foot. Standard anatomical sources were consulted regarding dermatome and myotome distributions.

### *Results:*

The dissection exposed nerves, vessels, and muscles with their attachments. Fascial planes were preserved where possible. In this specimen, the Sciatic Nerve followed the common route, exiting inferior to the Piriformis. The PFCN also exited inferior to the Piriformis, although more medially. PFCN is smaller, in a more boney confined space than the Sciatic Nerve, and appears greatly susceptible to external compression.

### *Discussion/Implications:*

Based upon anatomy and clinical presentation of Piriformis Syndrome, we propose the current definition of Piriformis Syndrome is insufficient. We propose two subcategories of Piriformis Syndrome based on anatomical variation. Variation one, the Sciatic Nerve pierces through the Piriformis, and variation two the Sciatic Nerve passes inferior to the Piriformis. For variation two, our dissection demonstrates that the deep gluteal rotators can compress the PFCN, which clinically produces posterior thigh pain above the knee. This common presentation for the purported Sciatic Nerve compression by the Piriformis muscle, we feel more accurately describes compression of the PFCN. The Sciatic Nerve may be involved in pain in its anatomic distribution, however, based on standard dermatome distribution the PFCN would cause paresthesia in the posterior thigh whereas the Sciatic Nerve would not.

## **Beneficial Effect of Resveratrol on Antioxidant Status in Brain of Obese Diabetic Mice**

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Resveratrol is a polyphenolic phytoalexin and known to exert anti-diabetic actions. Several studies have demonstrated a link between diabetes and Alzheimer's disease. The present study evaluates the neuroprotective action of resveratrol on obese (*ob/ob*) mice induced oxidative stress. Resveratrol was administered orally at the dose of 25 mg kg<sup>-1</sup> body weight daily for 3 weeks to lean and obese mice. The lipid peroxide was significantly increased in brain of obese mice. The enzymic antioxidants like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and non-enzymic antioxidants like tocopherol, ascorbic acid and glutathione were decreased in obese mice brain. Administration of resveratrol decreased the lipid peroxide levels and up-regulated the antioxidant activities in obese mice brain. These findings suggest the neuroprotective effect of resveratrol by improving the oxidative damage in brain tissue of obese mice.

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# AZCOPT

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## **Tear Film Osmolarity vs. Ocular Discomfort: A Dry Climate Analysis**

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*AZCOPT, Midwestern University, Glendale, Arizona*

This study analyzed the correlation between subjective dry eye symptoms and tear film osmolarity. After informed consent, Subjects were screened for corneal pathology using anterior segment slit lamp examination. Any pathology would exclude them from participation. Subjects were also asked not to wear contact lenses or use eye drops in the 24 hours prior to examination.

If eligible for the study, subjects then completed an Ocular Surface Disease Index (OSDI) questionnaire to assess for the presence of symptoms of ocular discomfort due to dryness. We then tested tear film osmolarity of the right and left eye using the TearLab instrument.

The site for this study was the Eye Institute at Midwestern University (MWU). We evaluated 16 subjects, which were recruited from the student body of the Arizona College of Optometry at MWU. One subject was disqualified after the first visit due to the finding of a small corneal abrasion during screening.

We used the higher osmolarity of each subject's two eyes for our analysis, comparing it to their OSDI score. Analyzing the data for 15 completed subjects, the Pearson correlation ( $r=0.06$ ) showed negligible relationship between our variables (OSDI and osmolarity). The low probability for statistical significance ( $p=0.82$ ) can be attributed to our small sample size. This study demonstrated no significant correlation between OSDI and tear film osmolarity.

# Utilization of Retinal Segmentation as an Early Biomarker Predictor of Diabetic Neuropathy and Retinopathy

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**Purpose:** To find biomarkers within the retinal layers that would serve as an early indicator of diabetic retinopathy and neuropathy and to assess the effects of diabetes on overall retinal thickness.

**Methods:** 40 control eyes and 8 diabetic eyes currently without retinopathy or neuropathy were analyzed using a Spectralis HRA+OCT. Each scan was then manually segmented using ImageJ segmentation software independently by three individuals. Segmentation results were obtained for the temporal and nasal retina of each eye approximately 1000 microns from the center of the fovea. The central foveal thickness for each eye, the mean retinal thickness along with the thicknesses for the RNFL, GCL, IPL, INL, OPL, ONL, Photoreceptor Layer, and RPE were also analyzed.

**Results:** There was very little variance between among three single blinded individuals, intraclass correlation coefficients (ICC) between all three segmentors, showed correlation of 0.912-0.947 with significance of  $p < 0.05$  for overall thickness and measurements of all layers both temporal and nasal. All but two layers had a  $p < 0.05$  correlation between control and Diabetes group. Outer plexiform layer in diabetic eye showed a 26% reduction in thickness on the nasal and 37% reduction in thickness on the temporal side as compared to the control group. Inner plexiform layer on the temporal side showed a 16% reduction as compared to the control group.

**Conclusion:** Manual segmentation as previously has been shown is a valid way to segment the retina and shows consistent results among different graders. Our preliminary data indicates that thickness of outer plexiform layer could be considered one of the biomarkers of the development of diabetes.

# **Retinal Function is Associated with Clinical Observations of Vessel Tortuosity in Diabetic Patients**

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## *Background*

In the US diabetes is the leading cause of blindness in adults aged 20 to 74 years of age. The majority of this vision loss is due to retinal neural and vascular changes known as diabetic retinopathy (ADA 2011). This loss can be measured powerfully by the multifocal electroretinogram (mfERG). Newer research also indicates that vessel changes appear early in diabetic eye disease. The objective of this study is to compare clinically observable changes in vessel characteristics in diabetic patients to mfERG implicit times.

## *Methods*

We compared mfERG findings in both control and diabetic subjects to 3 major vessel parameters measureable on a fundus photograph. The first being branching angles, then vessel thickness measured with standardized software, and finally the overall clinical tortuosity on a scale of 1-3. We analyzed the data with regression analysis and t-tests

## *Results*

As expected the diabetic group had on average a longer implicit time on mfERG ( $p=0.024$ ). It was also found that their arteries were significantly thinner ( $p=0.015$ ). Interestingly, implicit times increased as the overall tortuosity of the vessels increased ( $p=0.036$ ), as long as the patient's blood pressure was taken into account ( $p=0.014$ ). Other notable trends were that venules were thicker in diabetic patients and tortuosity was also more severe in the diabetic group.

## *Conclusion*

These findings are of interest for clinicians who actively look at a patient's vasculature during fundus examination. By assessing tortuosity it may be possible to draw conclusions about the overall health of their retina. More studies with large sample sizes are needed as follow up to this pilot data.

*All authors supported by Midwestern University Arizona College of Optometry*

## **Retinal Function is Associated with Ocular Perfusion in Patients with Systemic Vascular Disease**

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Background: Diabetic retinopathy, hypertension and coronary artery disease have the potential to cause loss of sight. These diseases alter blood flow throughout the body. Previous studies have demonstrated that patients with vascular disease and diabetes have reduced multifocal electroretinogram (mfERG) amplitudes and increased implicit times. These can be useful to help predict retinal health at a cellular level in order to preserve patients' sight.. The investigators seek to establish a link between ocular perfusion pressure, a measure of blood flow in the eye captured during a normal eye exam, and mfERG outputs.

Methods: 5 subjects with systemic vascular disease and 19 healthy subjects participated in a case-control study. Each group underwent a series of ophthalmic, neurological, and hemodynamic measurements. The results were analyzed using a t-test with unequal means and then used to create a multivariate model for ocular perfusion.

Results: The investigators found that the systemic vascular disease group had a higher age, higher total cholesterol, higher triglycerides, longer mfERG implicit time, and reduced mfERG amplitudes. There was no difference in ocular perfusion alone ( $p=0.282$ ), but when controlling for age there was an association between ocular perfusion and vascular disease ( $p=0.012$ ). The multivariate model for perfusion is:

$$\text{Perfusion} = \text{age} + \text{gender} + \text{vascular disease status} + \text{average mfERG amplitude.}$$

Conclusion: Ocular perfusion, which is a function of blood pressure and intraocular pressure, is associated with mfERG amplitude when age, gender, and vascular disease are taken into account. This also indicates that ocular perfusion may be a source of mfERG amplitude variation.

## **Survey of Optometry School Graduates class of 2012**

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Optometry is a rapidly changing field, with hundreds of optometry students graduating in the U.S. every year. With every year, we see new advancements in technology, new laws and regulations, and even new optometry schools. The future of optometry rests in the hands of its new graduates, which is why it is important to profile and analyze their status as they begin their careers. Our Capstone project took a deeper look into the circumstances and aspirations of these new graduate optometrists. We contacted the school coordinators for permission to survey the students with an on-line questionnaire. The survey was sent to 17 U.S. optometry schools. Survey topics include age, gender, school, salary expectations, post-graduate work, and modes of practice. 59 students from 2 schools responded to the survey. The survey results revealed that students' expectations differed from where they actually ended up. With this survey information, undergraduate students and current optometry students will be able to set realistic expectations and adjust for their future plans.

*Support for this project was given by Midwestern University College of Optometry.*

## **Development of Dry Eye in Second Year Optometry Students**

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Dry eye is a common clinical entity with potential to adversely affect one's quality of life. Ocular symptoms such as discomfort, blurry vision, or foreign body sensation may be present. The basis for these symptoms is unknown, but theories suggest tear hyperosmolarity and tear film break-up between blinks may be involved.

During the second year curriculum, optometry students learn many anterior and posterior segment ocular examination skills including fundus lens biomicroscopy, binocular indirect ophthalmoscopy, tonometry and gonioscopy. A high amount of visual concentration is required by the student doctor during the learning and execution of each of the procedures. The latter two procedures also require instillation of topical ophthalmic solutions, which can adversely affect the ocular surface environment.

The goal of this study was to examine development of dry eye signs in second year students as they complete their winter quarter of study. Students enrolled at the start of the quarter and a baseline symptom survey was taken. Baseline tear film osmolarity, tear break-up time, phenol red thread test, tear meniscus height, and corneal staining measurements were taken. Results were compared at the beginning and end of the quarter, after students practiced these procedures for between 4 and 10 hours each week. Significant changes indicating dry eye or worsening of dry eye ( $p < 0.05$ ) were seen in tear osmolarity, tear break-up time, and tear meniscus height measurement. These results suggest the need to carefully monitor for the development of dry eye in second year students during this quarter of study.

*All authors were supported by the Department of Optometry.*

# **A Clinical Correlation: Binocular Vision Testing and Symptomatic Dental Loupe Use**

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**Purpose:** A dental loupe is a magnification device used to see fine detail. Pre-clinical training with loupes often begins during the first year of dental school. Loupes have become commonplace in dentistry due to increased near magnification, improved working posture, and enhancement of certain procedures. Dental loupes employ convergent optics to form a stereoscopic image. Prolonged converging and focusing of the eyes can cause eyestrain. This study aims to find the visual tests that correlate with symptomatic loupe use. **Methods:** Thirty-three dental students from Midwestern University participated in this study. One subject was eliminated due to inconsistent results. Subjects were asked to report any problems while using loupes including eyestrain, headaches, or double vision. Each subject underwent nine tests to assess visual function: autorefractometry, visual acuity, stereoacuity, cover test, near point of convergence, negative relative accommodation (NRA), positive relative accommodation (PRA), vergences, and color vision. **Results:** The subjects were divided into two groups, those who reported visual symptoms when using loupes (N=11) and those who were asymptomatic (N=21). A two-tailed t-test showed the PRA values differed significantly between the two groups ( $p=0.03$ ). On multivariate analysis, no combination of tests performed better than PRA alone. Additionally, although not statistically significant there was a trend towards worse binocular visual acuity ( $p=0.19$ ) and abnormal near cover test ( $p=0.12$ ) in the group with symptoms. **Conclusions:** This study found a correlation between abnormal PRA and difficulty using loupes, suggesting that accommodative ability is an important factor for comfortable loupe use. Future studies should evaluate additional accommodative tests. A vision screening exam is recommended for all students entering dental school to identify individuals who may have symptoms with the use of loupes.

## Visual Performance of Multifocal Contact Lenses

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**Purpose:** The aim of the present study was to compare the visual performance of different multifocal contact lenses.

**Methods:** 10 subjects (habitual soft contact lens wearers) between the ages of 40-45 years participated in the study. Three different multifocal contact lenses were fitted within the same visit. Visual performance tests were performed that included low and high contrast acuity distance visual acuity, near visual acuity, contrast sensitivity (CSV-1000; OU; logarithmic scale for 3, 6, 12 and 18cpd), range of clear vision (OU) and through-focus curve (OU, low contrast). Visual performance was obtained with the subject's spectacles as well as with Acuvue, Airoptix and Biofinity low-add multifocal contact lenses. All the lenses were fitted based on the fitting guide.

**Results:** Mean logMAR visual acuity with spectacles, Acuvue, Airoptix and Biofinity for high contrast is -0.09 (SD=0.04), -0.08 (SD=0.03), -0.10 (SD=0.01) and -0.07 (SD=0.04), while that for low contrast is -0.05 (SD=0.05), -0.08 (SD=0.04), -0.06 (SD=0.04) and -0.02 (SD=0.06). Mean high contrast near visual acuity with spectacles, Acuvue, Airoptix and Biofinity is -0.09 (SD=0.02), -0.10 (SD=0.00), -0.10 (SD=0.01) and -0.10 (SD=0.01). Mean area under the curve for the contrast sensitivity test with spectacles, Acuvue, Airoptix and Biofinity is 26.51 (SD=1.50), 25.90 (SD=1.04), 26.61 (SD=0.76) and 26.28 (SD=1.26) respectively. ANOVA revealed no significant difference in high and low contrast distance visual acuity as well as near visual acuity and contrast sensitivity function between the 3 multifocal contact lenses and spectacles ( $p>0.05$ ).

**Conclusions:** There was no statistically significant difference between the three different low-add power multifocal contact lenses and the spectacles. Objective visual performance was similar between the 3 different types of contact lenses.

## **Case Study: Treatment of Cephalohematoma with Osteopathic Manipulative Medicine**

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*AZCOPT, Midwestern University*

**Context:** To present a case in which Osteopathic Manipulative Medicine (OMM) was used to treat an infant with a cephalohematoma.

**Patient and Method:** A 2-month-old male accompanied by his parents presented to the Osteopathic Manipulative Medicine Clinic for osteopathic evaluation and treatment of a persistent and previously diagnosed cephalohematoma. The patient presented with additional signs of distress and his parents complained of his restlessness, difficulty feeding, and decreased neck range of motion. A complete osteopathic examination was performed and somatic dysfunctions were found in multiple regions including the cranium. Several cranial strain patterns were noted in addition to condylar compression, asynchronous motion, and decreased amplitude of motion. These cranial osteopathic findings were treated, as were other areas of somatic dysfunction and restriction, using various osteopathic manipulative techniques. Over the course of three months, the patient was treated osteopathically 7 times. At each appointment, the size of the cephalohematoma was noted and his parents articulated any changes in his actions and behaviors.

**Results:** After a single treatment of OMM, the patient's parents noted improvement in his eating habits and sleeping patterns. Additionally, over the course of 7 treatments the diameter of the cephalohematoma decreased by 3cm.

**Conclusion:** Osteopathic Manipulative Medicine serves to enhance the patient's own innate self-healing mechanisms. This patient presented with a persistent hematoma, decreased feeding, disrupted sleep, and muscular strains. While there is little literature available on the direct impact OMM has on decreasing the size of cephalohematomas, OMM is frequently used to correct cranial and myofacial strains as well as relieve nerve entrapments, ultimately increasing circulation, innervation, and the body's capacity to heal. In addition to parent support and nourishment, OMM is a beneficial adjunctive treatment to improving the health status of an infant, and can aid in reducing the size of cephalohematomas. Further research and studies on the use of OMM in infants presenting with cephalohematomas is needed to determine the efficacy and help support the use of this treatment modality in future patient cases.

## **MWU Eye Institute: a marketing model for growing a healthcare practice**

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**Objective:** Determine which marketing strategies are most effective at increasing patient base for an optometric practice. The Midwestern University Eye Institute serves as a marketing model that can be applied to other types of practice settings.

**Methods:** Using administrative reports from MWU Eye Institute we collected data including marketing strategies implemented over a 28-month period (Dec 2010-Mar 2013) and patient surveys indicating referral sources. Total patient exams per week were tallied taking into account student intern academic and work schedules. Mathematically we analyzed the growth of the Eye Institute and correlated the marketing strategies to determine which strategies are most effective.

**Results:** Utilizing linear and logarithmic regressions to track weekly growth of patient base over 28 months, we describe the fraction of variability in patient visits being due to a growth trend ( $R^2 = 0.2715$ ). Two T-tests were used to obtain P-values which suggest significance for the linear ( $P = 7.48E-06$ ) and non-linear ( $P = 8.72342E-09$ ) regressions. During the Eye Institute's first two years, 76% of referred patients surveyed were gathered via no-cost marketing. The other 24% of referred patients surveyed were gathered via financed advertising. During this same time period, 43% of patients surveyed did not mark a referral source.

**Conclusions:** No-cost marketing such as word-of-mouth and referrals from neighboring clinics proved to be consistently more effective and less expensive than financed advertising such as radio ads, fliers, and magnets. Tracking marketing strategies and referral trends play an important role in increasing practice growth and limiting unnecessary expenses.

## **Isotretinoin induced systemic side effects and ocular side effects by different parts of the eye**

Justin Kim, Laroushna Pierre, and Wendy Harrison

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**Purpose:** Isotretinoin (Accutane) is a synthetic retinoid vitamin A derivative that is for treatment of acne. The efficacy of Isotretinoin in the treatment of acne has proven to be so remarkable that many physicians are willing to prescribe this medication to many acne patients more readily.

**Methods:** A literature review was conducted to investigate reported side effects of Isotretinoin. Over 50 articles were evaluated.

**Results:** The most frequently and widely reported systemic adverse reactions with usage of Isotretinoin are the mucocutaneous side effects on the lips, eyes, mouth, and other epidermal surfaces. Most data comes from two large trials (Trial 1: n=69, Trial 2: n=300) that studied the adverse events associated with Isotretinoin, over 98% of subjects, who were taking 1mg/kg in 2 doses, reported chapped lips in 4<sup>th</sup> week of the trial. Dryness or peeling of the skin was experienced by 91.6% of subjects at the same time point. Similarly, the symptom of dry eye is experienced in about 30% of Isotretinoin treated patients. One clinical trial study consisting of 60 male subjects on a daily dose of 0.5mg/kg, found a significant reduction in tear basal secretion at 3 months from the start of Isotretinoin treatment. In another clinical trial study consisting of 11 subjects dosed at 60 -120 mg/day, all of 11 subjects showed appearance of meibomian gland atrophy, a decrease in the volume of secretions. Also, Isotretinoin has toxic potential to affect retinal function and cause impaired night vision. Poor night vision and/or excessive glare sensitivity were reported by 3 subjects in a clinical trial consisting of 50 subjects dosed 1mg/kg/day for period of 4-5 months. Lastly, there are few case reports of Isotretinoin induced corneal steepening, papilledema, and optic neuritis.

**Conclusion:** Eye care professionals should be able to recognize Isotretinoin induced ocular side effects.

*All authors supported by Midwestern University Arizona College of Optometry*

## **Relationship Between Tear Osmolarity and Allergic Symptomatology**

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Dry, itchy, or red eyes are common symptoms which may prompt a visit to an eye care professional. Frequent causes of these symptoms are environmental allergies, which may affect people depending on the time of year and the climate in which they live. Seasonal allergic conjunctivitis has the potential to cause loss of corneal integrity. This integrity can be measured in part via tear film osmolarity. Increased tear film osmolarity may indicate decreased ocular surface integrity and/or increased tear evaporation.

The goal of this study was to examine the relationship between patient ocular signs and symptoms, environmental pollen counts, and tear film osmolarity values over a three week time course. Patient symptoms were obtained through the Ocular Surface Disease Index (OSDI) survey. Then, pollen counts for zip code 85308 were recorded and subjects' tear osmolarity was obtained. Finally, the subject's anterior segment was assessed including the presence or absence of injection and conjunctival papillae which are common in allergic conjunctivitis. These procedures were repeated approximately one month later.

Analysis of variance (ANOVA) modeling was used to compare OSDI values to tear osmolarity levels and no statistically significant relationship was evident at either time point. However, tear osmolarity showed an inverse correlation ( $r^2=0.85$ ) to pollen counts from all five recording dates through both a two-tailed t-test ( $p=0.010$ ) and ANOVA ( $p=0.029$ ). As environmental pollen levels increased, the tears of subjects became more dilute (decreased osmolarity). This could be attributed to reflex tearing in the presence of ocular allergens, which would serve to dilute the tear film.

*All authors were supported by the Department of Optometry.*

## **Twin Retinal Holes**

Jenny Mathew

*AZCOPT, Midwestern University, Glendale, Arizona*

Retinal Holes are atrophic holes described as small round lesions that are full thickness breaks in the retina caused by progressive thinning seen in less than 5% of the population. In this case, a young male presented with common retinal holes, that were found of identical size and shape right next to one another surrounded by cuffs of fluid, located in his peripheral retina. We used a variety of imaging tools to see what would allow for clear and easy monitoring of the holes.

*Jenny Mathew was supported by the Midwestern University Arizona College of Optometry*

## **Normative Values for Dynamic Visual Acuity using Burnell's Rotational Trainer**

Amanda Miskewicz-Zastrow, Eric Bishop, Alan Zastrow, Dan Mark Cuevas and Dr. William Rainey

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Burnell's Rotation Trainer was used to test Dynamic Visual Acuity (DVA) in 22 subjects, 10 Females and 12 Males, between the ages of 23 and 30. Only subjects with a refraction between +1.00 to -7.00 (spherical equivalence) were used. DVA was recorded at three different speeds (29 RPM, 24 RPM, and 16 RPM). At each speed starting with the fastest RPM values were recorded on accuracy and how long it took the subject to complete the nine lines. The subjects were asked to fill a questionnaire, which ask questions that may effect DVA. As the RPM decrease the subject's time decreased and their accuracy increased. The same trend was seen when you separated the males and females; however, the females' accuracy was lower and the time was greater in females when compared to the males.

This study is the first to use the Burnell's Rotation Trainer to quantitatively measure dynamic visual acuity. As such, it is important to set up a pilot study to both: a) establish normal trends, seen in the results; and b) to establish a well-defined methodology that is reproducible and able to produce quantitative data. Our results obtained shows that our procedure was able to produce a general trend in both the males, and females. In addition, it provides a strong foundation for future comparison tests, like measuring the difference between a normal population and a sports population. The procedure can also be used to compare the differences of dynamic visual acuity between subsets of populations. For example, a study can be developed where the DVA is measured against sports skill level.

# **Neovascularization of the Iris as the Presenting Sign of Complete Unilateral Intracranial Carotid Artery Occlusion**

Tina R. Porzukowiak OD, FAAO, Laura Addy, Matthew Kaminsky, Elizabeth Lyon, Ryan Anderson and Alicia Havens

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This case illustrates a rare presentation of ocular ischemic syndrome (OIS) where the primary ocular finding was neovascularization of the iris and angle. Complete, unilateral, intracranial carotid artery occlusion was diagnosed via magnetic resonance angiography (MRA). This case further supports the theory that uveal ischemia alone in the absence of retinal ischemia may be a significant contributor for the neovascularization noted in ocular ischemic syndrome.

*Tina R. Porzukowiak OD, FAAO was supported by the Midwestern University Arizona College of Optometry.*

# Comparison of Tear Break-up Times Using Fluress® and Fluorescein Strips

Bill Rainey, George Choueiry, Mark Malit, John Nguyen, Helen Park

*AZCOPT, Midwestern University*

## Purpose

To compare tear break-up times using two different fluorescein techniques, and to provide normalized tear break up times (TBUT) with the use of Fluress®.

## Methods

At the initial visit, thirty-seven subjects were given a McMonnies dry eye questionnaire, a thorough anterior slit lamp examination, a Schirmer 1 test, a Phenyl red thread test, and had three TBUT assessments with sodium fluorescein strips in each eye. Each exam component was tasked to a specific examiner. The subjects then returned in one week where they had a second anterior slit lamp examination and three more TBUT assessments per eye, this time using Fluress®. The TBUT assessments were all video recorded to provide a more accurate result.

## Results

Subjects showed an average TBUT of 7.27 ( $\pm 4.83$ ) seconds with fluorescein strips and an average TBUT of 14.65 ( $\pm 6.66$ ) seconds with Fluress® (Student T-test,  $p = 8.43e^{-18}$ ). All subjects had their 3 TBUTs averaged with each fluorescein strip assessment and each Fluress® assessment. The values were then plotted with x values as TBUT (seconds) with a fluorescein strip and y values as the TBUT (seconds) with Fluress®. A regression line was constructed and the regression equation was  $y = 0.2458x + 9.9586$ .

## Conclusion

TBUT assessments were different between Fluress® or fluorescein strips, and Fluress® TBUTs were typically longer. Since there was a difference between the two techniques, we set out to find a correlation between these two values. The regression line on the scatter plot gave the equation to compare the two values. As a rough clinical estimate, an examiner can take a TBUT reading with Fluress®, subtract 10 seconds, and then multiply by 4 to convert to its fluorescein strip correlate.

## **A Clinical Study to Evaluate the Efficacy of Assessing the Anterior Chamber using the Pulsar Tomographer**

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**Purpose:** The aim of this study was to assess whether the Sirius Pulsar Scheimpflug imaging system can accurately evaluate the anterior chamber.

**Methods:** The anterior chamber angles (ACA) and depths (ACD) of 20 healthy eyes of 10 normal subjects were evaluated using the respective gold standards for each measurement, gonioscopy and A-scan. Each eye was assessed under three pupillary conditions: normal, pharmacologically dilated, and pharmacologically constricted. The ACD results were evaluated using a Bland-Altman analysis, and ACA's with a simple agreement analysis.

**Results:** Compared to A-scan, the Pulsar consistently underestimated the ACD by 13%, 14% and 9% respectively for the natural, dilated, and constricted states. The Bland-Altman analysis showed a consistent negative bias for all states of the pupil. With regard to the ACA, the Pulsar was within the assessed gonioscopic range 42% of the time, over-estimated the angle 50% of the time, and underestimated the angle 9% of the time. The Pulsar was, on average, outside of gonioscopic range  $2^{\circ}$ ,  $11^{\circ}$ , and  $1^{\circ}$  respectively for the natural, dilated, and constricted states.

**Conclusions:** This study found that the Pulsar is a new tool that consistently underestimates ACD, and overestimates ACA 50% of the time. While it does have the benefit of patient comfort and ease-of-use compared to A-scan and gonioscopy, caution is advised with total reliance on this instrumentation. Further studies are warranted on repeatability and reliability of the findings. At this time, it is the opinion of this group that the Pulsar should not replace A-scan and gonioscopy for evaluation of the anterior chamber angle and depth.

## Reaction time, Defocus and Visual Performance in Presbyopes

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**Purpose:** The aim of the study was to assess the reaction time (RT) associated with visual performance (VP) tasks in the presence of defocus in presbyopic populations.

**Methods:** Nineteen subjects participated in the study between the ages of 35 and 50 years. Subjects were categorized as pre-presbyopes (35-39 yrs), early-presbyopes (40-45 yrs), and mid-presbyopes (46-50 yrs). The RT was measured when VP measurements were done at different defocus levels. VP tasks included monocular and binocular, high contrast (HC) and low contrast (LC) visual acuity at distance and near. Reaction time was measured with a precision timer. Defocus levels included 0D, 1D, 2D and 3D. Reaction time was compared between pre-presbyopes and presbyopes in the presence of defocus for different visual performance tasks.

**Results:** Mean RT for distance visual acuity for 0D, 1D, 2D and 3D was 1.46, 1.70, 1.70 and 1.84 secs for pre-presbyopes, 1.47, 1.70, 1.74 and 1.91 secs for early presbyopes, 1.48, 1.76, 1.82 and 1.99 secs for mid-presbyopes. Mean RT for distance visual acuity (LC) for 0D, 1D, 2D and 3D was 1.42, 1.53, 1.80 and 1.95 secs for pre-presbyopes, 1.49, 1.77, 1.76 and 2.01 secs for early-presbyopes, 1.49, 1.78, 1.82 and 2.06 secs for mid-presbyopes. Mean RT for near visual acuity (HC) for 0D, 1D, 2D and 3D was 1.54, 1.79, 2.04 and 2.37 secs for pre-presbyopes, 1.66, 1.95, 2.02 and 2.08 secs for early presbyopes, 1.58, 1.80, 2.07 and 2.27 secs for mid-presbyopes. Mean (SD) RT for near visual acuity (LC) for 0D, 1D, 2D and 3D was 1.70, 2.08, 2.23 and 2.99 secs for pre-presbyopes, 1.92, 2.06, 2.11 and 2.22 secs for early-presbyopes, 1.94, 2.10, 2.35 and 2.58 secs for mid-presbyopes. ANOVA revealed a significant difference for RT as a function of age only. There was a significant increase in RT for both HC and LC visual acuity for distance and near at different defocus levels. There was a significant effect of age for near visual acuity.

**Conclusions:** RT increased in the presence of increasing defocus for both distance and near visual acuity. Full correction of visual acuity at distance and near in presbyopes is warranted for critical tasks.

## Effectiveness of the King-Devick Test as a Concussion Screening Test in High School Football

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**Purpose:** To evaluate the effectiveness of the The King-Devick (KD) test in identifying people that may have sustained a concussion and should undergo further testing. Also, whether performing KD testing will increase awareness of concussion related symptoms in High School football players. **Methods:** Forty-seven high school football players were tested with KD test as a baseline prior to the beginning of football season. During the season, 3 Varsity students sustained concussions that were diagnosed by a neurologist. Students also took the exact same survey about their concussion awareness before and after the season. **Results:** Three students with a diagnosed concussion had their times for KD test significantly diminished by 41%, 100% and 143% respectively. When tested at the end of the season, 2 of the students times returned close to baseline times, while the third student's time still was 25% slower when compared to baseline. Test-Retest reliability was analyzed using Intraclass correlation coefficients (ICC) between baseline and end of the season data and was 0.873 with significance of  $p < 0.05$ . 95% limits of agreements (LoA) and their 95% confidence intervals were measured and showed very little variance in times among athletes before and after the season who did not have concussion. Paired sample t-test showed  $P > 0.05$  for the question of "I would say that my current knowledge level of concussions is very high." Student's awareness of concussions before the season compared to the end of the season was statistically significantly increased. **Conclusion:** This study showed that KD test, which is a simple 1 min test, can potentially be used to rapidly identify athletes who have a potential concussion.

*This research was supported by a grant from the Arizona College of Optometry.*

## **Triple Antibiotic Pulp Therapy for Primary Teeth: Review of Literature**

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**Background:** The quest to maintain a primary tooth until exfoliation has led to the development of a variety of pulpal treatments including indirect pulp therapy, direct pulp cap, pulpotomy, and pulpectomy. Medicaments used in primary pulpotomies vary from fixatives to coagulants to obturants. All have been demonstrated to be applicable, yet reported disadvantages including systemic toxicity, defects in succedaneous teeth, and high cost. Researchers continue to pursue other alternatives to offset these disadvantages.

**Review of Literature:** In the late 1980s, Japanese researchers developed a pulpal medicament comprised of three antibiotics in a carrier liquid. Clinically, Takushige et al. (2004) demonstrated 100% success for pulpotomies in primary teeth using this triple antibiotic paste following retreatment of 4 out of 87 total cases. Continuing research and case reports have supported the use of triple antibiotic pulpal therapy when compared to other medicaments; however, some clinicians have reported clinical success over time that was compromised by radiographically observed internal resorption. Although the triple antibiotic paste is more cost effective than MTA, it is not as of yet available in a commercial applicator and must be hand-mixed for each application.

**Conclusions:** Following the 2007 Joint Symposium of AAE and AAPD, MTA was the forerunner to replace formocresol as a pulpotomy medicament. With the observed clinical success and cost benefits of the triple antibiotic therapy for primary tooth pulpal treatment, continued research utilizing larger sample sizes, controlled environments, development of protocols for case selection and handling of the medicament, and prolonged follow-up will be helpful in determining the viability of this pulpal treatment.

## **Diagnostic Quality and ADA Guideline Compliance of Dental Radiographic Images Received by Midwestern University's Dental Institute**

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Dental radiographs of adequate resolution and diagnostic quality are imperative to identify dental pathology and formulate treatment plan. To minimize radiation exposure, all new patients attending dental institute are advised to request their previous dentists to electronically transfer their radiographs to the dental institute. In 2010, the American Dental Association issued specific guidelines about digital transfer of radiographs in dentistry. This study evaluated the diagnostic quality, and the ADA guideline compliance level of radiographic images received by the dental institute. Five observers consisting of two fourth year dental students, two general dentist faculty members, and one Oral and Maxillofacial Radiologist, evaluated images received for presence of technique errors. Also, the operators evaluated if the transfer method was DICOM compliant. 140 Full Mouth Radiographic series (FMS) received from outside providers were randomly selected for this study. The acquisition medium was determined to be CCD/CMOS solid state sensors (74 FMS), Phosphor plate (62 FMS), and conventional films (4 FMS). An average of 1623 errors were observed on a total of 2520 individual images with sensor/film position being the most common error at 40.3%, followed by blurriness at 23.9%, vertical angulation at 22.8%, horizontal angulation at 9.6% and 3.3% were missing images. For individual projections, lower premolar periapical radiographs showed more errors than any other projection. A common error (40.3%) with use of Phosphor plate system was scratched/dirty phosphor plate making the acquired image non-diagnostic. Less than 1% of all images received were transferred according to ADA recommended DICOM guidelines and without encryption making HIPPA compliance questionable. These findings point to the lack of awareness of practicing dentists about ADA transfer/ HIPPA compliance guidelines. This study highlights the need to establish quality control program in dental practices. Improved radiography technique will assist in overall reduction of absorbed radiation dose by the patients.

*Chelsea N. Baraff was supported by the Midwestern University Research Scholarship.*

## **A Survey of Radiation Safety Practices at US Dental Schools**

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In surveying radiography departments at dental schools, our goals were to determine methods currently being employed in dental schools to minimize radiation exposure and education of radiation safety procedures. This project was reviewed and approved by Midwestern University's IRB. A survey was emailed in October 2012 to Oral and Maxillofacial Radiology instructors at 58 U.S. dental schools. Forty-two out of a total of fifty-eight (72%) schools responded to the survey. It was found that a large number of dental schools use radiation badges/dosimeters for faculty (79%), staff (88%), pre-doctoral students (12%); pregnant pre-doctoral students (56%). Ninety-five percent of schools use leaded thyroid collars for intraoral radiography; 30% for extraoral radiography. The most common collimator used in schools is long & round with only 12% of schools using a long & rectangular PID. Phosphor plates are used in 55% and solid state direct digital are used in 50% of pre-doctoral radiology clinics. Of the 5 dental schools still using film in pre-doctoral clinic, only one school is using E-speed film and others are using F-speed. Twenty One percent schools provide didactic information in the use of handheld x-ray units and in 19% schools students operate handheld x-ray unit. Selection criteria is the primary basis for making need based radiographs, only two schools reported that radiographs were made routinely and not based on patient need. The results of this survey serve as a uniform source to review various radiation safety guidelines and to provide a general picture of how radiation standards are implemented and taught in dental schools across the country.

*Robyn Call was supported by the Midwestern University Research Scholarship.*

## **Atypical Gemination of Permanent Central and Lateral Incisors: Treatment Considerations**

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**Background:** Gemination is a rare developmental anomaly that occurs when a single tooth bud divides partially or completely and has an incidence of 0.47% without sex predilection (Clayton 1956, Kim & Jou 2000). Gemination can be difficult to differentiate clinically from fusion, which is the union of two or more enamel organs during development. Typically, counting the number of existing teeth is the best way to differentiate between the two clinically. Gemination occurs most often in the incisor region, and more often in the primary dentition. In these cases, treatment is not often necessary. The goal of treatment is to preserve tooth vitality, and if that is not possible, to preserve the tooth in position until replacement options become viable.

**Case Report:** A six-year-old Caucasian female was referred to the MWU Dental Institute for a second opinion and differential diagnosis. Radiographic findings revealed a vertical coronal defect that bisected tooth #9, and minor notching in the incisal edge of unerupted tooth #10. However, clinical findings revealed an asymptomatic tooth #9 with vertical enamel defect along both facial and lingual surfaces, with carious involvement.

**Discussion:** Due to the vertical nature of the defect, the root surface appears affected radiographically. Thus the prognosis of this tooth from both endodontic and periodontic aspects is guarded. A multidisciplinary approach including endodontics, periodontics, orthodontics, and prosthodontic restorations may be required to achieve a more favorable outcome.

## **Unerupted Permanent First Molar: Case Report and Treatment Considerations**

Rachel Ecker<sup>1</sup>, Hillary Key<sup>1</sup>, Robert Carpenter<sup>1</sup>, Kathryn Lawson<sup>2</sup>, Vijay Parashar<sup>1</sup>, Kimberly Patterson<sup>1</sup>, Cheryl DeWood<sup>1</sup>

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**Background:** Tooth eruption is a complex biological process that can fail for numerous reasons. This failure can be due to mechanical obstruction or to idiopathic causes as seen in Primary Failure of Eruption (PFE). PFE is a rare disorder that affects only 0.01% of first permanent molars (Palma, et al. 2003). Although CBCT is not routinely used for pediatric dental radiographic surveys, specific cases can benefit from a limited field of view scan.

**Case Report:** An 8 year old Caucasian American male presented to the pediatric suite of the Midwestern University's Dental Institute for an unrelated problem. The child's first dental examination had occurred 2 months prior, and the parent supplied radiographs. As tooth #19 was unable to be visualized clinically and with the radiographic survey provided, and as per ADA and AAPD guidelines, a panoramic radiograph was acquired revealing an unerupted #19 with roots near the inferior border of the mandible. CBCT imaging demonstrated the lack of lingual cortical plate near the unerupted coronal structure, approximation of the roots to both the inferior alveolar nerve, expansion of the mandibular cortex, and apparent perforation of the inferior aspect of the mandibular body by the root apices. The treatment plan included extraction of #19 to allow mesial drift of #18. The hard and soft tissue surrounding the follicle was also analyzed for histopathology and protein expression of matrix metalloproteinase's (MMPs), the enzymes responsible for tissue breakdown. Midwestern University IRB review and approval was received for this study.

**Discussion:** PFE poses challenges from diagnostic, surgical and orthodontic aspects. CBCT can provide valuable information about maxillofacial structures while observing the principles of ALARA, and should never be used as a screening tool. A multidisciplinary approach to cases involving PFE is advisable.

*Research supported by Midwestern University College of Dental Medicine Stipend*

## **Assessment of Predoctoral Pediatric Competency in U.S. Dental Schools**

Mindi D'Elia, Kimberly Patterson, Erin Nicholas, Ronald Hunt

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**PURPOSE:** The purpose of this study is to gain insight into how US dental education programs are assessing predoctoral pediatric competencies.

**METHODS:** Surveys were sent to directors/department chairs of predoctoral Pediatric Dentistry programs. Questions contained response categories ranging from multiple choices, multiple answer and fill in the blank. A cover letter emphasizing purpose of survey and anonymity of responses was sent via email to participants with the link to the survey. The surveys were distributed via Google Survey. Responses were recorded automatically into a spreadsheet. Results were analyzed using statistical software.

**RESULTS:** The survey garnered an 82% response rate. Currently, a rotation-based experience is twice as utilized as patient-based predoctoral pediatric experiences. Forty-one percent of predoctoral programs do not have specific requirements regarding pediatric experiences prior to graduation. To address the lack of pediatric patients, some institutions allow manikin-facilitated, computer-based examination, or OSCE examinations for pediatric competency assessment.

**CONCLUSIONS:** Meeting Commission on Dental Accreditation (CODA) requirements for dental school graduates is a mandate of all dental programs. With dental schools experiencing a limited number of pediatric patients, assessment of competence in delivering pediatric dental care is accomplished by a variety of methods.

*Mindi A D'Elia was supported by the Midwestern University College of Dental Medicine - Arizona*

## **An Orthodontic Case Demonstrating the Importance of Comprehensive Treatment Plan Consideration**

Dr. Cheryl DeWood, Nicole Kim, Charles Roberts, Yen Nguyen, and Katie DeWood

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In today's multi-specialty field of dental medicine, it is important for every practitioner to consider the overall goals for their patient's treatment. This case demonstrates that the conventional approach that a general dentist may take towards the treatment of a seemingly straight forward situation may not necessarily prove to be the most ideal treatment once the patient's comprehensive treatment needs are considered. In this case study, a 15-year-old male patient presented to a dental school with the complaints of a diastema between his front teeth and a toothache affecting one of his first molars. Clinical examination and radiographs revealed the potential need for endodontic treatment for three of the patient's first molars. Infected teeth showed signs of enamel defects. It can be noted that this situation is not uncommon, even in children and adolescents, due to the high prevalence of enamel defects affecting at least one tooth. The conventional approach to the patient's treatment would include first addressing the patient's emergency need, his toothache. Typically treatment would favor endodontic therapy rather than extractions of patient's first molars. The patient's orthodontic needs would be later addressed in future appointments. In this case, however, once this patient's orthodontic needs were considered, extraction of the patient's first molars became a more favorable treatment. This not only addressed the issue of pain and infection but also addressed his orthodontic issues. Furthermore, the patient benefited from receiving fewer dental procedures and less costly overall treatment. By keeping the patient's overall treatment needs in mind, his practitioners were able to provide the best possible care, even though that care defied the conventional approach.

## **Fusion Clinically Presenting as Gemination: Implications on the Developing Dentition**

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**Background:** Per Tannenbaum and Alling (1963), fusion is formation of two teeth from the same follicle with two root canals contained within one or two roots. Clinically, fusion typically results in fewer teeth present within the arch. Brook and Winter (1970) noted fusion occurring most commonly in the mandible, while gemination was observed more frequently in the maxilla. Either presentation can pose both cosmetic and developmental issues.

**Case Report:** A 9 year old Caucasian male presented to the pediatric suite of the Midwestern University Dental Institute for an ‘overall check-up’ and expressed an interest in orthodontic evaluation. Cosmetic concerns included the permanent maxillary central incisors erupting overlapped, and what clinically appeared to be a geminated primary maxillary right lateral incisor (tooth #D). Upon radiographic evaluation, tooth D was found to have two root canals indicating fusion. Tooth D was not undergoing physiologic root resorption, and erupting tooth #8 presented radiographically a distally dilacerated root with its eruption path partially blocked by fused #D. Evaluation by both Orthodontist and Pediatric Dentist confirmed the need for extraction of fused #D to promote proper eruption of #8, as well as provide a favorable eruption path for the unerupted and radiographically normally developing #7.

**Discussion:** While clinical presentation provides a basis for diagnosis, the use of radiographic evaluation augments both diagnosis and treatment planning. The nature of the root structure of primary teeth must be considered in the eruption sequence and subsequent orthodontic needs of patients. The universal principal of ALARA (As Low as Reasonably Achievable) must always be followed while imaging patients.

## **Screw-retained versus Cement-retained implant restorations. A literature review.**

Jennifer L. Martin

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Implant-supported restorations can be secured to implants with screws (screw-retained), or they can be cemented to abutments, which are attached to implants with screws (cement-retained). There are advantages and disadvantages to using a screw-retained versus a cement-retained implant crown. It is important for the clinician to be aware of the pros and cons of each type of restoration when deciding which prosthetic design is more appropriate for a particular case. This review presents a comparison of the retention methods with respect to ease of fabrication and cost, esthetics, access, occlusion, retention, incidence of loss of retention, retrievability, clinical prosthesis fit, restriction of implant position, effect on peri-implant tissue health, provisionalization, immediate loading, impression procedures, porcelain fracture, and clinical performance. When treating implant patients, choosing between a cement-retained and a screw-retained restoration requires a comprehensive decision during the treatment planning phase because in certain clinical situations it is better to use one method of retention rather than the other.

## Considerations In Treatment Planning for Geminated Permanent Maxillary Central Incisors

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*CDMA, Midwestern University- Glendale, AZ*

**Background:** Gemination and fusion are anomalies in size, shape, and structure of teeth. They occur more frequently in the primary dentition, but can occur in the permanent dentition, most often the incisor region. Clinically they can be distinguished from one another by counting the teeth present or radiographically observing the root structure. Properly distinguishing between the two anomalies will promote appropriate treatment, which typically is multidisciplinary in approach.

**Case Report:** A 12 year old Caucasian American male presented to Midwestern University Pediatric Suite for a second opinion regarding his “large front teeth.” Clinical findings include abnormally wide permanent maxillary central incisors with isolated enamel notching, and permanent maxillary lateral incisors erupted palatally. Radiographically, each central incisor presents with one root canal with an average width of 6+mm. A multidisciplinary evaluation confirmed endodontic treatment to facilitate esthetic recontouring would not be applicable due to the width of the central incisor roots. Also, the lateral incisors could be retained and relocated to the central position to maintain bone structure and lip support, whereby upon cessation of growth implant replacement of the maxillary lateral incisors could be a consideration.

**Discussion:** The prognosis of teeth #8 and #9 is poor due to the esthetic concerns as well as the physical form of the teeth. The palatally erupted maxillary laterals can be used to recapture an esthetic central incisor form, and their root configuration is radiographically favorable to orthodontic movement. A multidisciplinary approach incorporating Endodontics, Orthodontics, and Oral Surgery is recommended.

# **Incidental Endodontic Periapical Pathology Missed On Conventional Periapical Radiographs and Discovered On CBCT Scans**

Vijay Parashar and Nicholas Netzel

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Radiography plays a significant role in diagnosis of periapical pathology. Periapical radiography is standard two dimensional (2D) imaging modality used for diagnosis of endodontic periapical pathology. The use of 2D imaging for visualization of three dimensional (3D) objects can lead to geometrical discrepancy which, leads to imaging errors like distortion and magnification.<sup>1</sup> The 3D imaging capability of Cone Beam Computed Tomography (CBCT) allows for more accurate identification of oral and maxillofacial structures.<sup>2,3</sup> The American Academy of Endodontics in conjunction with the American Academy of Oral and Maxillofacial Radiology have issued a joint position statement describing the role of CBCT in endodontics.<sup>4</sup> This study evaluated the ratio of endodontic periapical pathology discovered on 2D vs. 3D imaging. Midwestern University's IRB review and approval was received for this study. 500 CBCT reports generated by Oral & Maxillofacial Radiologist for implant planning were reviewed for reporting of incidental endodontic periapical pathology (IEPP). The reports that informed of IEPP were selected and corresponding patient's radiographic form in Axium chart was reviewed for charting of periapical lesions on 2D radiographic interpretation. Of the 500 CBCT reports reviewed, 101 reports informed about presence of incidence of IEPP. The total number of IEPPs found was 147. When compared with the IEPPs identified on 2D periapical radiographs, only 60 of the 147 lesions were identified and reported in the patient's radiographic findings form in axium. Our findings show an increased incidence of IEPP on CBCT imaging. We found that 59% of the lesions identified on CBCT report were missed on 2D periapical radiographic interpretation in axium. These newly identified lesions were reviewed with endodontic faculty and appropriate treatments (Root canal/extraction/no treatment) were performed on previously missed lesions. The results of this study emphasize the need to review entire CBCT scan volume to identify potential incidental pathology.

*Nicholas Netzel was supported by the Midwestern University Research Scholarship.*

## **A Metameric Analysis of Six Esthetic CAD-CAM Materials Under Different Light Sources**

Anthony Royal, Christine Van Groesbeck, and Dr. Robert Kramer

*CDMA, Midwestern University, Glendale, Arizona*

The use of computer aided design and manufacturing (CAD-CAM) in dental offices and laboratories is becoming more prevalent every year as the technology matures and gains wider clinical acceptance. Dental CAD-CAM materials are produced by several manufacturers, in many compositions and with various properties, but ultimately serve the same purpose; the replacement of natural dentition with esthetically pleasing, yet highly functional, replacement structures. These materials are typically milled in-house by CAD-CAM systems that can produce a crown, for example, in about an hour and allow the delivery of the finished product to the patient without requiring a second visit. Prior to producing a crown, however, a proficient dentist must first select an appropriate shade and type of material for the finished restoration. Typically, this is done by comparing a shade guide to the patient's existing dentition and selecting the shade that is the closest match while the patient is seated in the operatory chair. However, human perception of color is affected by the source of illumination that is cast upon the object to be perceived. In order to analyze the metameric relationship of popular CAD-CAM dental restorative materials, ten samples each were fabricated from six different materials, in Vita A2 color. The samples were milled from one standard all ceramic crown design for a mandibular right first molar, utilizing the E4D Design Center, producing sixty crowns. A photo of each crown was taken, along with a Vita Shade Guide A2 tab, on a neutral gray background, using an iPhone 4S and Olloclip macro lens, utilizing six different sources of light and from a similar distance and angle. Each photo was sampled and analyzed in Apple iPhoto software. The histograms of each sample were compared. Variance was noted, leading to the conclusion that dental materials may appear different under different light sources.

*Anthony Royal was supported by the Midwestern University Fall Research Grant*

## **The Effects of a Dentigerous Cyst on Tooth Formation: A Case Report**

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*CDMA, Midwestern University, Glendale, AZ*

Dentigerous cysts are the most prevalent developmental cysts of the jaws and are associated with the crown of an unerupted or impacted tooth. The frequency of odontogenic cysts in children is relatively low. This case describes the diagnosis and treatment of a dentigerous cyst in an unusual location in a non-syndromic 13-year-old Caucasian male discovered via radiographic examination as prescribed by the FDA, ADA, and AAPD. Tooth #T was extracted and the cyst was removed with tooth #29 erupting into an unfavorable position. Not only does this case illustrate the importance of radiographic examination in preventing the deleterious effects of dentigerous cysts on the permanent dentition, it also highlights the need for a multidisciplinary approach in diagnosing and treating the pathology.

## **Examining Board Disciplinary Actions In The Areas of Arizona and Alberta.**

Cameron Stringham and Dr. David Rolf

*CDMA, Midwestern University – Glendale, Arizona*

This study is an examination of Arizona dental board disciplinary actions of Arizona dentists. These disciplinary actions have been categorized, and each category has been examined to understand the norms of board disciplinary action associated with each type of discipline. The goal of this study is to help dentists, and dental students understand areas most likely to result in state board discipline, and help them understand the related consequences associated with certain actions. The hope of this project is that an understanding of where dentists have made mistakes will prevent dental students and dentists from making similar mistakes in the future. The data for this project was gathered from the Arizona State Board of Dental Examiners (azdentalboard.us). This entire database was examined to find dentists in Arizona with dental board disciplinary actions. Of the thousands of dentists examined in Arizona 954 had at least one dental board disciplinary hearing. 563 of these hearing were not publicly listed, or were not within the scope of this study, which includes only current disciplinary actions from 2000-2012. The remaining 391 disciplinary actions within the time scope of this study were categorized as follows: Advertising (2 cases), Sexual Harassment (2 cases), Fraud (4 cases), Licensing Issues (12 cases), Non-compliance with boards requests (14 cases), Substance Abuse (57 cases), Problems with treatment planning/ documentation (89 cases), Unsatisfactory dental work (157 cases), Other areas of unprofessional conduct (54 cases). This project examined each of the above categories and discusses the average Arizona Dental Board disciplinary actions for each violation.

*Last years research project, an examination of potential discrepancies between American and Canadian, (specifically Arizona and Alberta) dental state/provincial board disciplinary actions, is also discussed.*

## **A Comparative Study of Color Consistency of Six CAD/CAM Dental Materials**

Christine Van Groesbeck, Tony Royal, and Dr. Robert Kramer

*CDMA, Midwestern University, Glendale, Arizona*

Color inconsistencies have been recognized among packable composite restorative materials however little research has compared the color consistency of milled restorative materials. Ten samples each of six different dental materials were milled in the E4D CAD/CAM system from the same All Ceramic Crown proposal: Empress Multi, Empress LT (leucite reinforced ceramic, Ivoclar Vivodent), e.max HT, e.max LT (lithium disilicate ceramic, Ivoclar Vivodent), Lava Ultimate (nanoresin zirconia filled polymer, 3M ESPE), and Paradigm MZ100 (nanohybrid composite, 3M ESPE) all following the same gold standard design of tooth #30. All materials were selected in the most common shade, A2, and verified using the VITA Easyshade® Compact colorimeter on a 45-degree angle to the buccal cusp. The restoration was placed on a neutral gray background during the color assessment. Colorimeter shade results were converted to numerical Munsell notation for hue, value, and chroma. The VITA Classic® shade guide was used as an industry standard constant for the A2 shade and verified by the VITA Easyshade® Compact colorimeter. Individual materials were compared graphically to the color components for A2. Graphically all materials performed differently. No material was completely consistent for A2. The range of shades recorded for all samples of all materials was: A1, A2, B1, B2, C1, D2. e.max LT had the most sample results for A2, as well as results for shades B2 and A1. Lava Ultimate produced the most consistent shade, B2.

*Christine Van Groesbeck was supported by the Midwestern University Fall Research Grant.*

### **Police Officers Health, Safety, Cognitive Abilities and Life Satisfaction Related to Shift Length**

Leonard B. Bell, PhD, Thomas B. Virden, PhD, Deborah J. Lewis\*, PhD, ABPP, Barry A. Cassidy\*\*, PA-C, PhD, Det.

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To determine the impact of alternate shift work on the performance, health and well-being of police officers, two precincts of the Phoenix Police Department participated in an eight month between methods design. The experimental precinct (900) was shifted to 13-hour, 20-minute (13+ hour) shifts for three days per week during the six month experimental phase, while the control precinct (700) remained on their usual 10-hour shifts four days per week. Precincts were assessed using the Quality of Life Inventory (QOLI), Stroop color-word test, Psychomotor Vigilance Test (PVT; reaction time), measures of cortisol, and Pittsburgh Sleep Quality Index (PSQI). Public service complaints and vehicular accidents were recorded as well as self report measures of caffeine intake and shift preference. Significant ( $p < 0.05$ ) decreases in reaction time, concentration, cognitive processing, and quality of life due to sleep deprivation were found in officers working 13+ hour shifts. Significant increases in errors and daytime dysfunction due to sleepiness were also found in the 13+ hour shift group. When officers working 13+ hour shifts returned to 10-hour shifts, variables returned to baseline levels relative to the control precinct. This study indicates that there are no apparent advantages and considerable liabilities associated with 13-hour, 20-minute shifts for police officers.

*Project supported by: Midwestern University, College of Health Sciences Research Incentive Grant and the Phoenix Police Department*

## **Epigenetic Regulation of Subtype Switching via Histone Acetylation in the SW13 Human Adenocarcinoma Cell Line**

Juliane Daggett, Agnes Pascual, Kimbal Cooper, Kathryn Leyva, and Elizabeth Hull

*Biomedical Sciences, College of Health Sciences, Midwestern University, Glendale, Arizona*

The human adenocarcinoma cell line SW13 has been reported to switch between two subtypes, one expressing vimentin (SW13+) and the other not (SW13-). We have found that vimentin expressing SW13+ appear to divide more slowly with the more spindly morphology characteristic of mesenchymal cells while vimentin negative SW13- cells appear to divide more quickly and are more rounded. Given the importance of metastasis in cancer progression and the induction of vimentin expression as cells adopt the mesenchymal or metastatic form, we have investigated the transition between the two SW13 subtypes further. Working with the hypothesis that the subtype switch is due to epigenetic regulation of gene expression via acetylation of histone octomers and the SWI/SNF chromatin remodeling complex, we first asked if histone deacetylase inhibitors could affect SW13 subtype switching. The results show that a broad-spectrum histone deacetylases inhibitor converts the SW13- cells to SW13+. More specific inhibitors with overlapping specificities were then used to identify which histone deacetylase enzymes were most important in subtype switching. The results suggest that histone deacetylase 1 and 3 are necessary for subtype switching and but that histone deacetylase 6 and 10 reduce the effectiveness the subtype switching initiated by 1 and 3. As histone deacetylases 1 and 3 affect acetylation of histone 3 and this histone is known to interact with the SWI/SNF chromatin remodeling complex, we assayed the acetylation level of histone 3. SW13+ cells had an increased levels of histone 3 acetylation at specific lysine residues shown to interact with the SWI/SNF complex and an increased expression of Brm, the ATP binding unit of the SWI/SNF complex, as compared to the SW13- cells. These results indicate that epigenetic regulation results in increased acetylation on histone 3 causes SW13- cells to transition to SW13+ cells possibly through involvement of the SWI/SNF complex.

*Juliane Daggett was supported by the Midwestern University Biomedical Sciences Program. Elizabeth Hull and Kathryn Leyva were supported by Midwestern University intramural funds.*

# Genome-wide analysis of primate and rodent protein-coding and associated non-coding nucleotide sequences

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Mammalian species have been characterized in the literature by genome-wide analysis of protein-coding sequences, but an analysis of the associated non-coding and regulatory sequences has been lacking. To fill this lacuna, we obtained the 5' and 3' UTRs, intron sequences, 5000 bases of the 5' and 3' flanking regions, as well as the coding sequences from Ensembl (<http://www.ensembl.org>) after determining the Ensembl IDs from InParanoid7 (<http://inparanoid.sbc.su.se>) for all known orthologs among four mammalian species (two primate and two rodent): human, chimpanzee, mouse and rat. There were 7,244 error-free sets of orthologs among all four species. Evolutionary analyses were done using in-house computer programs or by means of the program MEGA-CC. Homogeneity of the nucleotide substitution pattern between species was tested using the Disparity Index test, and tests of selection were done using the Z-test for coding sequences and Tajima's D for non-coding sequences. A majority of the genes (~ 71%) were found to be evolving neutrally, while almost all the rest were determined to be under purifying selection. A very small number (23) are undergoing adaptive evolution in the primate lineage. On average, these genes show a lower G+C content in all four species, but especially in the primates. In contrast, the genes under negative or neutral selection show high G+C content. Interestingly, while 327 genes were found to be evolving with a heterogeneous nucleotide substitution pattern between human and chimp, only two of them are under positive selection, while 140 are under purifying selection, and 185 are evolving neutrally. We present here the evolution of coding sequences across four mammalian genomes (two primate and two rodent), and discuss them in relation to the associated non-coding sequences.

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## **Blimp-1 Participates in Patterning during Pupal Eye Development**

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B lymphocyte-induced maturation protein (*Blimp-1*) was identified in *Drosophila melanogaster* nearly seven years ago, but its function remains largely unknown. Mosaic eye tissue (via the *ey-Flp/FRT* system) reveals a unique raised-glossy lens surface in mutant tissue in adults and significant interommatidial bristle patterning defects in both adults and pupae. Data from pupal eye discs with mutant *Blimp-1* clones reveals nonautonomous ommatidial patterning defects including loss and mispatterning of bristles (64% vs. 2% in control eye discs) and secondary pigment cells (17% vs. 0%), but not tertiary pigment cells. Analysis of cell adhesion molecules essential to ommatidial patterning reveals no apparent differential expression of N-Cadherin, Roughest or Kirre. However, reduced Hibris expression is observed in *Blimp-1* mutant tissue. This decrease in Hibris may explain the bristle patterning defects. Investigation of the adult raised glossy eye phenotype, the interaction between Blimp-1 and Crystallin, the main lens protein, is being studied through the use of *Cry-lacZ* reporters. When raised at 25°C, normal *Cry-lacZ* expression begins in primary pigment cells (PPC) sporadically distributed throughout the eye disc at about 35-40 hours after puparium formation (APF), and slowly ramps up into full production in all PPCs and cone cells (CC) by 45-50 APF, peaks at 60 APF, and then ceases by 65 APF. In striking contrast, by 35 APF at 25°C, all photoreceptors strongly express *Cry-lacZ*, and continue to do so through 50 APF in mosaic *Blimp-1* mutant eye discs. At 50-55 APF, PPCs appear to maintain a higher expression level of *Cry-lacZ* in the *Blimp-1* mutant cells. This misexpression pattern of *Cry-lacZ* might explain the raised lens phenotype observed in the adult mosaic eyes. CC-specific overexpression of *Blimp-1* significantly decreases *Cry-lacZ* expression in a cell-specific manner. These findings suggest that Blimp-1 regulates multiple pathways involved in late eye development.

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## **Calcium Transients in Collective Cell Migration of Keratocyte Sheets: Possible Intercellular Calcium Wave Mediated by Gap Junctions**

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Wound healing is a complex process involving a cascade of cellular responses that ultimately repair the injured site. Collective cell migration plays an important role in completing the re-epithelialization stage of this process. There are many key players in cell migration a current focus of research is calcium signaling which mediates some of the regulation of cellular movement. Research done on movement of single cells has characterized calcium transients associated with cell movement. We investigated this calcium-signaling phenomenon in the collective cell movement in zebrafish keratocyte sheets. A similar calcium transient burst pattern was observed between leader and single cells suggesting calcium may have a similar role in cell movement for collective cell and single cell migration. Calcium transients were not observed in follower cells which is consistent with the lack of adhesion and lamellae in these cells. An intercellular calcium wave was seen at the leading edge of the cell sheet and in the leader cells surrounding spontaneously formed holes, suggesting some sort of coupling. As coupling between cells is mediated by gap junctions composed of transmembrane proteins called connexins, we next investigated the role of connexin 43 (Cx43) as this connexin is upregulated during explant culture. Consistent with the lack of calcium transients in follower cells, this possible target was found to be present in those leader cells and not the followers using immunofluorescence. Cells were then treated with a Cx43 inhibitor that appeared to halt the coupling action seen with the calcium. These data suggest that calcium transients occur in certain cells of collective cell migration and a possible coupling between these cells through a gap junction protein may explain the communication between them. With these findings it is a step closer in explaining how these cells migrate in the wound healing process.

*Stacie Knight was supported by the Midwestern University Biomedical Sciences Program.  
Elizabeth Hull was supported by Midwestern University intramural funds.*

## Effects of nicotine on motor deficits and lifespan when given on different treatment days in a Parkinson's disease model

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In the US, Parkinson's disease (PD) affects about 13 out of 100,000 and is the second leading neuromotor degenerative disease. *Drosophila melanogaster* with *parkin* loss-of-function mutation exhibit similar pathology to patients with familial PD such as motor deficits, mitochondrial pathology and decreased lifespan, which makes it especially viable as a model for familial PD compared to other Parkin loss-of-function models, which do not exhibit these symptoms. Motor deficits may stem from mitochondrial pathology, which leads to indirect flight muscle degeneration. Epidemiological studies suggest a delay in the onset of PD in tobacco smokers and that nicotine has neuroprotective effects in models of sporadic PD. Previous data in our laboratory suggests that nicotine rescues diminished motor, viability and olfaction symptoms in *park*<sup>25/+</sup> *Drosophila* when given at day one post eclosion. This study was initiated to determine if nicotine can rescue symptoms when administered at later days by assessing climbing and flight assays on control and *park*<sup>25/+</sup> flies when exposed to nicotine at later days post eclosion. Results indicate that treatment with nicotine at 3 days post eclosion improves climbing and flight. Additionally, a new olfaction assay was created to address issues with the previous olfaction assay. A T-tube olfactometer was created and benzaldehyde, a chemorepellant, was used instead of food for a more acute response. Using the new assay, preliminary findings demonstrate that the *park*<sup>25/+</sup> flies do have olfaction deficits as observed before, but in a better method. These results will help clarify whether nicotine can be an effective treatment for familial PD when given to patients after they first start experiencing symptoms such as loss of olfaction.

## **Microstructure and cross-sectional shape of limb bones in Great Horned Owls and Red-tailed Hawks: how do these features relate to differences in flight and hindlimb usage?**

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The Red-tailed Hawk (RTH) and Great Horned Owl (GHO) are two species of raptor that are similar in body size, have generalized diets, and often occur sympatrically. The RTH is active during the day and the GHO is nocturnal. They also differ in primary flight style; the RTH uses static soaring and the GHO uses flap-gliding. Both species use their hindlimbs to catch prey, but the RTH uses rapid leg movements, whereas the GHO uses high force grip. The objectives were to characterize the microstructure and cross sectional shape of limb bones of these species and examine the relationship with flight and hunting behaviors. The mid-shaft of four limb bones (humerus, ulna, femur, tibiotarsus) from 6 individuals of each species was sampled and prepared histologically. The laminarity (proportion of circular primary vascular canals) and cross sectional parameters (amount and distribution of cortical bone: cortical area, second and polar moments of area) were calculated. As predicted, the forelimb elements and femur in both species exhibit higher laminarity than the tibiotarsus. The humerus and femur also exhibit higher polar moment of area, suggesting a higher resistance to torsional loading. The tibiotarsus has a larger relative cortical area than other bones, suggesting better resistance to compressional loads. Between species, the laminarity of the RTH femur is higher than that of the GHO. The femur of the RTH is more circular and the tibiotarsus is more elliptical than the GHO. Although the species use different flight modes, the microstructure and shape of forelimb bones is quite similar. Differences among hindlimb elements may reflect different methods of capturing prey

## Determining the Basis of Nicotine Rescue in Parkinson Disease Model Flies

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The *Drosophila melanogaster* Parkinson's Disease (PD) model based on homozygous loss of function of the *parkin* gene has been shown to have both flight muscle degeneration and dopaminergic neuronal loss in the brain. Our previous data also indicates that flies heterozygous for the *park*<sup>25</sup> null allele also experience motor function defects, olfaction loss and decreased lifespan. Interestingly, administration of nicotine to these flies improved or rescued all of the observed deficits. This study was initiated to determine the mechanism of this rescue by histological and genetic methods. The first method consists of histologically analyzing the indirect flight muscle and dopaminergic neurons in the brain to determine if the morphology or numbers of these tissues are affected by nicotine treatment. Results indicate that nicotine does not affect neuron numbers in 20-day-old *park*<sup>25/+</sup> flies, nicotine = 12.62 neurons/cluster (n=37) vs. no nicotine = 12.92 neurons/cluster (n=26). Initial qualitative analysis of muscle images tend to display less muscle integrity in both *park*<sup>25/+</sup> and control flies that were treated with nicotine. In addition to histological analysis, a genetic mechanism using RNAi to knock down Parkin in a tissue-specific manner was performed to determine the site of nicotine rescue. Ubiquitous knockdown of Parkin displayed a significant decrease in lifespan at 25°C, similar to that observed with *park*<sup>25/+</sup> flies. However, this cross does not show a deficit in climbing behavior at 25°C. When raised at 29°C, to increase the RNAi efficiency, the cross also displayed similar deficits to that of *park*<sup>25/+</sup>, indicating a successful phenocopy of the *park*<sup>25/+</sup> flies. However, the individual muscle- and neuron-specific knockdown of Parkin appears to have no effect in the behavioral assays. We are currently creating a fly cross to knock down both neuronal and muscle Parkin simultaneously to distinguish whether both tissues need to be involved.

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## **During collective migration, leader keratocytes generate tension in sheet and may become follower or individually migrating cells**

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Although the fish keratocyte is a well-established system for the mechanisms of single cell motility, little is known about the collective cell migration of these cells. Early in explant culture, leader cells begin to pull sheets of keratocytes from the scale at rates of  $\sim 145 \mu\text{m/hr}$  which results in follower cell spreading and tension within the sheet as demonstrated by the rapid retraction of the sheet after the addition of RGD but not control peptide. The rate of leading edge advance decreases to  $\sim 23 \mu\text{m/hr}$  after 24 hours in culture, possibly due to increased tension within the sheet as leader cells retain their ability to migrate rapidly after release from the sheet. Keratocyte cell types interconvert within minutes with changes in cell-cell adhesion. Leader cells migrate as single cells when they detach from the leading edge and single cells appear to become leader cells if they rejoin the sheet. Follower cells rapidly establish leader cell morphology during closing of spontaneously formed holes and revert to follower cell morphology after hole closure. Inhibition of Rho-associated kinase activity to disrupt supracellular contractility, releases leader cells from the sheet, halting the advance of the leading edge. The continued migration of released single cells suggests that greater contractility is necessary for maintenance of adhesions in the cell sheet than individual cell migration. These data establish fish keratocyte explant cultures as a collective cell migration system and underscore the importance of cell-cell interactions in determining the role of keratocytes within the migrating sheet.

## **Fluorescent *Pseudomonas* associated with cranberry (*Vaccinium macrocarpon* Ait.) roots and soils**

Scott D. Soby<sup>1</sup>, Sudhindra R. Gadagkar<sup>1</sup>, Zachary J. Hummel<sup>2</sup>, Alisha Holmberg<sup>1</sup> and Frank L. Caruso<sup>3</sup>

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Native and commercial cranberry bogs represent an ideal system for comparing differences in complex microbial population structures which may have arisen due to cultivation. Wild and domesticated cranberry bogs in southeastern Massachusetts are located in close geographical proximity, they share a common climate, and because many cultivated plantings are essentially wild accessions, native and cultivated plants are genotypically nearly identical. Differences in microbial populations can thus be confidently attributed to agricultural practices. As an initial part of a project to characterize wild and cultivated cranberry-associated microbial populations, soil and roots of native and commercial cranberry plants were aseptically sampled and examined for the presence of fluorescent pseudomonads. Isolates were characterized for a range of phenotypic and genotypic characters using standard and novel identification methods for plant-associated microbes, including Multi-Locus Sequence Analysis (MLSA), REP-PCR and MALDI-TOF MS. Forty-four characterized isolates were genetically and phenotypically diverse, and distinct from previously identified *Pseudomonas*. These isolates generally localized to three broad but distinct phylogenetic clades: the *P. fluorescens* and *P. putida* clades contain a number of well-characterized Plant Growth-Promoting Bacteria and commensals, and the third clade represents what may be a new group of *Pseudomonas* distantly related to the *P. syringae* group which contains a number of important plant pathogens. Detailed molecular analysis of a number of genetic characters within this group of fluorescent bacteria suggests that there are many unknown and uncharacterized bacteria associated with agronomically-important plants, and that the high level of genetic diversity within complex populations will require new ways of conceptualizing taxonomic groups.

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## **Exploring the Mechanism of Nicotine-Mediated Protection in Parkin Heterozygous Loss-of-Function *Drosophila melanogaster***

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Parkinson's disease (PD) is characterized by the death of dopaminergic neurons in the substantia nigra pars compacta, leading to motor and non-motor dysfunction. Epidemiological studies suggest that tobacco smoking can decrease incidence of sporadic PD and delay onset of motor symptoms; subsequent *in vivo* and *in vitro* studies have shown that nicotine can be neuroprotective in sporadic PD models. We have previously demonstrated that long-term nicotine exposure can rescue the pathological phenotype of heterozygous parkin loss-of-function mutant strain of *Drosophila melanogaster*. More specifically, nicotine increases median lifespan and improves motor and olfactory deficits in these flies. We sought to determine whether the protective effect of nicotine was mediated through activation of the *Drosophila* nicotinic acetylcholine receptor (DnAChR) or via DnAChR-independent mechanisms. To this end, we co-administered nicotine and mecamylamine, a nonselective nAChR antagonist, from day one, post eclosion and measured median lifespan, climbing, flying and olfaction. We have found that nicotine-mediated improvements in survival and climbing persist in the presence of mecamylamine, indicating that nicotine may be eliciting its protective in a DnAChR-independent manner.

## **One-Minute Paper: Student Perception of Learning Gains**

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**OBJECTIVE:** The one-minute paper is an assessment and learning strategy that facilitates students, through writing, to document the most important item or concept learned following class instruction and the item or concept that remained confusing or ‘muddy’. Despite academician support of the one-minute paper, research is lacking on student perceptions of the effect of this method on learning. The purpose of this descriptive survey study was to determine students’ perceptions of learning gains when using the one-minute paper in two healthcare graduate programs: nurse anesthesia (NA) and physical therapy (PT).

**PARTICIPANTS:** Thirteen respondents from the PT program and eighteen respondents from the NA program completed the online survey (n=31) following the use of the one-minute paper in three different class sessions within each of the programs. Mean age of respondents was 29 years (range 23-44 years). Eighty-one percent of the respondents were female.

**METHODS:** The data from each survey question was analyzed using means and frequency distributions. Non-parametric Mann-Whitney Tests were used to assess the significance of mean ranks of the survey question responses between the NA students and the PT students and to assess significance of age on the survey responses. The significance of students’ perceptions of learning gains between each of the five different teaching/learning methods was analyzed using the Friedman test.

**RESULTS:** Students’ perceptions of learning gains were significantly different between types of teaching/learning methods,  $X^2(4) = 18.05, p < .05$ . Wilcoxon tests were used to follow up this finding. Students reported less learning gains from participating in the one-minute paper as compared to attending lectures and listening to class discussions. No difference was found between participation in the one-minute paper and participation in group work or participation in class discussions. However, seventy-seven percent of students (n=24) indicated that completion of the one-minute paper resulted in good or great gains in connecting key class ideas with other knowledge. Seventy-four percent of students (n=23) indicated that completion of the one-minute paper resulted in good or great gains in applying what they had learned in class to other situations. No significant differences were found between the mean ranks of the responses from students in the different programs (NA and PT) on any of the survey items. Younger students ( $\leq 25$  years) reported significantly less learning gains from group participation than older students ( $> 25$  years).

**CONCLUSIONS:** Analysis of the data indicated that students perceived their learning gains to be less through participation in the one-minute paper and in group work than when involved in more traditional learning activities such as attending class and listening to discussions. However, students appeared to value the use of the one-minute paper in helping them to make connections with other course material and for application of knowledge. Future research should focus on student comfort and preferences for new active learning strategies. Use of the one-minute paper to enhance learning is supported.

*Debbie Anderson and Shari Burns were supported by CHS Midwestern University intramural funds.*

## **Student's Perceptions of the Efficacy of Prerequisite Requirements for Entry into the Nurse Anesthesia Program**

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Nurse anesthesia educators strive to identify factors that will lead to successful completion of the nurse anesthesia program and passing the National Certification Examination Using a ten question survey, we asked nurse anesthesia students to share their perceptions of the efficacy of the prerequisite requirements for entry into the Nurse Anesthesia Program. Out of 86 respondents, 10 (11.8%) were at the beginning of the program, 35 (40.7%) were at the end of the didactic phase, 16 (18.6%) were in the clinical phase, and 25 (29.1%) were recent graduates. Nearly 40% of the respondents indicated they considered success in the program as achieving a grade of 90-94% in each class. Twelve (14.8%) of the respondents considered a grade of greater than 95% as successful. The prerequisite requirements of anatomy/ physiology and pharmacology courses were perceived as “extremely helpful” (76.7 and 69.4% respectively). The majority of the students believed organic chemistry was considered “mostly helpful” (31.8%). General chemistry, basic research and statistics courses fell into the “slightly helpful” range (37.2, 49.4, and 45.9% respectively). Other non-required prerequisite courses the respondents “strongly or somewhat agreed” would be beneficial were a human anatomy cadaver lab (75.3%), a physical assessment course (73.8%), and a pathophysiology course (80.2%). Over 40% of the respondents “somewhat agreed” that their Bachelor’s degree education was sufficient. Students “strongly or somewhat strongly agreed” managing invasive lines and manipulating vasoactive drips are important in preparing them for their didactic experience (92.7%). Experience in the ICU and coronary care units were also highly beneficial for success in the didactic phase of the program (97.7 and 92.2% respectively). The students were asked how many years of critical care experience should be required for entry into the program. Seventy-one percent of the respondents considered “greater than two years” as the appropriate requirement.

*Rodney Fisher received no funding support for this study.*

## **Interdisciplinary Simulation: Perceptions of Graduate Health Science Students**

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Interdisciplinary education is supported by leading professional organizations around the world. Simulation training is standard practice in health science education. The diversity of programs within the College of Health Sciences provides opportunities for students training in specialties with a perioperative focus to engage in surgical simulation training together. Podiatrists require anesthesia services for many surgical cases and the cardiovascular perfusionist works very closely with anesthesia providers during cardiac cases.

Research on interdisciplinary simulation in these specialties has not been frequently reported. The purpose of this study is to assess the perceptions of graduate health science students before and after two collaborative simulation exercises.

The Interprofessional Education Perceptions Scale (IEPS) revised by McFayden and colleagues in 2007 is used in this pre-post survey of volunteer participants from the Nurse Anesthesia, Cardiovascular Science, and Podiatric Medicine programs at Midwestern University. Data collection is ongoing at the time of abstract submission.

*Generously supported by a Research Facilitation Grant from the College of Health Sciences.*

## **A Qualitative Study of Participant’s Experience in the NEW-R Weight Loss Program**

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Individuals with serious mental illness experience high rates of obesity, cardiovascular disease, and diabetes along with a significantly shortened life span when compared to the general population. These issues have led to the development of specialized weight loss programs for people with serious mental illness. A recent review found promising results; however, amongst participants there was great variability in terms of response to the intervention. This qualitative study used a phenomenological design to examine the personal experience of three individuals with serious mental illness who participated in the NEW-R weight loss program, but who had different levels of weight loss success. Purposive sampling was used to identify individuals with varying response to the program. Individualized and in depth interviews were conducted to examine the research question, “what are the facilitators and barriers to weight loss?” The interviews were transcribed and then analyzed by each of the researchers to identify relevant themes. The identified themes of motivators, strategies and outcomes were related to one another in a cyclical fashion that could be disrupted by barriers. Social support and encouragement was identified as an important motivator leading to the adoption of practical strategies for selecting foods, cooking and exercising. Feeling better and success with weight loss increased motivation. On the other hand, relatively minor barriers, such as not having a recipe or dealing with a flat tire on a bicycle, were difficult to overcome. Interestingly, the individual with the greatest weight loss had a less successful self evaluation than the individual with the least amount of weight loss. The results of this study point towards the importance of continuous support, approaches to reduce barriers, and the creation of habits that can lead to long term lifestyle changes.

*Catana Brown was supported with Midwestern University start-up funds.*

# **Efficacy of the NEW-R Weight Loss Program for People with Serious Mental Illness**

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Obesity rates among people with serious mental illness are higher than the general population particularly for women. Individuals with psychiatric disabilities have high rates of physical illness; yet, their general health condition tends to be neglected by health care providers. So egregious is the health care disparity that one study found decades of potential life lost for public mental health clients when compared to the general population, with the most common cause of death being cardiovascular disease. Weight loss programs are needed that address the specific needs of people with serious mental illness. This pilot study examined the feasibility and efficacy of the NEW-R (Nutrition and Exercise for Weight Loss and Recovery) intervention. NEW-R is an 8 week program meeting once a week for 2 hours that applies psychiatric rehabilitation principles to a nutrition and physical activity curriculum. 18 individuals with serious mental illness were recruited to participate in the intervention. 1 participant dropped out during the intervention and one participant dropped out at the 6 month follow-up. The average number of sessions attended was 5.8. The average weight at baseline was 229 lbs with an average BMI of 33. There was an insignificant mean weight loss of 3.02 lbs at 8 weeks ( $t = 1.84, p = .08$ ); however, participants continued to lose weight after the intervention with a statistically significant change in weight loss of 9.8 lbs at 6 months ( $t = 2.39, p = .028$ ). The drop-out rate was low and attendance rates were acceptable. The NEW-R program may be useful in helping individuals with serious mental illness begin and continue to lose weight.

*Catana Brown was supported with Midwestern University start-up funds.*

## *Physical Therapy Program*

### **The “Call It Like You See It” Games: A Novel Approach to Teaching Students Basic Electrocardiographic Rhythms**

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Rehabilitation professionals must be able to rapidly and correctly identify cardiac dysrhythmias in the presence of exigent clinical circumstances. The purpose of this project was to develop a game-oriented approach to enhance both ECG rhythm recognition speed and ECG rhythm interpretation accuracy. Forty-eight Midwestern University physical therapy students (23 males, 25 females; mean age  $24 \pm 2.1$  years) participated in the class activity. Students were divided into 8 teams of 6 with each group competing against all others in round-robin style. Specifically, two competing teams stood next to a projection screen upon which an ECG rhythm abruptly appeared. The team leader signified readiness to venture a “guess” by raising a cardboard placard. Should the answer be incorrect, the other team was given 10 seconds to also “guess.” This back-and-forth process continued until the rhythm was correctly identified. Class members were encouraged to cheer for their favorite teams. Competition ended with the awarding of gold, silver or bronze (plastic) medals. Approximately 80% of all ECG rhythms were correctly identified within 30 seconds and on the first try. AV block patterns presented the greatest challenge with 70% initially identified. Two ECG rhythms were not correctly identified by either team necessitating presentation of a second rhythm strip. We found the competition idea was readily accepted; students even developed team names like the “Bradycardic Bunch” and “The Heart Breakers.” We further noted, even under the stress of time, ECG rhythms were usually identified correctly. Last, incorrect guesses often generated class discussions serving to solidify concepts. Timed identification of ECG rhythms may be a superior method for training students than untimed training alone.

## **Evaluation of Students' Perceptions Regarding Their Learning within a Pharmaceutics Course Sequence**

Hillary Aphaisuwan and Bill J Bowman

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To determine students' perceptions regarding 1) how beneficial different aspects of a pharmaceutics course sequence were to their learning, 2) which course items were most and least beneficial, and 3) what should be added to improve student learning. During the 2010-2013 academic years (n=3), a survey instrument was distributed and collected at the conclusion of the pharmaceutics course sequence. The instrument asked students to rate how beneficial twenty different course items were to their learning using a four-point scale. The instrument also asked students to indicate which item was most beneficial, which was least beneficial, and what should be added to improve learning. The response rate was 98% (n=442). All course items were utilized by at least 97% of students except for supplemental readings (63%). Review questions ( $3.93 \pm 0.29$ ) and course instructor's knowledge ( $3.93 \pm 0.26$ ) were rated as having the highest mean level of benefit while supplemental readings had the lowest ( $2.90 \pm 0.26$ ). Compounding activities (n=125) and review questions (n=95) were most frequently indicated as most beneficial while pre-lab assignments (n=52) and supplementary readings (n=47) were most frequently indicated as least beneficial. Open lab sessions appear to be the most common theme with regard to what should be added to improve student learning. Students seemed to perceive the items that enabled them to practice the course material as being more beneficial to their learning. In addition, the lack of a comprehensive text for the pharmaceutics course sequence may be reflected in the lower perceived benefit of the supplemental readings.

## **Mitochondria-targeted Delivery of Dehydroascorbic Acid increases ATP Production in oxidatively stressed cells**

Brittany Boger and Volkmar Weissig

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Reactive oxygen species (ROS) -induced mitochondrial stress plays a key role in the pathogenesis of neuromuscular and neurodegenerative diseases. Ascorbic acid (AA) is well known to quench an excess load of ROS, but its transport into mitochondria is poorly understood. It was recently found that not vitamin C, but the oxidized form of vitamin C, which is dehydroascorbic acid (DHAA), enters mitochondria via facilitative glucose transporter 1 and accumulates in mitochondria as AA. Based on these findings we hypothesized that the direct delivery of DHAA to mitochondria cells will facilitate the mitochondrial accumulation of AA and thereby ameliorate oxidative stress. To prove our hypothesis we first induced oxidative stress in 4T1 cells via exposure to dimethoxynaphthoquinone (DMNQ), a redox-cycling agent that induces intracellular superoxide anion formation. We then measured the mitochondrial ATP production under different treatment conditions knowing that any impairment of mitochondrial function will inhibit oxidative phosphorylation. For mitochondria-targeted delivery, we encapsulated DHAA into mitochondriotropic STPP liposomes (1) and compared their effect on ATP production in stressed cells with DHAA encapsulated in plain non-targeted liposomes. While non-targeted DHAA did not show any effect on ATP production, cells incubated with DHAA encapsulated in mitochondria-specific STPP liposomes significantly increased cellular ATP levels.

*Brittany Boger was supported by the University Summer Fellowship Program. Volkmar Weissig was supported by Midwestern University intramural funds.*

(1) Boddapati, SV, D'Souza, GG and Weissig, V, Liposomes for drug delivery to mitochondria, *Methods Mol Biol.* 2010; **605**:295-303

## **Evaluation of Students' Perceptions Regarding their Development within an Introduction to Teaching and Learning Elective**

Bill J Bowman

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To determine students' perceptions regarding 1) how beneficial different aspects of an Introduction to Teaching and Learning elective course were to their development, 2) which course items were most and least beneficial, and 3) what should be added to improve student development. The elective was offered during the fall and winter quarters of the 2012/2013 academic year and enabled second year pharmacy students to serve as lab instructors for the pharmaceuticals courses and to participate within educational philosophy discussions. A survey instrument was distributed and collected at the conclusion of the elective. The instrument asked students to rate how beneficial various course items were to their development using a four-point scale. The instrument also asked students to indicate which item was most beneficial, which was least beneficial, and what should be added to improve student development. The response rate was 100% (n=16). Lab instructor keys ( $3.94 \pm 0.25$ ), pre-lab sessions ( $3.75 \pm 0.58$ ), and laboratory instruction ( $3.73 \pm 0.46$ ) were rated as having the highest mean level of benefit. Laboratory instruction (n=9) and pre-lab sessions (n=7) were most frequently indicated as most beneficial while the educational philosophy discussions (n=4) were most frequently indicated as least beneficial. Providing additional feedback on laboratory instruction was the most common theme with regard to what should be added to improve student development. Students seemed to perceive the course items that directly prepared them to teach as being more beneficial to their development than the items that explored teaching and learning from more of a philosophical perspective.

## **The use of stress ulcer prophylaxis at a county teaching hospital**

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Respiratory failure and coagulopathy/thrombocytopenia in the critically ill are significantly associated with stress ulcers. Appropriate stress ulcer prophylaxis (SUP) with pharmacotherapy may minimize risks and limit costs. A retrospective cohort study was conducted to evaluate the use and duration of SUP and discharge prescription for a SUP agent at Maricopa Integrated Health System (MIHS). Hospitalized adults were included if at least one dose of famotidine, pantoprazole, or sucralfate was received in June 2012 and excluded if continuation of home therapy, indication other than SUP, or active gastrointestinal bleeding. Invasive ventilation and coagulopathy/thrombocytopenia in the critically ill were defined as the most appropriate indications for SUP. Using a random number generator, information from selected patients was obtained using an approved data collection tool. 567 unique patients received at least one dose of an agent for SUP; 167 were screened; 50 random patients met inclusion, n=31 admitted to Internal Medicine (IM), n=14 to Intensive Care (ICU), n=5 other. Of the 29 patients started on SUP by IM, greater than 60% was inappropriate and via an IM general admission order set. Of the 17 started on SUP by ICU, greater than 90% was appropriate. Famotidine was initiated in 62% of patients; pantoprazole in 38%. Including IM and ICU, only 46% of the patients were started on SUP appropriately and the mean duration of SUP was 5 days. Annual extrapolated costs for inappropriate use may exceed \$12K. Continuation of therapy was prescribed at hospital discharge in 20% of patients. SUP is overused at MIHS increasing costs. Healthcare provider education about appropriate SUP, removal of SUP prompters from IM order sets, and inclusion of famotidine and pantoprazole criteria for use during prescribing will be implemented. Other hospitals should consider evaluation of SUP within their facility.

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# **Satisfaction with the Pilot Arizona Pharmacy Association Mentor Connection Program**

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The Arizona Pharmacy Association (AzPA) Mentor Connection Program was first piloted in the AzPA Health-System Academy with the vision of building relationships, furthering professional networks, and strengthening continuous professional development on behalf of student pharmacists (mentees) and practicing pharmacists (mentors). A survey was designed to determine the level of satisfaction with the inaugural mentor program, to identify programmatic revisions, and ensure continuous quality improvement based on participant feedback. After verifying eligibility, matched mentor and mentee pairs received instructions for participation in the pilot program (January 2012-July 2012). Participants (n=20) were expected to have meaningful contact. At the conclusion of the pilot program, participants completed an electronic satisfaction survey to capture quantitative and qualitative feedback regarding their experience with the MCP. Seven mentors and nine mentees (16 total) completed the survey (80% response rate). 94% and 100% of participants strongly agreed/agreed the MCP met expectations and was valuable, respectively. 94% would recommend the MCP to a colleague. Face-to-face was the most common and effective communication method, followed by electronic. Communication occurred at least once per month for greater than 45 minutes. Professional involvement, career choices, and residency training were the most common topics discussed. The overall strength cited for the MCP was a non-intimidating environment that allowed for mutually beneficial transfer of knowledge between the mentor and mentee. Suggested improvements included developing more structured topics and group activities, longer duration of formal program, and expansion of MCP to all academies. Satisfaction with the AzPA Mentor Connection Program was high. The pilot helped guide programmatic revisions at a grassroots level. Upon incorporation of continuous quality improvement, a 9-month expanded pilot program for the next mentoring cycle is in progress. Overall, the program was a success. Implementation of similar mentor programs in other state societies can be a valuable addition.

## **Satisfaction with an Elective Course in Rare and Interesting Diseases**

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This evaluative study reflects on the creation, implementation, and assessment of an elective course on the pharmacist's role in rare and interesting disorders. These disorders include methemoglobinemia, heparin-induced thrombocytopenia, neuroleptic malignant syndrome, immune/thrombotic thrombocytopenia, glucose-6-phosphate dehydrogenase deficiency, porphyria, therapeutic considerations in Jehovah's Witnesses, pharmacotherapy with organ donation, and others from the National Organization for Rare Disorders (NORD). Limited information regarding these rare and interesting disease (RAID) states is found in common pharmacy textbooks. The pharmacy academic literature did not retrieve any unique required or elective courses that focused on these disorders either. A 1.5 unit (15 hours total) elective course was implemented in a three year accelerated pharmacy program using case-based pedagogy and unique case-based andragogy. Student learning was assessed through short answer, essay, and fill in the blank quizzes; SOAP notes created from mock hard copy patient medical records; and an oral presentation on a rare disorder. Focused feedback on assignments was individualized for each student and disseminated electronically. All students enrolled in the course over a three year period (n=43) were eligible to provide quantitative and qualitative feedback regarding the elective. All students met or exceeded minimum competency levels on assignments. The majority of students (n=33) rated the course favorably on standardized evaluations (weighted mean 4.77, 5=strongly agree) and with qualitative comments. The RAID elective proved to be a popular course which filled a gap in the required curriculum by emphasizing the role of the pharmacist in rare and interesting disorders. Additional skill sets gained far exceeded the topics covered.

*No funding support was required for this project.*

## **An analysis of the cultivable bacteria associated with the internal thallus of the lichen *Acarospora socialis***

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Lichens are generally described as symbiotic organisms made up of a fungus (mycobiont) and green algae or cyanobacteria (photobiont). This classic description of lichens has recently come under question since researchers discovered that lichens also have diverse non-cyanobacterial prokaryote communities within their thalli. Despite these findings, there is still no definitive answer as to the functional roles of these bacteria. In this study, we show first results for the presence of culturable non-cyanobacteria associating with the internal thallus of the lichen *Acarospora socialis*. We also test these bacteria in order to infer possible functional roles within the symbiosis. The bacteria were isolated from the surface sterilized lichen samples and colonies with distinct morphologies were identified using 16S ribosomal DNA (rDNA) PCR and repetitive extragenic palindromic PCR (rep-PCR) genomic fingerprints with BOX and ERIC primers. The isolates were identified as members of the genera *Ralstonia*, *Sphingomonas* and *Methylobacterium*. Furthermore, isolates were analyzed for their biochemical reactivity on API 20NE test strips, antagonistic activity toward other microorganisms, indoleacetic acid (IAA) production, phosphate solubilization and dinitrogen fixation. Preliminary testing of the isolate's antagonistic activity was tested by the perpendicular streak plate method. IAA production was assayed using the Salkowski colorimetric technique. Phosphate-solubilization and dinitrogen fixation were tested qualitatively by plating the bacteria on National Botanical Research Institute's phosphate growth medium containing bromophenol blue (NBRI-BPB) and Burk's N-free agar, respectively. Results showed that none of the isolates showed antagonistic activity, they were not able to produce IAA or fix dinitrogen. The ability to solubilize phosphates was detected in all isolates of the genus *Ralstonia*. The exact function of these bacteria within the symbiosis needs to be tested further.

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# **Igniting the Fire Within: A Primer on Political Advocacy for Pharmacy Professionals**

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The need for a primer to convey the importance of political advocacy to the pharmacy profession and therefore promote interest amongst pharmacy professionals to become more involved was established after a literature search revealed limited recent articles on this topic. Pharmacists pride themselves on being patient advocates, but advocating for themselves and their profession may be overlooked. Many individuals believe they have neither the skills nor the time to dedicate to such a seemingly daunting, yet important task. Fashioned from interviews with experts in the field, personal experience, and the limited literature in existence, this resource serves as a how-to guide on becoming active in political advocacy. Based on the idea of “igniting a fire within” for political advocacy, this primer provides pharmacy professionals with information including websites, resources, and tips on how to be politically involved at any level, from beginner to master advocate. Each stage of expertise varies based on level of involvement, time commitment, and experience. The role of pharmacy in healthcare is expanding and it is imperative that pharmacy professionals not only advocate for their patients, but for the profession as well. This primer ignites the fire for political advocacy within pharmacy professionals by reinforcing the significant impact that advocacy has on our profession and by providing simple information on how to become more involved.

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## Isolation and Characterization of Potential Anti-Cancer Compounds from the Sonoran Desert Lichen *Acarospora socialis*

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Current anticancer treatments have multiple unresolved issues that limit the therapeutic efficacy of these drugs, including limited long-term effectiveness, severe adverse effects, and development of resistance of many cancers to first-line treatments. Therefore, more effective, alternative anticancer treatments are desired. Historically, a plethora of drugs, including anticancer drugs like paclitaxel, have been derived from natural products, making nature an ideal resource for the discovery of novel anticancer treatment. One untapped resource of potential anticancer compounds may be lichen, a symbiotic organism consisting of a fungal mycobiont and an algal photobiont. Previous research has demonstrated antiviral, antibacterial, antioxidant, and anticancer therapeutic properties in lichen secondary metabolites such as usnic, vulpinic, and pulvinic acids. *Acarospora socialis* is a species of lichen growing in the Sonoran desert that has not previously been investigated for potential anticancer compounds. However, our laboratory has previously identified antimicrobial activity against MRSA related to the pulvinic acid derivative epanorin while marginal anticancer effects of whole *A. socialis* extracts was observed in a MTT assay using MDA-MD 231 breast cancer cells. This research aims to expand investigation of *A. socialis* extracts to potential anticancer therapeutic compounds. Whole lichen samples collected from the Sonoran Desert were ground to a powder and compounds extracted by maceration with methanol overnight at room temperature. Filtered extracts were separated by preparative TLC using 170:15 toluene:acetic acid as the mobile phase. The crude compound and extract fractions were also separated using analytical HPLC into 96-well plates. Extract fractions (10, 30, 100 mcg/mL) will be tested for anticancer activity using an MTT assay by incubating into 12-well plates or bulk fractions in 96-well plates to test for preliminary anticancer activity in HL-60 (leukemia), Jurkat (T-cell lymphocytes) and MDA-MB-231 cells. Active compounds will be sent to Arizona State University for structural analysis and identification.

*Karlie Grate was supported by the Midwestern University College of Health Sciences Masters of Biomedical Sciences Program.*

# Development of Guidelines for Accurate Measurement of Small Volume Parenterals Using Syringes

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**Background:** Syringes are commonly used in pharmacy compounding for the measurement of small volumes, especially related to preparation of sterile products for injection and infusion. However, there are no current guidelines for the proper use of syringes in measuring small volumes. Therefore the purpose of this project will be to determine the accuracy and precision of commercially available syringes in measuring small volumes during compounding of sterile product preparation in the pharmacy setting.

**Hypotheses:** Given manufacturer specifications, we hypothesize that small volume measurements using syringes will deliver within  $\pm 5\%$  of the desired volume.

**Methods:** In order to assess precision and accuracy of syringes, three separate investigators measured 5%, 10%, or 20% ( $n = 30$  each) of the indicated volume of a 1, 3, 5, 10, or 20 mL syringe with an attached 18 gauge, 1 1/2" needle by drawing Sterile Water for Injection, USP from a vial. Delivered volumes were measured gravimetrically using an electronic balance, and converted to volume using the specific gravity of water (1.0). Accuracy and precision are represented as mean absolute and root mean squared error, respectively. Measurements are represented as mean and coefficient of variation (CV) and differences are assessed using a one-way analysis of variance (ANOVA) with Bonferroni adjustments and significance set at  $p < 0.05$ .

**Results:** The results from this study will be used to develop and publish a set of recommended guidelines for the use of syringes in measurement of small volumes in the preparation of compounded sterile products.

*Melanie Jordan and Lynn R. Patton were supported by a Midwestern University College of Pharmacy Intramural Grant.*

## **Assessing Accuracy and Impact on Clinical Decision-Making of Community-Based Blood Pressure Monitoring**

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Recent evidence has shown that monitoring blood pressure in the home or community setting is as important, if not more, than readings from a clinical setting when managing hypertension. As a result, clinicians often encourage patients to monitor their blood pressure outside of a clinical setting. Many patients often decide to utilize blood pressure monitoring equipment offered in the community setting, as it is typically free of charge and conveniently located in pharmacies, grocery stores, and other community settings. Although the equipment appears easy to use, inaccuracies may occur due to user error and non-adherence with maintenance recommendations per the manufacturer. In order to assess the accuracy of these blood pressure devices, blood pressures were monitored by a pharmacist using a validated automatic blood pressure machine in addition to a recently installed community-based blood pressure machine. The results of this study showed a significant difference between the two methods of blood pressure measurement when looking at all participants in the study for both systolic blood pressure ( $p=0.006$ ) and diastolic blood pressure ( $p=0.012$ ). In addition, this variance between the two readings would result in a change in treatment (or initiation of blood pressure medication) in 36% of patients. As a result of these treatment implications, this study examined provider practices related to the influence of these readings on clinical decision-making. These results are still pending while we await survey return. In addition to the importance of blood pressure control and monitoring, the role of community-based pharmacists in improving patient health and welfare were also emphasized with participants.

*Nicole Kitts, Samantha Karr, Mary Gurney and Dawn Knudsen Gerber were supported by Midwestern University intramural funds.*

## **Description of Collaborative Diabetes Services in a Family Medicine Clinic**

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This study characterized outcomes of collaborative diabetes services offered by pharmacists and student pharmacists in a university-based outpatient family medicine clinic. The primary endpoint was the impact of collaborative services on glycemic control as measured by the mean change in hemoglobin A1C over a 3 month period. This study was approved by the Institutional Review Board and involved a retrospective chart review of all patients with diabetes referred for collaborative pharmacy management between July 2008 and April 2012. Baseline patient demographics were evaluated and reported. The mean number of clinical encounters and the types of consult formats were quantified for up to one year from referral; with the mean change in hemoglobin A1C calculated for patients with baseline and 3-month measurements. Of 109 patients referred, 85 patients met criteria for inclusion. Most patients had type 2 diabetes (97.7%) and 34.1% were referred for collaborative care at their initial diagnosis. The mean number of concurrent chronic medical conditions was 3.7 at baseline. Upon referral, the most common antihyperglycemic regimens were oral medications (61.2%), oral plus insulin (18.8%) and insulin (11.8%). Of 853 total encounters, 62.3% were telephone-based and 21.5% were combined pharmacist-physician follow-up visits. A baseline and 3-month hemoglobin A1C was reported for 50 patients. The mean baseline and 3-month hemoglobin A1C measurements were 9.10% (range 6.1 – 15.7%) and 6.99% (range 5.5 – 9.8%), respectively, a difference of 2.11% ( $p < 0.001$ ). Collaborative diabetes services provided by pharmacists in a family medicine clinic positively impacted glycemic control through reduction in hemoglobin A1C.

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## **An assessment of Arizona pharmacy students' attitudes and experiences with pharmacists providing emergency contraception**

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With the advent of over-the-counter (OTC) emergency contraceptives, pharmacists are positioned in a unique way to be accessible to the public and provide contraceptive services. Our research aims to characterize Arizona pharmacy students' experiences with pharmacists' refusals or provisions of emergency contraception (EC). The influence of these experiences on pharmacy students' attitudes towards EC is also evaluated. A cross-sectional survey of 352 pharmacy students was conducted to assess experiences with pharmacists providing EC. Students were also surveyed on personal beliefs towards EC and future willingness to provide EC. Information from the survey was collected using Qualtrics software and analyzed with SAS® statistical software. One hundred and sixty-four students responded to the survey. Among the respondents, 13% witnessed EC refusals to men and 12% witnessed EC refusals to women. Themes for EC refusals to men included age verification of female partners. Female students were more willing to provide EC, regardless of the reason, if EC was stocked ( $p < 0.01$ ). Sixty-seven percent of students disagreed that their experiences with pharmacists providing EC influenced their views and practice. No statistically significant differences were found when evaluating age, political party, religion, or year in school. Many Arizona pharmacy students have experienced a practicing pharmacist dispense or refuse to provide EC, thus these interactions with practicing pharmacists may influence student attitudes toward EC provision or refusal. Refusals to men were commonly identified for reasons other than regulatory requirements, indicating that knowledge gaps exist among students and pharmacists that signifies a potential need for additional education and training in EC.

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# Targeted Delivery of Glutathione to Mitochondria in Oxidatively Stressed Cells

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\*Both authors contributed equally to this work

Mitochondrial glutathione is an essential component of the intra-mitochondrial antioxidant system. Under physiological conditions, in its reduced form glutathione contributes to reducing the load of reactive oxygen species (ROS) formed during cellular respiration. Pathological processes, among them ischemia-reperfusion injury, glucose-6-phosphate dehydrogenase deficiency and anticancer chemotherapy may cause a significant increase in the mitochondrial production of ROS thereby depleting the mitochondrial pool of glutathione which eventually may lead to apoptotic cellular death.

We hypothesize that the targeted delivery of glutathione to mitochondria within oxidatively stressed cells will alleviate ROS caused mitochondrial and cellular damage and subsequently prevent apoptosis.

In a previous study (1) we investigated the encapsulation of glutathione into mitochondria-targeted STPP liposomes (2). Here we present first preliminary data in support of our hypothesis. 4T1 cells were stressed with dimethoxynaphthoquinone (DMNQ), a redox-cycling agent that induces intracellular superoxide anion formation and depending on the concentration apoptosis or necrosis. DMNQ does not react with free thiol groups, a feature of particular interest when working with glutathione. Following the induction of oxidative stress, cells were treated with glutathione encapsulated in liposomes and thereafter the production of cellular ATP was measured. Any increase in ATP production over appropriate controls indicates a rescuing effect of liposomal antioxidant treatment.

*Volkmar Weissig was supported by Midwestern University intramural funds.*

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## Mitochondria-Specific Lipidic Nanocarriers of Genistein

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One principal aim of chemotherapy is specific eradication of cancer cells, mostly through the induction of apoptosis. Genistein, the major soy isoflavone, has been shown to induce apoptosis and arrest cancer cell growth, both *in-vitro* and *in-vivo*. Specifically, genistein has been recently reported to induce apoptosis mediated by activation of caspase-9, and -3 and was associated with a decrease in mitochondrial trans-membrane potential and cytosolic release of cytochrome *c*, in different solid and hematological cancer cells.

Owing to the lipophilic nature of genistein, it was an ideal candidate for loading into liposomal, (Lip), nanoemulsion (NE) and micellar (Mic) nanocarriers (NCs). The nano-encapsulation of genistein would afford not only improved delivery of the drug into cancer tissues due to improved solubilization but it also offers enhanced means of intracellular translocation of this partially ionic compound, within interstitial and intracellular pH milieu. Based on different recognized QSAR model-driven criteria for mitochondrial selectivity, genistein, as free drug molecule would be a highly likely mitochondriotropic candidate compound. Hence, lipidic-membrane incorporation of mitochondria-specific ligand would be quite feasible.

Therefore, our current work investigated the mitochondria-specific pro-apoptotic activity of genistein-targeted lipid nano-emulsified particles (mean size of 20 and 160 nm for Mic and NE, respectively). Specifically, NCs, with high membrane-incorporation of genistein molecules, in order to facilitate specific delivery to mitochondria of model cancers. The mitochondria-specific accumulation of FITC-labeled genistein NCs, was confirmed microscopically, in different cancer cells. Such targeted delivery strategy would accelerate and enhance the mitochondria destabilization effect of genistein, as well as further induce more caspase-dependent apoptotic cancer cell death.

The enhanced mitochondrial-specificity and pro-apoptotic activity of genistein-NCs can serve as a promising drug delivery platform for lipophilic anticancer chemotherapeutic drugs, to augment their anti-neoplastic effectiveness.

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# Liposomal Delivery of Ascorbic Acid to Oxidatively Stressed Cells

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\*Both authors contributed equally to this work

An excess of mitochondrial generated reactive oxygen species (ROS) is highly detrimental to cellular function and may lead subsequently to apoptosis or necrosis. Mammalian cells and mitochondria are well equipped with endogenous antioxidants, but under pathological conditions associated with for example ischemia –reperfusion, stroke, arthritis and atherosclerosis this natural defense system against ROS becomes easily overwhelmed. Therefore, the idea of administration of exogenous antioxidants to cells suffering from increases oxidative stress has arisen long time ago. Numerous attempts have been made to deliver natural and synthetic antioxidants into cells and mitochondria. But currently explored strategies focus on the delivery of one or at the most two different antioxidants and hence actually neglecting the interdependence of intracellular redox systems. Liposomes (1) present themselves as ideal pharmaceutical nanocarriers for a combination of antioxidants with different physic-chemical properties. Here we present data in continuation of our efforts (2,3) to raise the level of intracellular antioxidants by utilizing liposomes. In particular, we present preliminary data involving the delivery of antioxidants to cells stressed with dimethoxynaphthoquinone (DMNQ) in deviation of our earlier protocols, which were based on hydrogen peroxide-induced stress.

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## **Emergency department utilization in the elderly prescribed oral clonidine as needed for hypertension in the ambulatory care setting**

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Clonidine can cause rebound hypertension and requires tapering if used daily for more than four days. Clonidine also causes “orthostatic hypotension and CNS adverse effects (drowsiness, 35%; dizziness, 16%).” All of these clinical situations are of special concern in the elderly population. Clonidine is listed in the 2012 American Geriatrics Society Updated Beers Criteria for Potentially Inappropriate Medication Use in Older Adults as “high risk of adverse CNS effects; may cause bradycardia and orthostatic hypotension; not recommended as routine treatment for hypertension.” One of the investigators noticed, during her seven years of practice at her ambulatory practice site, that emergency department usage seemed to be higher in elderly (>65 years old) patients prescribed oral clonidine as needed for hypertension in ambulatory care setting compared to elderly patients diagnosed with hypertension not prescribed oral clonidine as needed for hypertension. In order to investigate this hypothesis, a chart review of Banner Health Center’s medical records will be completed evaluating ED usage in elderly patients prescribed as needed oral clonidine for hypertension compared to clonidine (oral and transdermal) scheduled, in ambulatory care setting for any chief complaint. The results are pending at this time

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## **Rational Design of 2-Oxoglutarate Mimics for Treatment of CNS Disease**

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Reactive oxygen species (ROS) are often implicated in a range of CNS disease states, ranging from stroke and epilepsy to neurodegenerative disease including Alzheimer's Disease (AD). We sought to design novel blood-brain barrier penetrating antioxidants based upon the hydroxytetronimide ring core. While chemical antioxidants have struggled to produce significant results, the concept for this project was to design enzyme inhibitors that would induce expression of enzyme antioxidants, resulting in catalytic rather than stoichiometric suppression of ROS. Inhibition of prolyl-4-hydroxylase was targeted, due to the potential up-regulation of HO-1 and erythropoietin (EPO). This ring core mimics prolyl-4-hydroxylase substrates ascorbic acid and 2-oxoglutarate for enzyme inhibition, and is expected to exploit the Vitamin C transport mechanisms for blood-brain barrier (BBB) penetration. These compounds have been demonstrated to inhibit Fat Mass and Obesity (FTO) and Lysine Histone Demethylase (KDM), and have potent in animal activity in the pharmaco-resistant animal model for epilepsy. Inhibition of other enzymes is currently being evaluated. These results demonstrate that the hydroxytetronimide ring core can be modified to provide a range of different molecules, with different enzyme inhibition and anticonvulsant properties. The good activity, demonstrated blood-brain barrier penetration, and low toxicity of the lead compounds suggests application to disease states beyond epilepsy, including stroke and AD. A number of closely related 2-oxoglutarate utilizing enzymes to prolyl-4-hydroxylase are involved in epigenetic regulation, including FTO, TET, and KDM. These enzymes may be the actual target of these compounds, suggesting that this ring core may have activity through modulating epigenetic activity in the CNS.

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*Joie Rowles was supported by Midwestern University intramural funds.*

*Mark Olsen was supported by Midwestern University intramural funds.*

## **Polysomes: Polymerized Lipidic Vesicles for Selective Intracellular Delivery of Macromolecules**

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The cellular uptake of proteins, oligo-nucleotides and colloidal supra-molecular systems represents a major technical hurdle for the biologic effectiveness and therapeutic success of these macromolecules. Despite several advantages of conventional cationic liposomal carriers of macromolecules, their flexible and un-modified membranes create some limitation for efficient intracellular delivery of their sensitive cargos. Hence, our re-invented concept of partially polymerized liposomes, “Polysomes” offers structural improvements to traditional cationic liposomes. Moreover, pH-responsive membrane-modification of our polysomes can exploit the patho-physiologically altered pH-micro-environment of cancer. Such modular design, along with endosomal escape of these pH-sensitive polysomes therapeutic cargos, would provide for selective drug targeting.

In our current work, remodeling polymerized liposomal membrane structure creates a block-polymerized backbone that would allow for enhanced loading, stability and intracellular delivery of test protein, BSA. The pH-sensitive polysomes were obtained by addition of stearyl-PEG-poly-sulfadimethoxine, which exploits the acidic character of sulfadimethoxine (pKa of 6.1) to guarantee pH-induced hydrophilic/hydrophobic switching consequence of protonation in the tumor. Such structural modification is expected to alter the surface properties of the polymerized nano-vehicles, which consequently induce intracellular drug release.

Block polymerized liposomes (Polysomes), were prepared using modified freeze-dry-rehydration method (Composed of 35 mol% Cholesterol, 55 mol% of DiynePC, and <10 mol% of stearyl-PEGSDM<sub>11</sub>, when applicable), demonstrated homogenous size range (approx. 200 and 400 nm, for FITC-PE and FITC-BSA loading respectively). Plain and pH-responsive polysomes demonstrated good stability (<15% particle size change over 30 days), compared to non-polymerized controls. Furthermore, epi-fluorescent microscopy revealed intracellular localization of FITC-PE acid sensitive liposomes associate with cells four times more efficiently at pH 6.5 as compared to pH 7.4, compared to controls, within 4 hrs of co-incubation with murine breast cancer (4T1) cells.

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## **Thrombospondin-1 (TSP1) Impairs Vasorelaxation via Signal Regulatory Protein (SIRP)- $\alpha$ -Mediated Activation of NADPH Oxidase 1 (NOX1)**

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The secreted matricellular protein TSP1 acutely controls vascular tone by inhibiting relaxation of vascular smooth muscle cells (VSMC). However, the exact mechanism involved remains unknown. TSP1 is the soluble ligand for the cell surface receptor CD47, whereas CD47 has been reported to be a ligand for signal inhibitor regulatory protein SIRP- $\alpha$ . Both CD47 and SIRP- $\alpha$  are expressed in VSMC and SIRP- $\alpha$  was associated with reactive oxygen species (ROS) production in macrophages. This suggested that TSP1, via SIRP- $\alpha$ , inhibits vasodilation by promoting ROS production in VSMC. We herein show that TSP1 binds to SIRP- $\alpha$  and activates its intracellular signal transducer Src homology 2 domain-containing tyrosine phosphatase-1 (SHP-1). Knocking down CD47 or antibody blockade of CD47 in VSMC does not alter TSP1-mediated activation of SIRP- $\alpha$ . TSP1 phosphorylates the p47<sup>phox</sup> subunit of Nox1 and increases O<sub>2</sub><sup>•-</sup> production in VSMC, which is decreased following siRNA silencing of SIRP- $\alpha$ . In functional bioassays TSP1, in a SIRP- $\alpha$ -dependent manner, inhibits vasorelaxation through Nox1-stimulated O<sub>2</sub><sup>•-</sup> production. These data identify the TSP1-SIRP- $\alpha$  signaling axis as a novel activator of Nox1-derived O<sub>2</sub><sup>•-</sup> in VSMC for inhibition of arterial vasodilation, and further suggest that targeting SIRP- $\alpha$  may offer therapeutic potential to mitigate pathologic ROS and enhance tissue blood flow in cardiovascular disease.

## **Novel FTO Inhibitor Modulates MicroRNA**

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Genetic variation in FTO has been linked with Alzheimer's Disease (AD) in human studies, and patients with variant FTO are also associated with decreased brain volume. FTO is a highly expressed 2-oxoglutarate utilizing enzyme in the brain involved in the demethylation of RNA N6-methyladenosine (m6A) residues. m6A residues are the most common mRNA modification in humans, and are associated with microRNA binding sites in the 3'UTR of mRNA transcripts. We have synthesized a novel blood-brain barrier penetrating FTO inhibitor, demonstrated a significant increase in cellular mRNA m6A residues, and investigated the modulation of microRNA by microarray analysis. Numerous microRNAs were either up-regulated or down-regulated by the novel FTO inhibitor. Up-regulated microRNA include miR-505, 575, 4444, 4505, 4638-5p, 4732-5p, and 4753-5p. Down-regulated microRNA include miR-34b-3p, 362-3p, 486-3p, 4485, 4701-5p, 4717-5p, 6509-3p, 6514-3p, and 6722-5p. Analysis of targeted mRNA indicates modulation of numerous genes, including mitochondrial transporters and CNS receptors. The pattern of microRNA modulation suggests that mitochondrial transport may be altered in treated cells relative to control. Future studies investigating the modulation of microRNA in CNS cell types may be useful in evaluating the potential of a FTO inhibitor in CNS disease states, including epilepsy, epileptogenesis, and AD.

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