# 57th Annual AOA Research Conference—Abstracts, 2013

This issue of *The Journal of the American Osteopathic Association (JAOA)* features abstracts from the posters that will be presented at the 57th Annual American Osteopathic Association (AOA) Research Conference. These posters represent the most recent work of numerous osteopathic medical clinicians, researchers, educators, and students.

This year's abstracts are organized into 6 groups:

series F—fellowships (page e1) series P—osteopathic manipulative medicine/osteopathic principles and practice (page e2) series C—clinical studies (page e10) series B—basic sciences (page e28) series ME—medical education (page e46) series H—health policy (page e47)

To enhance the readability of this special feature to the JAOA, the abstracts have been edited for grammar and basic JAOA style. The content of these abstracts has not been modified; neither the AOA Council on Research nor The Journal assumes responsibility for the abstracts' content.

This year's AOA Research Conference, "From Bench to Bedside," will take place in Las Vegas, Nevada, from Monday, September 30, to Wednesday, October 2, during the AOA's 2013 Osteopathic Medical Conference & Exposition (OMED 2013), "Know the Science, Practice the Art."

For more information on the AOA Research Conference or other programs taking place during OMED 2012, access the conference's website at http://www.osteopathic.org/omed. The AOA Research Conference program can be accessed at http://www. osteopathic.org/conference-schedule.

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 Abstract entered in the SOMA Student Poster Competition.

# Fellowship

### ♦F1

# Progressive Teaching Model to Evaluate Pelvic Landmark Localization by Medical Students

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**Introduction:** Using only student-mentor teaching in the training of psychomotor skills is no longer adequate in the modern educational system. Current teaching trends support the use of simulators, standardized patients, and objective competency-based outcomes to assure safety and consistency in skills.

**Hypothesis:** To use calibrated anatomic pelvic models, human volunteers, and consensus training in a 3-stage study to determine the level to which 4 osteopathic medical students localize pelvic landmarks.

**Methods:** In all stages, 5 pelvic landmarks were examined: anterior superior iliac spine (ASIS), pubic tubercle (PT), iliac crest (IC), posterior superior iliac spine (PSIS), and ischial tuberosity (IT). Positions were quantified into X-Y-Z coordinates by a 3-dimensional position-capturing camera system. Examiner and landmark order were randomized for each evaluation. In stage 1, pelvic models were evaluated. The models' hip bones were set to various degrees of rotation and shear to create coronal plane landmark asymmetries of 1 to 6 mm. In stage 2, 19 volunteers were examined. In both stages, consensus training routinely occurred. In stage 3, 40 volunteers were examined without consensus training. The degree of consistency in landmark localization among the students was assessed by the average deviation of the 4 examining digits in the coronal plane for each stage, landmark, and left or right side combination. A random intercepts model was used to compare the consistency across stages. Institutional review board approval from A.T. Still University was obtained.

**Results:** Mean localization of all landmarks was most precisely performed in stage 1 (1.1-2.3 mm), was worst in stage 2 (2.5-8.4 mm), and improved in stage 3 (2.2-7.1 mm). For ASIS and PT, changes between stages 1 and 3 were not statistically significant (P=.10 and 0.99, respectively). For IC and PSIS, localization was significantly worse from stage 1 to 2 (P≤.0001 for both) and did not statistically improve in stage 3. For IT, each stage was significantly different (P<.0001): stage 2 was worse than stage 1, and stage 3 was better than stage 2 but worse than stage 1.

**Conclusion:** Student localization of all landmarks was most accurate in stage 1. Accuracy decreased in stage 2 with the introduction of human volunteers but improved in stage 3 for some landmarks. Landmarks for ASIS and PT showed lesser changes between models and humans; PSIS had the greatest changes. Consensus training and objective feedback appeared to help refine student localization skills during stage 3.

 Abstract entered in the SOMA Student Poster Competition.

# Osteopathic Manipulative Medicine/Osteopathic Principles and Practice

### ♦P1

# Effects of Osteopathic Manipulative Treatment on Self-Perceived Stress in First-Year Osteopathic Medical Students

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**Introduction:** Medical students experience high levels of stress in their preclinical training. Osteopathic manipulative treatment (OMT) has been cited as a modality for stress reduction; however, its application in a medical student population has not been evaluated.

**Hypothesis:** It was hypothesized that OMT would provide a reduction of self-perceived stress in first-year osteopathic medical students.

Methods: Thirty study participants were de-identified and randomly assigned to 1 of 3 groups: control, osteopathic manipulative treatment, and non-directed treatment (NDT). Study participants with contraindicated medical conditions (eg, recent surgery), or prior exposure to OMT were excluded prior to enrollment. Participants self-reported as single and white, with an age range of 21 to 29 years. Participants in OMT and NDT groups received 20 minutes of therapy once a week for 4 weeks. OMT was a direct treatment focused on treating core stress areas: rib raising, anterior cervical fascia release, sternocleidomastoid inhibition, levator scapulae inhibition, and occipitoatlantal release. NDT was a nondirect treatment focused on noncore stress areas: lower extremity balanced ligamentous tension and unwinding, upper extremity balanced ligamentous tension and unwinding, and pelvic neuromuscular release. Groups rested supine for 10 minutes after treatment. All groups completed electronic Self-Perceived Stress Scale (PSS) questionnaires at weeks 0 (pre), 2 (mid), and 4 (post). Scores of the PSS were analyzed retrospectively. **Results:** Average PSS scores for the OMT and control groups were reduced between the presurvey and midsurvey: 1.8 and -2.3, respectively (P=.02). There was no significant change in average PSS scores over time for either the OMT or NDT groups (P>.1). The control group showed a significant increase in PSS scores from presurvey to midsurvey (P=.09), but had no significant change in scores over the course of the study.

**Conclusions:** Participants receiving a treatment regimen focused on core somatic dysfunctions demonstrated a decrease in stress from the presurvey to midsurvey when compared with those in the control group, however, no significant trends between OMT and NDT scores were established. Curriculum changes during the study and use of self-employed stress reduction techniques may have influenced the data. Future studies with a larger sample size, broader demographic population, and longer treatment period are warranted.

## ♦P2

## Reducing Medical Student Fatigue With Osteopathic Manipulative Treatment

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**Background:** The rigors of medical school often induce fatigue, which can negatively impact academic performance and mental health. The psycho-behavioral model of osteopathic manipulative treatment (OMT) suggests utility to reduce fatigue; however, no prior studies have assessed this treatment modality. **Hypothesis:** The hypothesis of this study was that OMT would reduce self-reported medical student fatigue.

Methods: This was an institutional review boardapproved blinded longitudinal pilot study. Firstyear medical students from Lake Erie College of Osteopathic Medicine-Bradenton were recruited by general interest survey. Participants with psychiatric condition, contraindicated health concern, or prior exposure to OMT were excluded prior to enrollment. Thirty students were randomly selected, deidentified, and assigned to 1 of 3 groups: control, nondirected treatment (NDT), or OMT. All participants reported as white and single, with average age of 24 years, and both sexes equally represented. Those in the OMT and NDT groups received 20 minutes of therapy weekly for 4 weeks. Somatic dysfunction related to fatigue is assumed to be primarily in the core. The OMT group treatment focused on the core and included rib raising, anterior cervical fascia release, sternocleidomastoid and levator scapulae inhibition, and occipitoatlantal release. The NDT group treatment included upper and lower extremity balanced ligamentous tension and unwinding, as well as pelvic neuromuscular release. After treatment, groups rested for 10 minutes. The control group received no treatment. Participants completed an electronic Epworth Sleepiness Scale survey at weeks 0, 2, and 4. Data were analyzed retrospectively.

**Results:** There was a significant difference in average Epworth Sleepiness Score (ESS) in the OMT group compared with the control group between surveys 1 and 2 (2.4 and -1.1, P=.01) and surveys 1 and 3 (2.8 and -1.2, P<.01). A significant difference was noted in ESS in the OMT group compared with the NDT group between surveys 1 and 2 (2.4 and -0.56, P=.07) and surveys 1 and 3 (2.8 and -0.1, P=.02). There was no significant difference among any groups between surveys 2 and 3 or between NDT and control groups.

**Conclusions:** This pilot study demonstrates a significant decrease in ESS for the OMT group compared with the control and NTD groups, indicating the potential use of OMT to reduce self-perceived fatigue in medical students. Because of this study's small sample size and lack of participant diversity, further studies are warranted.

### **P3**

### Musculoskeletal Disorders in Medically Underserved Regions in Ben Tre Province, Vietnam

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**Introduction:** Understanding and elucidation of acute and chronic musculoskeletal somatic dysfunction in medically underserved areas of Vietnam has yet to be achieved and remains a priority for the World Health Organization. Existing literature for this area poorly characterizes musculoskeletal conditions, specific to body region and type of pain.

**Hypothesis:** Anecdotal data indicate that poor and underserved Vietnamese are experiencing increases in work-related musculoskeletal disorders and that there is an expanding need for interventions that target improved health and management of somatic dysfunction.

**Methods:** To evaluate acute and chronic musculoskeletal dysfunctions in underserved communities in persons aged 18 to 88 years (mean [standard deviation], 59 [14.65] years) receiving medical care during an NSU-COM outreach trip to the Ben Tre province of Vietnam in December 2012, 295 patients completed an anonymous 1-page questionnaire that ascertained generalized and specific musculoskeletal acute (past 7 days) and chronic (past year) pain. Survey items also included age, sex, height, weight, occupation, general health, number of physician visits in the past year, pain in the past year, and pain in the past 7 days. The survey was offered in Vietnamese and took approximately 10 minutes to complete. Data were analyzed using SPSS v.20 statistical software.

**Results:** The sample (N=295) consisted of 69% women and 31% men. Twenty-four percent had not seen a physician in the past year, 33% saw a physician once, 16% saw a physician twice, and 13% saw a physician 3 times. Twenty-five percent reported their health status to be "not too bad." Associations were found between gender and chronic pain in the shoulder, upper back, wrist/hand, and knees, as well as acute pain in the wrist/hand and knees. Women reported more chronic neck than men (56% vs 44%), shoulder pain (53% vs 47%), upper back pain (79% vs 21%), hand/wrist pain (85% vs 15%), and knee pain (80% vs 21%). Chronic pain in the lower back and knees was statistically significantly associated with older age.

**Conclusion:** Data from this study will assist in determining which treatment modalities, including osteopathic principles and practice, will be most beneficial when considering the development of targeted health care services and generating guideline protocols specific to age and gender for future medical providers practicing in this region.

## P4

## Peripheral Skin Blood Flow Changes After Different Controlled Cervical Osteopathic Mobilizations: A Pilot Study

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Background: Modulating peripheral sympathetic nervous system (pSNS) function may be a mechanism by which osteopathic manipulative treatment (OMT) impacts numerous disorders, particularly visceral disorders and possibly peripheral skin blood flow (pSkBF). Increased pSkBF has been associated with a hypoalgesic effect on musculoskeletal pain after different therapeutic modalities. Published outcomes on pSkBF changes after OMT are scarce, have not considered a dose-related effect, and have conflicting results, although OMT is usually associated with a short-term sympathoexcitatory effect. Objective: To investigate short-term doseresponse effects of non-noxious pressures by means of standardized OMT applied on the cervico-thoracic junction on pSkBF changes in the upper limbs and on pulse rate variability (PRV), an indirect measurement of pSNS function.

**Methods:** This project was approved by our institutional review board. The proposed methodology recorded and analyzed pSkBF and PRV data without motion artifacts as in previously published studies. We used a crossover design on 4 asymptomatic participants who underwent 4 different procedures on 4 different days: control (no touch), placebo (touch), low-pressure OMT, and high-pressure OMT. The OMT was the same cervical mobilization applied rhythmically with different non-noxious pressures. Using a Biopac MP36 system, we collected pSkBF data as percent changes from baseline and PRV data as the low to high frequency ratio of PRV spectra (LF/ HF). Pressure data (kPa) were recorded with a Novel Pliance-X system.

**Results:** Our pilot data revealed a positive, though nonsignificant, relationship between normalized pSkBF and raw kPa, but the same OMT was associated with a decrease or increase in pSkBF. The PRV data had no clear pattern. However, we observed opposite changes in the LF/HF ratios after low-pressure and high-pressure OMT.

**Conclusion:** Meaningful pSkBF, PRV, and pressure data were collected in this study. Although not significant, our data may suggest opposite short-term changes in pSkBF and the LF/HF ratio depending on the amount of non-noxious pressure used to perform the same OMT. A larger sample may provide more data to specifically address these trends.

### P5

# Relationship Between Sacral Base Position, Sacral Sulcus Depth, Gender, and Body Mass Index as Measured by Ultrasonography

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**Introduction:** Musculoskeletal ultrasonography is an emerging clinical tool and has been successfully integrated into the curriculum at A.T. Still University-Kirksville College of Osteopathic Medicine (ATSU-KCOM). It provides students with the understanding of musculoskeletal anat-

omy and helps advance palpatory skills.

**Hypothesis:** Normative values for many musculoskeletal structures are yet to be established. Ultrasonography can be efficiently used to identify musculoskeletal landmarks and validate clinical findings related to somatic dysfunction of the sacrum.

**Methods:** First-year osteopathic medical students at ATSU-KCOM used musculoskeletal ultrasonography to obtain bilateral sacral base position (SBP) measurements and calculated the sacral sulcus depth (SSD) by subtracting the measurement to the posterior superior iliac spine from the SBP. Data were analyzed with respect to age, sex, body mass index (BMI) (normal, 18 to <25; overweight 25 to <30).

Results: First-year osteopathic medical students successfully acquired ultrasonographic images and useful data for the initial stage of establishing normative values for sacral landmarks. Sixty-nine participants (37 men [54%], 32 women [46%]; mean [standard deviation (SD)] age, 25.0 (2.6) years; mean [SD] BMI, 23.3 [2.6]), were measured by 87 students. The SBP was not significantly different between the left and right (mean [95% confidence interval (CI)] left, 3.36 [3.24, 3.47] cm; right, 3.38 [3.27, 3.50] cm; P=.77). The SBP was significantly different between BMI categories (normal, 3.21 [3.10, 3.32] cm; overweight, 3.73 [3.56, 3.90] cm; P<.001) and between males (3.29 [3.16, 3.41] cm) and females (3.65[3.49, 3.81] cm) (P<.001). The SSD was not significantly different between the left and right (left, 1.69 [1.59, 1.79] cm; right, 1.72 [1.62, 1.82] cm; P=.33). There was significant interaction of BMI and sex (P=.05) where for women, as BMI increased, SSD increased, but not for men. The SSD was not significantly different between BMI categories (normal, 1.68 [1.60, 1.80] cm; overweight, 1.74 [1.64, 1.85] cm; P=.38).

**Conclusion:** Introduction of ultrasonography into the osteopathic manipulative medicine

course allowed for research and education. The research aspect yielded normative values for SBP and SSD and their relation to BMI and sex within the studied age group. The establishment of these normative values allows for future studies involving somatic dysfunction and ultrasonography. The educational aspect helped students gain confidence in palpatory skills as related to somatic dysfunction, as well as musculoskeletal ultrasonographic image acquisition and interpretation.

### P6

# Reliability of the Diagnosis of Thoracic Outlet Syndrome

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**Hypothesis:** This study assessed the reliability of the special tests used to diagnose thoracic outlet syndrome (TOS) by comparing them with Doppler ultrasonography (US).

**Research Design:** Thoracic outlet syndrome refers to a condition of obstruction of the neurovascular bundle, including the subclavian artery and brachial plexus at the thoracic outlet. All or some of these structures can become compressed resulting in numbness and weakness in the upper extremity. Adson, Halstead, and Wright tests can be used to diagnose TOS and assess its impact on blood flow to the upper extremity based on diminished pulse intensity. Using Doppler US, blood flow velocity (BFV) changes, particularly an increase in BFV, suggest that the subclavian artery is narrowed.

**Methods:** Thirty-one individuals with TOS enrolled in the study. The participants had the 3 special tests performed on them while a sonographer concurrently performed Doppler US on the subclavian artery. Blood flow velocity during these test positions were compared with BFV with the upper extremity in a resting position. Positive tests were compared with BFV readings from Doppler US to analyze whether a positive test confirmed the presence of a narrowed vessel in participants with clinically diagnosed TOS.

Results: When evaluating the reliability of Adson test performed in the left upper extremity it was determined that there was an overall 42%agreement that a positive test would also exhibit an increase in BFV on Doppler US and a negative test would exhibit no change or decreased velocity comparatively. Kappa was at 0.035, depicting that agreement between Adson test and Doppler US was no better than expected by chance. Right Adson test depicted slightly better agreement at a 52% with a Kappa coefficient of 0.064. Left Halstead test demonstrated a 48% agreement and a Kappa of -0.053 while right Halstead performed similarly. Wright test exhibited 32% agreement on the left upper extremity and 68% on the right with a Kappa of -0.254and 0.23 respectively.

**Conclusion:** The special tests used to diagnose TOS: Adson, Halstead, and Wright tests demonstrate poor reliability when compared Doppler US. It may be that the condition of TOS does not compress on the subclavian artery enough to actually alter the pulse intensity unless in severe cases, further studies may determine this. These tests are taught widely in osteopathic principles and practice curriculums and used by clinicians to diagnose TOS. However, poor reliability suggests that TOS may be better as a diagnosis of exclusion remaining as a syndrome.

### P7

## Somatic Dysfunctions Correlated With Weight Bearing and Leg Length Inequalities

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**Objective:** To investigate whether certain somatic dysfunctions of the pelvis, sacrum, and lumbar spine are correlated with weight bearing and leg length discrepancies between the lower extremities.

**Research Design:** Leg length discrepancies and weight bearing asymmetry may contribute to the development of somatic dysfunctions in the pelvis, sacrum, and lumbar spine and vice versa. However, there is minimal literature to identify the specific somatic dysfunctions that can lead to a long or short leg or lead to weight bearing discrepancies between the lower extremities.

**Methods:** Ninety-eight young, healthy participants without soft tissue or osseous injury or previous osteopathic manipulative treatment (OMT) were enrolled in the study. Each participant's somatic dysfunctions were diagnosed by an osteopathic physician with 10 years of experience. The participants' leg lengths were measured with measuring tape, and weight bearing was assessed through each lower extremity using a specialized quadruped scale.

**Results:** Descriptive statistics found that the most common pelvic dysfunction is a superior shear

(25%) where the most common sacral dysfunction was a left on left sacral torsion (34%). There was a significant association between right anterior innominate rotation dysfunctions and weight bearing (P=.02). A higher percentage of patients with a right anterior innominate dysfunction bear more weight on their left (45%) than on their right (27%) or neither (27%). Of those participants with right anterior innominate dysfunctions, most (75%) exhibited a shorter leg when measured in supine on the left. Furthermore the participants with a left superior shear exhibited a significantly shorter left leg in supine (P=.05). For sacral somatic dysfunctions, participants with a left on left sacral torsion tended to exhibit a shorter left leg in standing, and this finding was statistically significant (P=.02).

**Conclusion:** In healthy individuals, osteopathic physicians performing an osteopathic structural examination and OMT can expect that specific pelvic and sacral somatic dysfunctions may exist with minor leg length discrepancies. Furthermore, certain somatic dysfunctions may exist with specific weight bearing differences through the lower extremities. This may be a helpful tool to guide clinicians to not only manage somatic dysfunction but to also address whether leg length discrepancies exist or remain with their treatment approach.

### **P8**

# Persistence of Lumbar Somatic Dysfunction and Its Association With Bone Mineral Density

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<sup>1</sup>Department of Osteopathic Manipulative Medicine, A.T. Still University-Kirksville College of Osteopathic Medicine, Missouri; <sup>2</sup>A.T. Still Research Institute, A.T. Still University, Kirksville, Missouri; <sup>3</sup>Department of Neurobehavioral Sciences, A.T. Still University-Kirksville College of Osteopathic Medicine, Missouri **Hypothesis:** Significant somatic dysfunction, if left untreated, should persist over time and be associated with objective measurable findings.

**Objective:** To investigate the persistence of lumbar somatic dysfunction over 8 weeks and its association with lumbar bone mineral density (BMD) T scores.

**Methods:** Individuals were assessed at 0, 4, and 8 weeks for the presence and severity of paraspinal tissue texture abnormalities (TTA), vertebral rotational asymmetry (RA), anterior motion restriction (MR), and tenderness from L1 to L4. Participants received a dual energy x-ray absorptiometry scan of the lumbar spine at 0 and 8 weeks. Persistent somatic dysfunction findings found at all 3 examinations were compared with the BMD T scores obtained at 8 weeks and to the change in the BMD T scores from 0 to 8 weeks. The local institutional review board approved this project.

Results: Forty-eight individuals (38 women [79%], 10 men [21%]) participated in the study. The mean (standard deviation [SD]) age was 30.1 (6.4) years with a range of 20 to 40.8 years, and the mean (SD) body mass index was 26.3 (5.2). The incidence of persistent somatic dysfunction varied by lumbar level and ranged from 44% to 83% for TTA, 63% to 79% for RA (52%-69% left RA, 0%-4% right RA), 10% to 56% for MR, and 2% to 13% for tenderness. Vertebrae with persistent MR had higher mean BMD T scores (95% confidence interval) than those without persistent MR (0.6 [0.4, 0.8] vs 0.2 [0.1, 0.4]; P=.02). There was a significant difference between on the change from the initial to final vertebral BMD T scores between vertebrae that demonstrated persistent tenderness and those that did not (-0.2 [-0.4, 0.1] vs 0.1 [0.0, 0.1]; P=.04). There was a significant increase in the vertebral BMD T scores for those vertebrae that demonstrated persistent TTA (0.1 [0.0, 0.2]; P=.02) and for those vertebrae that demonstrated persistent moderate to severe TTA (0.3 [0.0, 0.5]; P = .02).

**Conclusions:** A persistence of predominately left lumbar rotation was identified and is consistent with Zink common compensatory pattern of somatic dysfunction in the lumbar areas. Persistent vertebral MR was shown to have an association with the final lumbar BMD T scores, and persistent TTA and tenderness were associated with the change in the BMD T scores over 8 weeks. **Acknowledgment:** This study was supported by the National Institutes of Health–National Center for Complementary and Alternative Medicine, grant no. 1R01AT00305-1, and by the American Osteopathic Association, grant no. 00-04-505.

## P9

# Demonstration of Increased Vascular Characteristics Using Bilateral, Multiparameter Sensors After Controlled Vertebral Osteopathic Manipulative Treatment

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**Introduction:** Vertebral somatic dysfunction is described by sympathetic hyperactivity, which affects related innervated visceral structures including blood vessels. This study investigates the influence of osteopathic manipulative treatment (OMT) on upper extremity vascular characteristics at selected vertebral segments.

**Hypothesis:** This study evaluates the hypothesis that OMT, using balanced ligamentous tension (BLT) at selected vertebral levels, affects upper extremity digital vascular flow, as well as electro-cardiographic (ECG) characteristics.

Methods: Cardiovascular parameters were record-

ed in a continuous, noninvasive manner from clinically asymptomatic individuals (13 women, 4 men; age, 19 to 58 years), who were recruited to participate in an institutional review boardapproved study. Signals from both the right and left hands for 28 total sessions were acquired by attaching the Photo-pulse-Plethysmogram (PPG) and Laser Doppler Flow (LDF) sensors on the index and middle finger tips, respectively. A 3-lead electrocardiogram (ECG) signal was recorded simultaneously. For active sessions, participants lay supine and BLT was performed by the osteopathic physician at sequential vertebral levels, OA-C2, T1-T4, T8-L2, and L5-sacrum. A quiescent phase of approximately 8 to 12 minutes followed each region treated as the subject continued to lie passively. Data were acquired for these sessions lasting between 100 to 150 minutes. For baseline sessions, the subject was instructed to lie supine without disturbance for 45 to 75 minutes, and the same vascular parameters were acquired. Time domain analyses (peak-peak [PK-PK] and root-mean-square) and frequency domain power spectral density (PSD) post-analyses were performed. The ECG data were analyzed to asses heart rate variability.

**Results:** A statistically significant difference in the root-mean-square values was noted for the LDF and PPG signals (.003<P<.05), during the OMT phases, compared with corresponding phases from baseline sessions. Comparing active OMT and baseline sessions, active PSD demonstrated a higher magnitude of harmonics. Analysis of ECG data showed the heart rate during OMT to be statistically different from baseline sessions (.001<P<.05.)

**Conclusion:** This study successfully demonstrated increased peripheral flow (PPG and LDF), as well as changed heart rate after BLT compared with baseline sessions, under continuous bilateral multi-parameter recording.

# **Clinical Studies**

## C1

# Hyperglycemia Induces High-Density Lipoprotein Oxidation: Preliminary Evidence From an Ex-vivo Study

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Introduction: The failure of antioxidant vitamins to influence clinical outcomes may have many explanations, including the inability of vitamin E to prevent the formation of oxidized phospholipids and other lipid oxidation products of the myeloperoxidase pathway. Oxidized high-density lipoprotein (HDL) has reduced capacity for cholesterol efflux, but the role of oxidized HDL in the pathogenesis of cardiometabolic disease has not been fully demonstrated. In a recent study in nondiabetic subjects with dyslipidemia, using an antibody against oxidized apoAI, we have shown that high serum glucose levels may contribute to HDL oxidation, irrespective of HDL cholesterol levels. This finding suggests that serum glucose in the normal range can lead to the oxidative modification of HDL, which would be expected to render it less functional and therefore may be associated with cardiovascular disease events.

**Hypothesis:** We hypothesized that glucose per se induces oxidation of HDL.

**Methods:** Sera from 8 healthy volunteers were incubated under steryle conditions at 37°C for 4 days, in the presence of 0 (no addition), 20, and 40 mmol/L glucose, and antiproteases. Oxidized HDL was measured by ELISA, with Ab No.7D3 as previously described.

**Results:** A significant, stepwise increase in oxidized HDL was found in 5 cases, up to a 40% increase over the control with no added glucose. The other 3 participants showed no difference.

Conclusions: We present proof of principle evidence that mimicking hyperglycemia for a period compatible with HDL half-life can significantly increase oxidized HDL in most but not all cases. This finding suggests a new pathophysiological mechanism for hyperglycemia-induced HDL functional dysfunction. Glycation of apoAI and of phospholipids has been previously shown by us and others. Glycation generates ROS locally, and apoAI is a target of oxidation. Our findings also point to the existence of resistance to HDL oxidation in some cases. Is this a result of interindividual differences in HDL subclasses? Is this a result of interindividual differences in PON1 activities? Larger studies are being conducted to confirm these findings.

### C2

## New Method to Assess Apo-lipoprotein Profiles in Native Low-Density and High-Density Lipoprotein Subclasses

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Introduction: High-density lipoprotein (HDL) cholesterol (HDL-C) is a crude surrogate for cardiovascular risk that works well epidemiologically (low values correlate with increased risk); however, high values do not necessarily indicate protection. Indeed, the HDL-C hypothesis has been challenged by recent mendelian randomization studies. Classicgradient gel electrophoresis followed by lipid staining identifies 5 HDL subspecies separable on the basis of particle diameter: HDL3c (7.2 to 7.8 nm), HDL3b (7.8 to 8.2 nm), HDL3a (8.2 to 8.8 nm), HDL2a (8.8 to 9.7 nm), and HDL2b (9.7 to 12.9 nm). Much more information can be obtained by analyzing the individual apolipoproteins across the spectrum of particles. There are only a few studies using elaborate MALDI-MS proteomics on HDL purified by ultracentrifugation. We developed a practical method to detect the major apolipoproteins in HDL subclasses in human serum.

Methods: Serum HDL subclasses are separated by native gradient gel electrophoresis (4%-12%, Biorad), which is followed by transfer and sequential Western blot to detect apolipoproteins AI, AII, B, E, CII, and CIII and lp(a). In this way distinct apolipoprotein profiles across HDL subclasses are obtained. In a pilot study we tested our method on purified HDL and LDL as well as in serum from survivors of myocardial infarction (n = 72, all men, aged 47-51 years) recruited in the cardiology unit of the Hospital Universitaride Sant Joanand gender and age-matched healthy controls. High-density lipoprotein subclasses were also analyzed for comparison and control purposes using the Lipoprint HDL system (Quantimetrix).

**Results:** Our method allows for detection of the major apolipoproteins in native HDL subclasses and shows a striking differential distribution in different subjects, independent of the HDL-C. Moreover, differences in sdLDL, apoE, CII, and CIII are striking when AMI survivors and control subjects are compared.

**Conclusions:** Our procedure is more practical for routine use than MALDI-MS proteomics on purified HDL by ultracentrifugation, which also has the disadvantage of high ionic strengths that lead to loss of surface proteins. It uses 20  $\mu$ L of serum compared with the several milliliters needed for HDL preparation by ultracentrifugation. Our method may thus become a practical tool

for studies that aim to unravel specific patterns of apolipoproteins in HDL that may be associated to the functionality of HDL and help predict coronary artery disease risk.

### **C**3

# Rethink the Ink: Demographic Profile and Motivation of Adult Patients Receiving Laser Tattoo Removal

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**Hypothesis:** Modalities aimed at altering body image such as permanent tattooing are perceived to be associated with greater risk-taking behavior. As a result, with extensive literature emphasizing the psychological perceptions and implications of body art, the investigators sought to determine the demographic profile and motivation of adult patients seeking to improve selfimage through laser tattoo removal.

**Research Design:** The authors conducted an observational retrospective study, approved by the institutional review board, to review data from clinical medical records from 4 separate Dr Tattoff offices in Southern California between 2004 and 2012.

**Methods:** A retrospective medical record review of 5000 randomly selected charts revealed 1041 patients meeting predetermined study parameters. Parameters included age older than 18 years, tattoo age of more than 6 weeks, and more than 5 laser tattoo removal sessions. Demographic data including age, sex, ethnicity, Fitzpatrick skin type, tattoo age, tattoo location, and motivation for tattoo removal were recorded.

**Results:** Data analysis of the 1041 patients revealed a sex prevalence of 33.2% male and

66.8% female, with an average patient age of 31.6 years. Ethnicity was predominantly Caucasian (53.6%), followed by Hispanic (27.8%), Asian American (8.7%), and African American (5.8%). A small subset of Filipino, Middle Eastern, Native American, and Pacific Islander patients made up the remaining 4%. Fitzpatrick skin types II and III dominated the data, consistent with the high percentage of Caucasian patients. The average tattoo age was 8.6 years with the most common location for removal being the arm/forearm followed by the hand/wrist and the back. Finally, motivation for tattoo removal was dominated by the desire to restore self-image and improvement of professional image (42%) followed by aversion to the tattoo appearance (34%) and termination of a relationship (7%).

**Conclusion:** The overwhelming interest in laser tattoo removal may reside within the psyche of individuals wishing to transform social perception, self-identity and image. Given the implications of economic difficulty, many individuals are beginning to rethink their ink in an effort to appear more marketable and professional and to renovate self-image. As a result, many individuals are choosing to remove tattoos from visible locations of the body such as the upper extremity to achieve this goal.

### ♦ C4

## **Evaluation of Early-Stage Biomarkers of Cadmium Nephrotoxicity**

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**Background:** Chronic cadmium (Cd) exposure results in proximal tubule injury characterized by polyuria and proteinuria. Traditional biomarkers such as metallothionein, Cd, Beta 2-micro-

globulin, and *N*-acetyl-Beta-D-glucosaminidase (NAG) are not capable of identifying early stages of Cd-induced kidney injury. Emerging biomarkers such as cystatin-C, clusterin, and neutrophil gelantinase-associated lipocalin (NGAL) have yet to be evaluated in the context of Cd toxicity.

Hypothesis: We postulated that emerging biomarkers of kidney injury might outperform traditional biomarkers in the early identification of Cd nephrotoxicity. We also proposed that emerging Multiplex (MagPix) technology will be a more sensitive tool capable of detecting earlier excretion of urinary biomarkers compared with standard enzyme-linked immuno-sorbent assays (ELISA).

Methods: Male Sprague-Dawley rats were given daily subcutaneous injections of Cd (0.6 mg/kg, 5 d/wk). At 3, 6, 9, and 12 weeks, urine samples were collected and analyzed for protein, pH, creatinine, and the panel of biomarkers. The levels of the various biomarkers were determined using standard ELISA (Beta 2-microglobulin, Kim-1, cystatin-C, Alpha-GST, and Pi-GST) and/or by state of the art Luminex xMAP Technology using Millipore MagPix xPONENT 4.1 system with proprietary reagents (Beta 2-microglobulin, Kim-1, cystatin-C, clusterin, NGAL, and albumin). Representative animals were euthanized and kidneys were processed for histopathological analysis. The animal treatment protocol was in compliance with the National Institutes of Health guidelines and was approved by Midwestern University's Animal Care Committee.

**Results:** Traditional ELISA did not detect (with statistical significance) biomarker excretion until the development of proteinuria and polyuria (week 9). Cystatin-C, NGAL, and Beta 2-microglobulin biomarkers detected by MagPix technology appeared 3 weeks prior to the overt onset of renal dysfunction (with statistical significance). Pearson correlation analysis provided evidence that the MagPix and ELISA yielded generally similar results.

**Conclusion:** MagPix provides more robust results with earlier statistical significance than traditional ELISA. All of the emerging biomarkers examined neither outperformed nor underperformed traditional biomarkers.

**Funding:** Midwestern University's Biomedical Science Program and Department of Pharmacology supported the present study.

### C5

## Improvement in Glycemic Control With Saxagliptin as Monotherapy or Add-on Therapy in Patients With Type 2 Diabetes Mellitus

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**Introduction:** Dipeptidyl peptidase-4 inhibitors are a newer class of oral antihyperglycemic medications that raise prandial levels of the incretin hormones, producing a glucose-dependent increase in insulin secretion.

**Hypothesis:** Saxagliptin (SAXA) is effective in reducing glycated hemoglobin (HbA<sub>1c</sub>), fasting plasma glucose (FPG), and postprandial glucose (PPG) when used as monotherapy or add-on therapy in patients with types 2 diabetes mellitus. **Methods:** Efficacy of SAXA 5 mg/d in reducing HbA<sub>1c</sub>, FPG, and 120-min PPG at week 24 in 5 phase 3 studies (SAXA monotherapy [NCT00121641], SAXA QAM or QPM monotherapy [NCT00316082]; SAXA add-on to metformin [MET; NCT00121667], glyburide [GLY; NCT00313313], or thiazolidinedione [TZD; NCT00295633]) was analyzed.

**Results:** Across studies, SAXA significantly improved all efficacy outcomes vs placebo (PBO). In the monotherapy studies, the PBO-subtracted

difference in adjusted mean change from baseline HbA<sub>1c</sub> (95% CI) was -0.64% (-0.93% to -0.36%, P<.0001), -0.40% (-0.69% to -0.12%, P=.006 [QAM]), and -0.35% (-0.63% to -0.07%, P=.016 [QPM]). In the add-on therapy studies, it was -0.83% (-1.02% to -0.63%, P < .0001 [+ MET], -0.72% (-0.88% to -0.56%, *P*<.0001 [+GLY]), and -0.63% (-0.84% to -0.42%, P<.0001 [+TZD]). For FPG (mg/dL), the PBO-subtracted difference in adjusted mean change from baseline (95% CI) as monotherapy was -14.7 (-25.5 to -4.0, P=.007), -14.0 (-26.4 to -1.6, P=.027 [QAM]) and -11.2 (-23.2 to 1.2, P=.076 [QPM]) and as add-on therapy was -23.3 (-30.3 to -16.3; P<.0001 [+MET]), -10.3 (-16.9 to -3.8; P=.002 [+GLY]), and -14.5 (-22.7 to -6.3; P=.0005 [+TZD]). For 120-min PPG (mg/dL), the PBO-subtracted difference in adjusted mean change from baseline (95% CI) as monotherapy was -37.3 (-59.2 to -15.4; P=.0009), -30.5 (-56.0 to -5.0; P=.019 [QAM]), and -26.6 (-52.4 to 0.8; P=.044 [QPM]) and as add-on therapy was -40.3 (-56.4 to -24.1; P<.0001 [+MET]), -41.7 (-52.8 to -30.6; P<.0001 [+GLY]), and -50.0 (-66.2 to -33.8; P<.0001 [+TZD]). Across studies, the percentage of patients reporting 1 or more adverse events for SAXA vs PBO was 58% to 76% vs 47% to 77%, respectively, with low rates of all reported (2.7%-14.6% vs 1.4%-10.1%) and confirmed (ie, symptoms with glucose  $\leq 50 \text{ mg/}$ dL) hypoglycemia (0-0.8% vs 0-0.7%).

**Conclusion:** In patients with type 2 diabetes mellitus, SAXA is effective across multiple glycemic outcomes and is generally well tolerated when used as monotherapy or add-on therapy.

### ♦ C6

# Acute Coronary Syndromes in Patients Without Elevated Low-Density Lipoprotein (LDL) Cholesterol Not Predicted by Serum Phytosterols

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Introduction: Phytosterols are recommended by the American Heart Association as a dietary supplement in a heart healthy diet because of their established low-density lipoprotein (LDL)-lowering effect. On ingestion, their levels in serum rise in proportion to amount ingested and amount absorbed. Although the effect of phytosterols in the blood is not yet understood, there is a rare disorder called phytosterolemia in which elevated phytosterols leads to premature atherosclerosis and typically early coronary heart disease, often without an accompanying elevation in cholesterol. Statins have been shown to be less effective in patients with high serum phytosterols who have higher absorption and lower synthesis of cholesterol.

**Hypothesis:** It was hypothesized that phytosterols could be a biomarker for increased risk of acute coronary syndromes (ACS) in patients with LDL lower than 100 mg/dL.

**Methods:** To determine whether elevated serum phytosterols could be an important risk predictor of ACS in those meeting their LDL goal, 18 ACS participants with LDL less than 100 mg/dL and non-HDL less than 130 mg/dL at the time of their acute coronary event were evaluated 1 month after stabilization from their acute events, which are ischemic EKG events including ST segment elevation or depression, elevated troponin levels, or unstable angina with a coronary lesion of more than 70% stenosis. Exclusion criteria included cardiogenic shock or a percutaneous intervention within the last year. Ninety control

 Abstract entered in the SOMA Student Poster Competition. participants evaluated at baseline were not overweight, not diabetic, and had no cardiovascular disease at NCEP-ATP III goals.

**Results:** Phytosterol levels of sitosterol and campesterol were assessed. Lathosterol, a marker of cholesterol synthesis was also evaluated, as were ratios of total cholesterol to each of these sterols. No correlation was found between elevated serum phytosterols or their ratios to cholesterol and presentation of ACS in subjects with LDL lower than 100 mg/dL.

**Conclusion:** Our study suggests that elevated serum phytosterol is not a good predictor of acute coronary syndromes in patients meeting LDL cholesterol guidelines. However, limitations on study size may have decreased the power of a relationship between serum phytosterols and acute coronary events. It is also possible that a subset of patients unidentified in our cohort may present with elevated serum phytosterols accompanying their acute coronary syndromes, as do those presenting with phytosterolemia.

### C7

### Dapagliflozin as Add-on Therapy in Type 2 Diabetes Mellitus

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**Introduction:** Patients with type 2 diabetes mellitus (T2DM) usually require multiple medications to achieve and maintain glycemic control. Dapagliflozin (DAPA) is a selective inhibitor of sodium-glucose co-transporter 2 and reduces plasma glucose by inhibiting renal glucose reabsorption. **Hypothesis:** Dapagliflozin is consistently effective when used as add-on therapy in T2DM, with a low incidence of adverse events. Methods: Patients with T2DM were treated with placebo (PBO) or DAPA 10 mg/d as addon to metformin  $\geq$ 1500 mg/d (MET, N=546, NCT00528879), glimepiride 4 mg/d (GLIM, N=596, NCT00680745), or sitagliptin (SITA) 100 mg/d  $\pm$  MET (N=451, NCT00984867). Exclusion criteria included significant cardiovascular, hepatic, or renal disease. The primary end point was adjusted mean change from baseline in glycated hemoglobin (HbA<sub>1c</sub>) after 24 weeks of treatment.

Results: Adjusted mean changes from baseline in HbA<sub>1c</sub> at week 24 were DAPA+MET, -0.84% vs PBO+MET, -0.30% (P<.0001); DAPA+GLIM, -0.82% vs PBO+GLIM, -0.13% (P<.0001); DAPA+SITA, -0.45% vs PBO+SITA, +0.04% (P < .0001). The proportions of patients achieving HbA<sub>10</sub> < 7% at 24 weeks were DAPA+MET, 41% vs PBO+MET, 26% (P=.006); DAPA+GLIM, 32% vs PBO+GLIM, 13% (P<.0001); DAPA+SITA, 28% vs PBO+SITA, 19%. Adjusted mean changes from baseline in fasting plasma glucose were DAPA+MET, -24 mg/dL vs PBO+MET, -6 mg/dL (P=.006); DAPA+GLIM, -29 mg/dL vs PBO+GLIM, -2 mg/dL (P<.0001); DAPA+SITA, -24 mg/dL vs PBO+SITA, +4 mg/dL (P<.0001). Adjusted mean changes in body weight with DAPA were -2.9 kg as add-on to MET (vs -0.9 kg with PBO, P < .0001); -2.3 kg as add-on to GLIM (vs -0.7 kg with PBO, P < .0001), and -2.1 kg as add-on to SITA (vs -0.3 kg with PBO, P<.0001). Mean placebo-corrected decreases in systolic blood pressure of up to 5 mm Hg were observed. Across studies, the incidence of adverse events was slightly higher with DAPA vs PBO. Hypoglycemic events were infrequent and occurred in a similar proportion of patients with DAPA and PBO in the add-on to MET (4% vs 3%) and add-on to SITA studies (3% vs 2%). Hypoglycemia was more frequent with DAPA (8%) than with PBO (5%) in the add-on to GLIM study. Across studies, there were increased genital (7%-9% vs 0.4%-5%) but not urinary tract (5%-8% vs 4%-8%) infections with DAPA compared with PBO. **Conclusion:** DAPA produced improvements in glycemic control and body weight without propensity for hypoglycemia and was generally well tolerated when used as add-on therapy to MET, GLIM, and SITA.

### **C**8

## Comparison of Reported Adverse Events Among Different Adjustable Gastric Banding Systems

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Introduction: According to the US Centers for Disease Control and Prevention, 35.7% of people in the United States are obese. The adjustable gastric banding systems represent a few of the bariatric surgery options available on the market for obese patients. Currently, 2 adjustable gastric banding devices are available in the United States: the LAP-BAND system and the REAL-IZE Adjustable Gastric Band.

Hypothesis: According to the US Food and Drug Administation's device approval records, the REALIZE Adjustable Gastric Band has been implanted in Europe since 1996. By contrast, the LAP-BAND system has been implanted in Europe since 1993, thus allowing for maturity and revisions of its design. It is hypothesized that because the LAP-BAND system has been on the market for a longer period, the reported adverse events will be less overall with the LAP-BAND than in the REALIZE Adjustable Gastric Band. **Objective:** To examine the adverse events associated with the LAP-BAND system and the REALIZE Adjustable Gastric Band.

**Methods:** A literature review and meta-analysis were performed. This research conducted a comparison in 3 different categories of adverse events between 2 types of adjustable gastric banding systems: (1) LAP-BAND with OMNIFORM Design and (2) REALIZE Adjustable Gastric Band.

**Results:** There were 1770 digestive-related adverse events reported from using either REAL-IZE or LAP-BAND system. For digestive-related adverse events, there were 508 reported events (29%) from using the REALIZE system and 389 reported events (22%) from using the LAP-BAND system (odds ratio [OR], 1.04; P=.68). For infection-related adverse events, there were 122 reported events (7%) from using REALIZE system and 41 reported events (2%) from using the LAP-BAND system (OR, 2.5; P<.0001). For pain-related adverse events, there were 150 reported events (8%) from using REALIZE system and 45 reported events (3%) from using the LAP-BAND system (OR, 2.9; P<.0001).

**Conclusion:** Patients using the REALIZE system have the highest odds of reporting pain-related adverse events rather than infection or digestive related adverse events when compared with the LAP-BAND system. Patients using the REAL-IZE system have statistically significant higher odds of reporting infection and pain related adverse events when compared with the LAP-BAND system.

Take Home Message: Patients using the REAL-IZE system are more likely to report pain-related adverse events when compared with patients using the LAP-BAND system.

### C9

# Comparisons of Perceptions Between Parents With Bipolar Disorder, Major Depression, and Psychiatric Comorbidity

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**Introduction:** Numerous research studies have found various personal, financial, psychological, and societal costs result from mental disorders. It has been suggested that patients diagnosed with bipolar disorder experience particularly problematic occupational and social outcomes.

**Objective:** This current project expanded the investigation of the costs resulting from mental disorders to those of specifically individuals with parenting roles with a particular focus on bipolar and depressive disorders.

Hypothesis: It was hypothesized that parents diagnosed with bipolar and depressive disorders would show problematic attitudes, perceptions, behaviors, and emotions in the context of their parenting role, and that differences would exist between parents with bipolar vs depressive disorders.

**Methods:** The participants in this study were men and women with a diagnosis of bipolar disorder, depressive disorder, or both who are parenting at least 1 child and a comparison group of parents without any psychiatric diagnosis. A structured interview comprised 70 questions related to various aspects of parenting behaviors and attitudes was administered to the participants who rated their responses to the questions using a 5-point Likert scale. The Midwestern University institutional review board provided approval for the project.

**Results:** Factor analysis for the responses on the structured interview produced 7 components with Eigenvalues greater than 1 that explained

83.2% of the total variance. The factors were labeled parenting effectiveness, relational functioning, emotional recognition, inter-generational influences, occupational functioning, coping with anger, and social compliance. Independent samples t test indicated that parents with bipolar or depressive disorder had greater dysfunction in parenting effectiveness (t=3.93, P=.001), relational functioning (t=3.78, P=.000) and occupational functioning (t=-2.41, P=.019). Chi-square analysis indicated that parents with bipolar disorder had greater difficulty coping with anger ( $\chi^2$ =20.67, P=.000) and with social compliance ( $\chi^2$ =7.06, P=.008). Comparing parents with bipolar disorder, depressive disorder, or comorbidity showed significant differences on occupational functioning (F=10.92, P=.001) and social compliance (F=3.61, P=.031).

**Conclusions:** Compared with parents without psychiatric diagnoses, parents with a mental disorder showed significantly diminished parenting effectiveness, relational functioning, occupational functioning, difficulties coping with anger, and poor social compliance. Parents with bipolar disorder exhibited the highest levels of occupational dysfunction and poor social compliance. Results suggest a need for parenting support services and programs to promote effective parenting, as well as occupational and relational functioning among persons with mental disorders.

# C10

# Cryoneurolysis of the Infrapatellar Branch of the Saphenous Nerve, a Novel Treatment for Pain and Impaired Function From Osteoarthritis of the Knee

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**Introduction:** Management of osteoarthritis (OA) of the knee is aimed at reducing pain and

improving function.

**Hypothesis:** One novel investigational therapy is the application of focused cryotherapy to the infrapatellar branch of the saphenous nerve (ISN) with the goal of producing a reversible neuroablation.

Methods: Adults (aged  $\geq 18$  years) with confirmed OA of 1 or both knees and an average pain score of 4 or higher on a 0-to-10-point visual analog scale (VAS; 0=no pain) were enrolled in this prospective, multi-center, open-label study. The location of the ISN was determined with bony landmarks and palpation. Following anesthetizing the area with lidocaine and epinephrine, the Focused Cold Therapy device (myoscience) was inserted perpendicularly to the ISN and the nerve was exposed to a temperature below -20°C for 60 seconds. During the study, the effect on pain was assessed with the VAS and the effect on interference with function was assessed with the Western Ontario and McMaster University Osteoarthritis (WOMAC) index.

Results: The first 10 patients enrolled at this study site (mean age, 55 years; range 38-68 years; 4 women) had 16 knees treated. Mean (standard deviation) pain score declined from 6.1 (1.7) at baseline to 1.2 (1.0) in the postprocedure period and remained improved at days 7 (2.2 [1.4]) and 30 (2.3 [1.7]). The mean (SD) WOMAC interference with function score improved from 88.4 [19.03] at baseline to 22.6 (21.0) on day 7, representing a 75% improvement. On day 7, 15 of 16 (94%) and 14 of 16 (88%) treated knees demonstrated 30% or more and 50% or more improvement in WOMAC function score, respectively. On days 7 and 30, 90% of patients responded that they would have the treatment again. The positive response rate was 80% on day 56. Two treatment-emergent adverse events were reported by 1 patient who experienced numbress in both legs; the numbness was deemed to be definitely related to treatment and moderate in severity. The numbness resolved in 1 leg after 164 days and improved in the other leg. Anticipated observations included bruising (31% of knees at day 7), tingling (13%), redness (13%), local pain (50%), crusting (44%), and hyper- (13%) or hypopigmentation (13%). Only tingling and 1 instance of bruising did not resolve within 56 days.

**Conclusion:** Treatment of patients with OA of the knee with cryoneurolysis of the ISN was well tolerated and reduced pain and improved function in all treated knees. Further research is warranted to more fully characterize the safety and efficacy of this investigational therapy for patients with OA of the knee.

### ♦C11

# HIV Awareness and Testing in the Lowcountry: A Survey-Based Study of Beaufort, Jasper, and Hampton Counties in South Carolina

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**Introduction:** A surveillance report on human immunodeficiency virus (HIV) done by the US Centers for Disease Control and Prevention ranked South Carolina as ninth in prevalence of AIDS during 2010, with a rate of 15.5 per 100,000 people. The present study was conducted to examine whether the rural patient population of the South Carolina Lowcountry is aware of the local prevalence of HIV and is being adequately screened for the disease.

**Hypothesis:** The population of South Carolina's Lowcountry has a lack of perceived knowledge and awareness of HIV in their community and the majority are not being offered HIV screening tests.

**Objectives:** Assesses willingness to get tested for HIV, perceived knowledge of HIV transmission, awareness of HIV prevalence, awareness of personal HIV status, and prevalence of healthcare workers actively offering HIV testing.

**Methods:** Anonymous surveys were provided to patients of Beaufort-Jasper-Hampton Comprehensive Health Services (BJHCHS) through 5 clinical settings. The 12-question survey including 5 demographic questions. Patients were made aware of the surveys either by a receptionist or a medical student mentioning them or by noticing them on the table in the waiting room. They were told that the surveys were anonymous and voluntary. No further information regarding the study was given so as to alleviate any bias. Exact 95% confidence limits were calculated using SAS 9.3 (SAS Institute Inc).

**Results:** Fifty-five surveys were used for analysis. Of the respondents, 81.13% would be willing to take an HIV test (0.6803, 0.9056), 98.1% claim to know how HIV is transmitted (0.8974, 0.9995), 53.7% believe HIV is a significant problem in their community (0.3961, 0.6738), 56.6% don't know their HIV status (0.4228, 0.7016), and 64.8% have never been offered an HIV test by a provider (0.5062, 0.7732).

**Conclusion:** Of the population surveyed, more than half do not know their HIV status. Most would be willing to take an HIV test, yet the majority has never been offered an HIV test by their health care provider at this health center. On the basis of our results, we recommend that increased efforts be made to educate patients of BJHCHS regarding the significance of the prevalence of HIV in these counties. Additionally, we recommend expansion of efforts to train employees of this health center to offer HIV testing to each patient and also teach patients how to reduce their risk of acquiring HIV.

# C12

## Examination of Wound Healing Rates and the Costs Associated With the Amount of Treatments Needed

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**Introduction:** Obesity is acknowledged to increase the risk of many diseases and health conditions. Specifically, healing wounds and preventing amputation in diabetic patients is challenging for physicians and hospitals. The costs associated with diabetic wound healing are high because of prolonged hospitalization, rehabilitation, and a need for home care and social serviced disabled patients. Per capita spending for the obese is \$1429 higher per year than for someone of normal weight. Medicare expenditures for lower-extremity ulcer patients were on average 3 times higher than those for Medicare patients in general.

**Hypothesis:** The hypotheses were that severity of obesity affected total cost of wound care and the number of doctor visits. Different types of insurance and diabetes status may also affect the cost of wound care.

**Methods:** This is a retrospective cohort study. One hundred medical records of wound care patients were randomly selected from the patient database of a private practice in Rutherford College, North Carolina. Demographic information, body mass index (BMI), social history, number of doctor visits and cost per visit, insurance type, phases of wound, diabetic status, and glycated hemoglobin level were collected.

**Results:** Ninety-one patients had inflammatory initial phase of wound. The average total cost to

treat severely obese patients with lower extremity ulcers was \$3218 ( $\pm$ \$1764), which was significantly higher than that of the other BMI categories (P=.029). Severely obese patients had a significantly higher average number of visits (50 $\pm$ 30.1) compared with that of other BMI categories (P=.049). The average cost of wound care for patients who paid by private insurance or self-paid was \$1832 ( $\pm$ \$509), which was higher than that of patients who paid by Medicare or Medicaid by \$1032 (P=.028). The average cost of wound care to treat patients with type 2 diabetes mellitus was \$1523 ( $\pm$ \$425), which was higher than that of nondiabetic patients by \$409 (P=.39).

**Conclusion:** The results showed that being severely obese had a significant effect on the average total cost of wound treatment and number of visits. Patients with Medicaid or Medicare had significantly less total cost for their wound treatment compared with private insurance/self-paid patients. Diabetic and nondiabetic patients had an insignificant difference in cost of wound care and number of visits. This finding could be because the diabetic patients were able to control their conditions and had in home wound care treatments during the visits.

### C13

# Inappropriate Prescription of Antibiotics to Patients at Ambulatory Medical Care Centers in the United States

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**Introduction:** An inappropriate prescription of antibiotics can lead to the development of

antibiotic resistant. Antibiotic resistance is the greatest cause of death for children and young adults—13 million people throughout the world die every year because infectious diseases have become resistant to antibiotics. Some antimicrobial resistance may have resulted from inappropriate use of antibiotics. As antibiotic resistance increases, our ability to treat bacterial infections will be severely diminished. Antibiotic resistance is inevitable, but if health care professionals and their patients are properly educated on the topic of antibiotic resistance, then the rate that bacteria acquire resistance may be controlled.

**Hypothesis:** The hypotheses were to compare the number of antibiotics prescriptions received for a cold among the following groups: (1) male vs female; (2) outside metropolitan area vs inside metropolitan area; (3) age 44 years or younger vs 45 years or older; and (4) African American vs Caucasian.

Methods: This is a retrospective cohort study to review the antibiotic prescribing habits of health care professionals working at an ambulatory medical care center and to make comparisons of antibiotic prescription trends between race, age, sex, and location. The data used in this study were collected in a report via a National Probability Survey of Non-Federal, General, and Short-Stay Hospitals conducted by the US Centers for Disease Control and Prevention's National Center for Health Statistics, Division of Healthcare Statistics. The data were collected from patients who visited an Ambulatory Medical Care Center in the US and received an antibiotic prescription for a cold.

**Results:** The odds of receiving unneeded antibiotics for a common cold was 6.98 times higher for a person younger than 44 years compared with a person older than 44 years (P<.0001); 1.294 times higher for a male person compared with a female person (P<.0001); 1.371 times higher for a person living outside compared with a person living within a metropolitan area (P=.003); and 1.497 times more likely for an African-American compared with a Caucasian person (P<.0001). **Conclusion:** The odds of receiving antibiotics for a common cold are higher if a patient is male, African American, aged 44 years or younger, and lives outside the metropolitan area.

### C14

# Effects of Geographic Location on Cancer Mortality in Virginia

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**Introduction:** This research was conducted to gain a better understanding of how underserved areas in Virginia are affected by a lack of access to health care. Lower education levels, rural counties, minority populations, and income and economic disparities are factors associated with poor health.

**Hypothesis:** To compare the cancer mortality rising, stable, and falling rates of underserved counties to non-underserved counties in Virginia.

Methods: This is a retrospective cohort study. The data were obtained from the National Cancer Institute (NCI) "State Cancer Profile" and Virginia Department of Health from 2005 to 2009. Mortality data were obtained from the National Vital Statistics System public. Mortality rates were calculated by the NCI using SEER\*Stat. The data for underserved counties in Virginia were provided by the Virginia Department of Health. Death data were provided by the National Vital Statistics System public use data file. Death rates were age-adjusted to the 2000 US standard population. Virginia Medically Underserved Areas (VMUA) represents counties or cities that have inadequate access to health care defined by the Virginia Department of Health.

Results: For all cancer, we observed underserved counties had a 5% (10%-5%) higher rising cancer mortality rate likelihood than non-underserved counties (P=.74); which was not significant. Underserved counties had a 6% (60%-54%) higher stable cancer mortality rate likelihood than non-underserved counties (P=.004); and a 9% (40%-31%) lower falling cancer mortality rate likelihood than non-underserved counties (P=.0007), which were both significant. For lung cancers, we observed underserved counties had a 3% (20%-17%) higher rising cancer mortality rate likelihood than non-underserved counties (P=.20); and a 21% (75%-54%) higher stable cancer mortality rate likelihood than non-underserved counties (P=.08), neither of which were significant. Lastly, underserved counties had a 24% (29%-5%) lower falling cancer mortality rate likelihood than non-underserved counties (*P*<.0001).

**Conclusion:** We concluded for all cancer that living in a VMUA increases the likelihood of having a stable cancer mortality rate and decreases the likelihood of having a falling cancer mortality rate in comparison with living in a non-underserved area of Virginia. We also concluded that living in a VMUA decreases the likelihood of having a falling lung cancer rate in comparison to a non-underserved area.

#### C15

### Analysis of Drug Abuse and Mental Health in Appalachia

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Introduction: The Appalachian region includes 13 states that lie along the Appalachian Mountains in the eastern United States. The Appalachian economy depends highly on the industry of coal mining. Research indicates that there are several health disparities, including drug abuse, within the region. Drug abuse within the Appalachian region is more prevalent among the coal mining counties than the rest of the Appalachian population. This study includes individuals of all ages who are living in the Appalachian region, specifically coal mining areas, as defined by the Appalachian Regional Commission. This includes 410 Appalachian counties with 176 coal mining counties.

**Hypothesis:** The purpose of this study is to determine which types of drugs, and consequently which mental health disorders, are at a heightened risk of abuse in the coal mining regions of Appalachia opposed to the non-coal mining regions of Appalachia.

**Methods:** This is a cross-sectional study that used a survey of the Appalachian population to determine the prevalence of certain types of drug abuse. Data from this survey come from 2 federal data sets, including the Treatment Episode Data Set (TEDS) and the Health Care Utilization Project's (HCUP) National Inpatient Stay (NIS) data set, which included patient discharge information from substance abuse treatment centers and community hospital records, respectively, within the Appalachian region. Data were further stratified between the coal mining and non–coal mining counties of the region, as defined by the National Coal Resources Data Set (NCRDS).

**Results:** The results suggested that there is an increase in the odds of heroin use, opiate and other synthetic drug abuse, and hospitalization because of a cognitive disorder of people within coal mining counties of Appalachia vs people outside of coal mining counties within Appalachia. The odds of heroin abuse are 1.52 times higher for people within coal mining regions (P<.0001). The odds of opiate abuse are 1.90 times higher and the odds of hospitalization because of a cognitive disorder are 1.12 times higher for people within coal mining regions.

**Conclusion:** Despite its socioeconomic disadvantages, drug and alcohol dependence and abuse among the adult population in the Appalachian region as a whole compares favorably to the rest of the nation. The coal mining regions of Appalachia, a valuable subset of this population, exhibit substantial disparities in terms of illicit drug abuse and mental health disorders.

### C16

# Health Indicators Among Public Safety Workers

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**Introduction:** Although many studies have demonstrated the existence of poor health among public safety workers, few have sought to establish correlations between work intensity or number of calls for service answered per shift and poor health indicators. With a constantly growing population and declining economic state, the use of public safety services—police, fire, and emergency medical services—has increased. As the demand for these services grows, the stresses placed on these workers increases as well.

**Hypothesis:** The aim of this study is to determine if there was a correlation between an increase in poor health indicators and an increase in the volume of emergency responses. If a correlation is determined, this study could provide the basis of policy changes that could ultimately contribute to an overall increase in the well-being of public safety workers and the public they serve.

Methods: Public safety workers from Town of Richlands Department of Fire/Rescue, Virginia Tech Rescue Squad, Fauquier County Sheriff's Department, and Loudon County Fire and Emergency Medical Services Department were recruited to complete an anonymous, computer-based, electronic survey concerning a number of lifestyle habits including substance use, sleepiness, and posttraumatic stress disorder symptoms.

**Results:** Thirty-six public safety workers (8 women and 28 men) responded to the survey. Sixteen workers (44%) were younger than 21 years and 1 worker was older than 60 years. Seven workers were combined firefighters and emergency medical technicians (EMTs), 23 workers (64%) were EMTs, 4 workers were firefighters, and 1 was a law enforcement officer. Number of calls on each shift was significantly associated with smokeless tobacco (r=0.63, P<.0001), body mass index (r=0.49, P=.0024), number of cigarettes (r=0.44, P=.0228) but was not associated with life satisfaction, alcohol consumption, or Epworth Score of sleepiness.

**Conclusion:** An increasing number of calls for service answered per shift correlated with poor-

er health indicators. This study allowed public safety administrators to examine worker work intensity and balance that against poorer worker health as well as the poorer safety that has been associated with it. This study made progress toward identifying specific factors of emergency services work that leads to poor health outcomes in public safety workers and indicates directions for future investigation.

### C17

# Assessment of Nutritional Status and Knowledge in Honduran Children During a VCOM Education Mission Trip

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**Introduction:** Edward Via College of Osteopathic Medicine postbaccalaureate students offered nutrition education sessions to Honduran children in selected communities in underserved areas.

**Objective:** To improve the children's nutrition knowledge.

**Methods:** Anthropometric and knowledge data were collected on children in a remote mountain school (MS) and boys in a rural orphanage (O). **Results:** Data indicated that for children in the MS group (25 boys, 20 girls), the average age was  $9.0 \pm 2.2$  years; height,  $125.2 \pm 12.1$  cm; weight,  $26.0 \pm 6.6$  kg; and waist circumference,  $59.0 \pm 5.5$  cm. Boys in the O group (N=47) were aged  $12.3 \pm 3.3$  years; height,  $145.2 \pm 16.0$  cm; weight,  $39.7 \pm 12.1$  kg; and waist circumference,  $66.1 \pm 7.0$  cm. Stunting (-2 height for age *z* scores) was observed in 23% of the MS children and 7% of the O children. Pre- and posttest questions were

administered after a 20-minute nutrition education session, and paired t tests were conducted to determine significant differences. Children (MS, N=59; O, N=42) selected responses from multiple choice questions on food items that were good sources of protein, calcium, iron, and vitamin A. Results showed variable improvements in children's knowledge. Generally, the O subjects had a higher nutrition knowledge base than the MS subjects. In the MS group 92% initially recognized milk as a good source of calcium while 76% of O responses selected milk. Initially, MS children selected milk (41%) and fish (41%) rather than meat (2%) as a good source of iron; beans (66%) as a good source of protein rather than meat (18%); and rice (67%) as a good source of vitamin A rather than tomatoes (20%). McNemar<sup>2</sup> tests indicated that MS children tended to improve their knowledge of calcium food sources (P=.057), while O children significantly improved in response to food sources of protein (P < .000) and tended to improve in response to food sources of vitamin A (P=.057). For some questions, the selection of the correct food item to provide a nutrient declined from pre- to posttest.

**Conclusions:** The improvements in knowledge were not as encouraging as anticipated because of language barriers, challenging physical environments, teaching strategies, and learning distractions. These factors influenced assessment results and will be considered to improve future educational mission trips. The need for nutritional education was confirmed by the high rates of stunting reported.

### C18

# Teaching Substance Abuse Prevention to Children in Honduras

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**Introduction:** Postbaccalaureate students from the Edward Via College of Osteopathic Medicine provided an education program on substance abuse for children aged 5 to 18 years in 2 Honduran communities.

**Objective:** The objective of the study was to determine whether the children's knowledge improved after participating in the educational sessions.

Methods: The program was assessed in Guajire (n=54; average age, 9 years) and Jovenes en Camino orphanage (n=47; average age, 12 years). Children's knowledge was assessed with a pre-/post intervention test with 3 questions about substance abuse: El alcohol deteriora tu juicio? (Does alcohol impair your judgment?), Olger pega es peligroso para tu cuerpo? (Is huffing glue dangerous for your body?), and Puedes convertirte dependiente a las drogas? (Can you become dependent on drugs?). The 20-minute presentation consisted of photographs of celebrities and famous athletes who were substance abusers. The children used a pair of Drunk Busters Twilight Vision Goggles (Drunk Busters of America, LLC) to experience a simulation of the physical effects of binge drinking.

**Results:** In Guajire, the pretest results indicated that 74% of children knew that alcohol would impair their judgment and 85% knew that huffing glue was dangerous. In the orphanage, responses to pretests were higher with 96% and 98% responding correctly regarding the dangers of alcohol and huffing glue, respectively. In both communities there was no significant change

in their knowledge after the interventions. The pretest response to drug dependency in both communities was lower than responses to questions on alcohol and glue with 23% in Guajire and 46% in Jovenes responding correctly. Posttest responses were both significantly improved to 46% (P=.008) in Guajire and 49% (P=.001) in Jovenes.

**Conclusions:** Children's prior knowledge of substance abuse was better than anticipated, indicating an awareness of substance abuse in Honduras even in remote areas and in young children. In Guajire, although parent assistance was welcomed, it might have been a confounding factor in assessments of children's knowledge. The findings indicated that future substance abuse programs should investigate how knowledge of substance abuse correlates with current and future substance use behaviors. Anecdotal findings suggested that future assessments should also consider pilot testing culturally appropriate questions.

## C19

# Effect of Preventive Health Education on Children's Personal Hygiene Knowledge in 2 Communities in Honduras

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**Introduction:** The leading causes of death in Honduras are infectious and parasitic diseases. Because of the lack of preventive measures, diseases such as dengue fever and influenza are becoming more prevalent. **Objective:** An educational session was developed to emphasize the importance of personal behaviors in preventing the spread of disease, and testing was used to determine knowledge gained.

Methods: The sessions were presented in 2 communities: a mountain community of Guajire (N=53; average age, 9 years) and an all-boys orphanage, Jovenes en Camino (N=43; average age, 12 years). Methods included a presurvey that assessed the children on personal hygiene habits and knowledge, interactive teaching games, and a posttest that contained the same questions as the pretest to assess the change in knowledge. The main method of teaching used a germ simulating lotion that could only be seen on the children's hands under ultraviolet light. After the game, the children washed their hands with assistance in proper technique. The questions were as follows: When should you wash your hands? How should you cover your mouth when you sneeze? Which of the following is a common place to pick up germs or pass them along on your hands? What is the most common way for disease to spread? After you've lathered up with soap, how many seconds should you spend vigorously washing your hands? Results: Knowledge gained on how to properly cover a sneeze changed from 20% to 91% (P < .0001) because of the education intervention for Guajire and changed from 70% to 84% (P=.057) in Jovenes due to the education intervention. Knowledge gained on what the most common way for disease to spread changed from 26.4% to 64.2% (P<.0001) due to the education intervention in Guajire and changed from 60.5% to 58.1% (P=1.00) in Jovenes. Knowledge gained on proper length of time for hand washing changed from 77.4% to 88.7% (P < .146) as a result of the education intervention in Guajire and changed from 48.8% to 76.7% (*P*<.017) as a result of education intervention in Jovenes.

**Conclusions:** The educational intervention for the mountain community children in Guajire signifi-

cantly improved knowledge of how to properly cover a sneeze and the most common way disease is spread and in the Jovenes orphanage on proper hand washing time. Some questions and teaching strategies and distractions should be reviewed to improve assessment of knowledge in future programs.

# C20

# Violence Prevention Education Among Youth Groups in Tegucigalpa, Honduras

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**Introduction:** Youth violence is the number 1 health and safety issue in Honduras. Educating youth about violence in Honduras may help lower the rate of violence.

**Objective:** The goal of the study was to assess the knowledge gained by children who were provided an education program on personal violence.

**Methods:** Boys and girls aged 5 to 18 years living in 2 communities in Tegucigalpa were studied: the rural community of Guajire (GC) (n=50; average age,  $9 \pm 2.2$  years) and a boys orphanage, Jovenes (JO) (n=42; average age  $12.3 \pm 3.3$ years). Postbaccalaureate students presented educational sessions on violence. The children interacted with the presenters by answering questions, participating in a matching game, and expressing how they felt on the topic. The educators also included a role-playing scene to teach the effects of bullying. Five survey questions on pre- and posttests were administered to the children to assess knowledge gained. Results: Children's knowledge of avoiding danger when encountering strangers improved from 53% to 68.3% (P=.01) in GC and improved from 38% to 43% (P=.51) in JO. The children's knowledge of differentiating between self-defense and violence behavior improved from 71% to 87% (P=.12) in GC. The children's knowledge on how to properly react to conflict improved from 78.3% to 90% (P=.18) in GC and from 79% to 83% (P=1.0) in JO. The children's knowledge on how to identify types of bullying improved from 34% to 43% (P=.41) in GC and from 33% to 40% (P=.42) in JO. The children's knowledge on defining sexual abuse improved from 20% to 47% (P=.004) in GC and from 11% to 98% (P=.75)in JO.

Conclusions: Children in GC gained more knowledge compared with boys in JO. The pretest scores in JO were higher than in GC suggesting that the boys in JO had prior knowledge on the topic of violence prevention. Both communities gained the most knowledge on sexual abuse, suggesting that sexual abuse may not typically be discussed in these communities. Study limitations included language barriers hampering interaction and communication and preventing educators from explaining survey questions thoroughly. Such language barriers may have encouraged some children to guess the answers. Another limitation included the conversation among children during testing, possibly influencing one another's test responses. As presenters, we felt satisfied that the educational experience was worthwhile.

#### C21

# Assessing Nutrition Knowledge in Honduran Children

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**Introduction:** Malnutrition is a substantial problem throughout Honduras with growth stunting and wasting affecting many children. Postbaccalaureate students at Edward Via College of Osteopathic Medicine traveled for a week offering a day-long program of health education sessions in children's centers.

**Objective:** The goal of this project was to assess nutritional knowledge of children using pre- and posttests administered to children of varying ages in selected children's centers in Honduras; a rural, mountain-top community outside of Tegucigalpa (N=59; average age, 9 years) and an orphanage an hour's drive outside of Tegucigalpa (N=42; average age, 12 years).

Methods: Each child and parent signed the appropriate assent and consent forms and subject confidentiality was maintained with unique numerical identifiers as per the protocol approved by the institutional review board. A cumulative pretest for all health education sessions was given before the educational sessions were presented and a post test was given before the end of the day. The health education sessions included a presentation with visuals (ie, US Department of Agriculture MyPlate flyers and food models), drawing commonly consumed foods, and discussing the content for the pretest questions. Questions were adapted from previous US population survey questions. Study outcomes were the subjects' pre- and posttest responses to the nutrition questions, demographic data collected (age, educational level, socioeconomic status, etc) and anthropometric measures (ie, height, weight, waist and wrist circumferences).

**Results:** There was no significant improvement in correct responses to pre- and posttest questions in the mountain-top community. Data from the orphanage showed no significant change between pre- and posttest responses to food sources of calcium, iron, and vitamin A. However, questions pertaining to protein sources and water consumption recommendations showed a significant improvement (P=.001 and <.001, respectively).

**Conclusions:** The absence of statistical significance to confirm improvements in nutrition knowledge were unexpected. The findings and observations made by the educational team suggest improvements for future educational knowledge assessment include revising the protocol for a more culturally appropriate experience, practicing using the educational materials, optimizing the environment for presenting and testing, and using methods of delivery and assessment that focus on the selected nutritional content.

# ♦C22

# Impact of Fluctuating Coal Production on Indicators of Social Determinants of Health in Southwest Virginia

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**Introduction:** Environmental impacts of coal mining, the industry that has historically supported the economy in the southwest Virginia, have recently been heavily scrutinized for its relationship to poor health. These studies have been the topic of debate regarding their meth-

ods, analysis, results, and unsubstantiated conclusions. Poverty is known to be associated with poor health outcomes, but no literature has been published that investigates the economic impact of declining coal production on poverty rates in this region. It is imperative to understand on a county by county level the impact of changes in coal production on counties that have been in economic decline and on those that are economically improving.

**Objective:** The objective of this descriptive study was to determine whether counties in southwest Virginia in Appalachia experiencing a change in coal production between 1980 and 2008 had also experienced a change in poverty.

**Methods:** Percent change was calculated for 6 counties using 4 data points for poverty (1980, 1990, 2000, 2010) and annual data points for each year from 1980 to 2008).

**Results:** Lee County reported a decrease in poverty between 1980 and 2010. Lee County maintained its level of coal production between 1980 and 2008. Tazewell County reported the largest decrease in coal production (64.7%) and was second in poverty increase (18.1%) after Wise (31.2%). Buchanan, Dickenson, and Russell all reported decreases in coal production with accompanying increases in poverty levels since 1980. All changes in values between 1980 and 2008/2010 were statistically significant (P<.0001).

**Conclusions:** Maintaining coal mining production in Lee County may have provided an economic infrastructure which may be a significant contributor to the stabilization of poverty. This relationship can be considered when contrasting Tazewell County's coal production and poverty status. However, coal production alone cannot be the only variable of interest when considering the level of poverty in a given county. Although coal production may have a relationship, the multifactoral nature of poverty status requires

more in-depth and complete county-by-county analysis. This may involve improving the "granulation" or analysis on a more intimate and microscopic level. Using this method may provide a more accurate illustration of the affects that coal production have on poverty, health, and the environment in southwest Virginia.

# **Basic Sciences**

### ♦B1

# PON1 Lactonase Activity and Differential Distribution in High-Density Lipoprotein Subclasses in the Postprandial Phase: A Pilot Study

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Introduction: Paraoxonase-1 (PON1) is a promiscuous esterase carried by high-density lipoprotein (HDL), which protects low-density lipoprotein (LDL) from oxidation. Evidence is accumulating, indicating that the lactonizing/lactonase activity of PON1 may be physiologically the most significant. Although most static lipid studies are done in the fasting state, we spend most of the day in the postprandial phase. Given the importance of oxidative stress in the postprandial period, studies should be undertaken to elucidate the role of PON1 in this phase. Studies on postprandial excursions of PON1 are scarce and contradictory in some regards. Some studies show an increase, some a decrease, and some no change. One major limitation of all the studies on postprandial excursions of PON1 is that they measure the esterase activity, not the physiological lactonase activity. No studies exist that focus on postprandial excursions of PON1 physiological activity as a lactonase. No studies exist that focus on puta-

 Abstract entered in the SOMA Student Poster Competition. tive changes in PON1 distribution across HDL subclasses in the postprandial period. This study addresses these knowledge gaps.

**Hypothesis:** PON1 lactonase activity changes in the postprandial period.

**Methods:** Healthy volunteers were recruited for this study. A fasting (12 hour) and 3 postprandial blood samples (at 1, 2, and 5 hours) were taken from finger pricks after a standardized mixed meal containing 700 calories, 32 g fat (11 g saturated fat), 85 mg cholesterol, 85 g carbohydrates (43 g sugar), and 24 g protein. PON1 lactonase and arylesterase activity, glycemia, and triglycerides were measured in these samples. Subclasses of HDL were separated by native gradient gel electrophoresis (4%-12%, Biorad), and PON1 activity was detected using our method.

**Results:** We recruited 24 volunteers. Because of blood sample volume, we observed the lactonase activity for 14 individuals: 10 women and 4 men. Lactonase activity significantly steadily decreased in 6 individuals, including 4 women and 2 men, and significantly steadily increased in 5 individuals, all women. Arylesterase activity did not show significant changes.

Conclusions: This is the first study that shows that PON1 physiological lactonase activity varies after a mixed meal. Moreover, arylesterase activity, which correlates closely with PON1 concentration, does not show significant change. This finding suggests a change in function rather than in quantity. Interestingly, the changes in lactonase vary among individuals-some increase and some decrease. Does this behavior correlate with lipoprotein changes? Is this behavior a marker of postprandial protection against dietary oxidized lipids in some individuals and not in others? Further studies are being conducted to confirm these findings. We posit that in some individuals PON1 is redistributed to small HDL in the postprandial phase, which increases its activity. In addition, further studies are needed to elucidate the possible metabolic differences that may contribute to the variations of lactonase activity between individuals in the postprandial state.

## ♦B2

# Novel Genistein-Loaded Lipidic Nanocarrier Adjuvants Enhance Anticancer Efficacy and Overcome Cancer Resistance to Chemotherapy

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Introduction: Genistein, a biologically active flavonoid found in soy, is recognized as an important inhibitor of multi-drug resistance protein transporters in cancer and a potent apoptosis inducing agent in various tumors. Despite evident anti-cancer activity of genistein, the clinical potential is restricted by its lipophilic nature, extensive metabolism, and poor bioavailability. Our work describes the development of new lipid-based nanocarriers, namely liposomes (Lips), nanoemulsions (NEs), and polymeric phospholipid micelles (Mics), as drug delivery vehicles for genistein, either alone or in combination with doxorubicin (DOX), in various types of cancers.

**Hypothesis:** The enhanced mitochondrial-specificity and pro-apoptotic activity of genisteinloaded lipidic nanocarriers can augment the antineoplastic effectiveness of lipophilic anticancer chemotherapeutic drugs.

**Methods:** Optimized Lips, NEs and Mics loaded with genistein were tested in vitro against murine sensitive breast carcinoma (4T1) and resilient colon cancer (C26). Inhibition of drug-efflux in human ovarian and DOX-resistant (NCI/ADR-RES) cancer cells was assayed, using drug-combination ratios with liposomal DOX.

**Results:** Genistein-loaded vesicles showed high drug incorporation (NE>Mic>Lip) and stable nano-scale pharmaceutical formulation. Microscopy indicated enhanced uptake and significant induction of morphological apoptosis, in C26 and 4T1, respectively, with genistein-loaded Lip and Mic. Superior cytotoxic effects were demonstrated for genistein Mic and Lip, in all tested cancer cell models, compared with all treatment and vehicle controls. Most importantly, the half maximal inhibitory concentration (IC50) values for genistein/DOX Lip were at least 3- to 6-fold less than DOX-Lip in NCI/ADRRES, showing significant synergistic 1:1 drug ratio, and additive 1:2, 2:1, and 3:1 drug ratios, respectively.

**Conclusion:** Genistein-loaded nanocarriers demonstrated enhanced delivery of genistein to both naïve and drug-resistant cancer cells, showing strong potential as therapeutic platforms using this potent pro-apoptotic nutraceutical, to augment the anti-neoplastic effect of doxorubicin and similar drugs in resistant tumors overexpressing drug efflux pump.

### **♦**B3

# Metabolism of Volatile Anesthetics: Implications for Postoperative Cognitive Dysfunction

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**Introduction:** While the human liver can metabolize all volatile anesthetics, the amount of isoflurane that has been shown to undergo biotransformation is on the order of 0% to 0.2%. The metabolic pathway is thought to involve cytochrome P450 enzymes, yielding trifluoroacetic acid, 2 free fluoride ions, carbon dioxide,

HCl, and water. Our laboratory has previously shown that glyceraldehyde 3-phosphate dehydrogenase (GAPDH) binds to volatile anesthetics. We were interested in examining whether GAPDH played a role in the metabolism of volatile anesthetics.

Hypothesis: GAPDH, which we hypothesize to be a target of anesthetics, is a diverse protein that exhibits many nonglycolytic functions that appear associated with oligomeric dynamics. This multifunctional enzyme exhibits diverse catalytic properties, such as peroxidase activity. We hypothesize that GAPDH contributes to the chemical transformation of inhaled anesthetics. Methods: We oxidized GAPDH using an in vitro Fe/ascorbate system prior to exposure to isoflurane and observed substances showing spectroscopic identifiers that suggest the appearance of

alkenes.

**Results:** GAPDH-reactive cysteine (ie, Cys-149) undergoes stepwise oxidation from a thiol group to sulfenic acid, then to sulfinic acid, and finally to sulfonic acid. GAPDH shifts from oxidoreductase to peroxidase activity upon modification. We observed that sulfenated GAPDH increased on protein oxidation (P<.05). Additionally, the monomeric form of GAPDH predominated in oxidized GAPDH as evidenced by changes in elution patterns in size exclusion chromatography. Lastly, we observed a byproduct upon incubation of oxidized GAPDH and isoflurane, as indicated by greater absorbance at wavelengths associated with oxo-dienes, which are capable of further modifying the protein.

**Conclusion:** The results from this in vitro study showed that oxidized GAPDH contributes to the metabolism of isoflurane supporting our hypothesis. We also saw that GAPDH may be further modified by this putative byproduct. Because GADPH is so abundant, these processes may have ramifications for anesthetized patients, though this remains to be shown in an in vivo system. Because these chemical modifications are associated with cellular decline as it occurs in the oxidative stress of aging, we propose that tissues, such as the aging brain in humans, may provide a chemical environment suitable for enhancing the metabolism of isoflurane. The metabolic products suggested by our in vitro findings may contribute to the further decline of these tissues, suggesting a mechanism for postoperative cognitive dysfunction in the elderly population.

### ♦B4

# Comparison of Substrate Specificity of *Escherichia coli* p-Aminobenzoylglutamate Hydrolase With Pseudomonas Carboxypeptidase G

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**Hypothesis:** *Escherichia coli* p-aminobenzoylglutamate hydrolase can cleave aminopterin and methotrexate.

Background: Methotrexate (MTX) is a potent chemotherapy drug used in the treatment of various cancers, rheumatoid arthritis, and psoriasis. When patients accumulate toxic levels of MTX, they are treated with intravenous Glucarpidase (carboxypeptidase G2), an enzyme isolated from Pseudomonas. Although efficient at cleaving MTX and aminopterin, Glucarpidase also markedly breaks down endogenous folates, which are important for human health. We compared Ecoli p-aminobenzoylglutamate hydrolase (PGH) to Pseudomonas carboxypeptidase G (CPG) in enzyme assays measuring cleavage of folate, MTX, aminopterin, leucovorin (5-formyltetrahydrofolate, an alternative treatment for MTX overdose), and 5-methyltetrahydrofolate, the circulating form of folate in humans. Carboxy-

peptidase G is a close relative of CPG2 that is readily available.

**Methods:** We isolated PGH from *E coli* cells transformed with a plasmid that expressed high levels of PGH. We then performed kinetic assays on a spectrophotometer to study the rate of enzymatic degradation of a variety of folates and antifolates. The rate of cleavage was monitored using wavelengths and extinction coefficients obtained from the literature (folate [303 nm, 8,000 M-1cm-1]; aminopterin [310 nm, 6,000 M-1cm-1]; methotrexate [320 nm, 8300 M-1cm-1]; 5-formyltetrahydrofolate [312 nm, 5000 M-1cm-1]).

**Results:** Our results indicated that the ability of PGH to cleave glutamate from MTX and aminopterin was lower than that of CPG; in addition, while assay limitations made measurement of Km values, or rates of substrate cleavage, impossible, the Km values for PGH were much higher than those of CPG, as we were unable to saturate PGH for folate and anti-folate substrates. P-aminobenzoylglutamate hydrolase demonstrated no ability to cleave reduced folates, in sharp contrast to CPG.

**Conclusion:** In conclusion, PGH is potentially a useful enzyme in that it would preserve leucovorin and 5- methyltetrahydrofolate while cleaving MTX and aminopterin. However, as a result of its low affinity for the substrates, PGH would need to be genetically modified to increase its efficiency before being considered for use in clinical practice.

#### **♦**B5

Quantitative Analysis of Structural Changes of Growth Cone in *Xenopus laevis* Retinotectal Axonal Navigation James Ha, BS

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**Introduction:** During early stages of the embryo, axonal tracts are required for the proper development of motor and sensory functions. Growth cones, which are the motile tips of growing axons, are responsible for navigating and helping with the formation of synaptic connections. Here, we will focus on mathematical analysis of the shape of outer boundaries of the growth cone and the shape of the juncture between the axon's shaft and the growth cone (axonal wrist). This analysis will help determine possible trends that may affect retinotectal axonal navigation of *Xenopus laevis*.

**Hypothesis:** We hypothesize that growth cones will change shape and structure during axonal navigation through the optic tract of *Xenopus laevis*.

**Methods:** The perimeter and area of the peripheral domain of 2 retinotectal axons were measured from an in vivo time-lapse video of *Xenopus laevis* embryo recorded by Sonia Witte of Cambridge University. Using Image J, the boundaries of the growth cones were traced to determine the area and perimeters. Image J was also used to determine the angle formed at the juncture of the central domain and the axon's shaft.

**Results:** The growth cone's structure and size were variable. The measurements demonstrated no correlation between the perimeter, angle, or area of one growth cone to another, indicating that growth cones changed in sizes independent to one another. However, both axons showed a significant variability of the changes in the size for the growth cones as it progressed toward the

tectum. As the axons reached the tectum, the growth cones showed trends of decreased area, as well as perimeter. The axonal wrists' angles also showed a significant reduction as the axons reached the tectum.

**Conclusion:** The shape of the overall growth cone reflects the behavior of axons and also indicates how proximal they are relative to the tectum. A larger area, perimeter, and angle indicate that the axons are still in earlier development of the optic tracts and require axonal guidance and navigation. Once the axon reaches its destination at the tectum, axonal navigation is no longer required which leads to the reduction of growth cone size. Understanding the variability of axonal growth cone sizes of *Xenopus laevis* allows a better understanding of how axonal navigation for the optic tracts can be regulated through mechanisms that involve adjusting the size of growth cones.

### **B6**

# Role and Expression of Insulin-Like Growth Factor-II (IGF-II) mRNA-Binding Protein-2 in the Nervous System of the Mouse

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**Introduction:** Insulin-like growth factor-II (IGF-II) mRNA-binding protein-2 (IMP2) is 1 of 3 homologues (IMP1-3) that play important roles in the posttranscriptional regulation of gene expression in several tissues. IMP1/ZBP1 (zipcode binding protein) has been shown to facilitate in axon guidance and regeneration by regulating the localization and translation of specific mRNAs. However, the function of IMP2 is least understood, largely because an isoform-specific antibody was not available, which makes the conventional techniques to locate protein expression unfeasible.

**Hypothesis:** It is hypothesized that IMP2 is present in the central and peripheral nervous system throughout all stages of life.

Methods: Primary neuron cultures of dorsal root ganglia were designed to serve as an in vivo model for extensive studies of IMP2 by immunocytochemistry. The dorsal root ganglia were removed from embryonic, postnatal, and adult mice. Tissues from the cerebral cortex, spinal cord, and dorsal root ganglia were used to examine IMP2 by means of Western blot analysis and immunohistochemistry. The tissues were used from embryonic, postnatal, and young adult thy-1-YFP-16 mice.

**Results:** Using a custom-made IMP2-specific antibody, IMP2 expression was observed in the brain, spinal cord, and dorsal root ganglia of the mouse nervous system of the embryonic, postnatal, and adult mice. IMP2 was seen within the cell body and the axons of the cells along with the surrounding supportive neuronal tissues. When examined closer, IMP2 was also present within the growth cones of the neurons.

**Conclusion:** It was found that IMP2 is expressed in both the central and peripheral nervous systems and is developmentally expressed in all stages of life, unlike that of IMP1 and IMP3. Ongoing experiments are aimed at further understanding of IMP2 expression patterns during injury and assessment of its role to facilitate mRNA localization during axon regeneration in the adult nervous system.

## B7

# Impact Injury Progression to Posttraumatic Osteoarthritis in a Female Rat Model

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**Introduction:** Posttraumatic osteoarthritis is a complication of joint injuries. Our long-term goal is to determine the efficacy of osteopathic manipulation and exercise in preventing the onset and advancement of posttraumatic osteoarthritis. Osteoarthritis may set in years or even decades after the initiating injury, so we used a rat model because osteoarthritis progresses more quickly in the rat.

**Hypothesis:** Rats injured with a drop tower device that delivered an impact to the joint have worse osteoarthritis scores than controls.

**Methods:** Experiments were done with the approval of the Animal Care and Use Committee. Because osteoarthritis is more common in women, we used female rats. Impact injury was delivered to anesthetized rats using 90, 120, or 160 g weights (n=10 or 11) and impact force was recorded. Controls received no impact. Rats were sacrificed 4 weeks later. Contralateral and ipsilateral distal femurs were harvested and processed for histology. Transverse sections stained with hematoxylin and eosin were scored from 0 to 5 by 2 blinded investigators. Scoring criteria were chondrocyte density, depth of stain of matrix and chondrocytes, cartilage width, and clustering

or stacking of chondrocytes. The study was powered to detect a difference in score of 1.5 standard deviation with 0.80 power ( $\alpha$ =.05).

**Results:** The greatest weight that delivered an impact without frequently fracturing the femur was 160 g. In the first ~100 slides, 17 were revisited because of divergent scores, and in the next ~150 slides only 10 were revisited, as consistency between scorers improved. More distal sections of the femoral condyles scored worse than proximal sections (P<.0001). Rats impacted with 120 g scored worse than uninjured control rats but the effect was not statistically significant. Unexpectedly, rats impacted with 160 g scored better than any other group (P<.02 vs 120 g). There was no difference in score between contralateral and ipsilateral. Impact force did not correlate with score.

**Conclusion:** We developed a drop tower device and standardized scoring between investigators. Worse osteoarthritis scores on the more distal cartilage may reflect normal patterns of wear. The lack of a consistent effect of impact on score may reflect inadequate time for progression of osteoarthritis, so we are performing a timecourse study.

### **♦**B8

# Differential Effects of Cyanobacterium Anabaena Lipopolysacchride on Brain Microglia Classical and Alternative Activation

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**Introduction:** Although cosmopolitan Gram-negative cyanobacteria may contaminate freshwater by releasing toxins such as lipopolysaccharide (LPS), which may affect environmental and human health, current knowledge of the neuro-

immunotoxicology of cyanobacterial LPS is limited. We recently reported that cyanobacterium Anabaena sp. lipopolysaccharide (AnaLPS) elicited release of superoxide anion, thromboxane B2, and matrix metalloproteinase-9 by neonatal rat microglia (BMG) in vitro (The Toxicologist CD 132 [S-1]).

**Hypothesis:** We hypothesized that BMG treated managed with cyanobacterium AnaLPS in vitro would induce classical and alternative activation cytokines and chemokines.

Methods: AnaLPS was prepared by hot phenol/ water extraction. BMG were isolated and treated with 0.1-100,000 ng/mL AnaLPS for 18 hours at 35.9°C. Escherichia coli (EcLPS) 026:B6 from Difco Lab, Detroit, Michigan, was used as a positive control. Cytokines and chemokines were determined using a Milliplex MAP rat cytokine/ chemokine multiplex immunoassay. Adherence to National Institutes of Health guidelines on the use of experimental animals and protocols was approved by Midwestern University's Research and Animal Care Committee for the isolation of BMG.

**Results:** EcLPS and AnaLPS stimulated BMG to release statistically significant quantities of the following: (a) proinflammatory cytokines: interleukin (IL)-6 >IL-1 $\beta$ >Tumor necrosis factor  $\alpha$ ; (b) pro-inflammatory chemokines: Macrophage inflammatory protein (MIP)-2>MIP-1  $\alpha$ >MCP-1; and the (c) anti-inflammatory cytokine: IL-10, at >1 ng/mL and 10,000 ng/mL, respectively.

**Conclusion:** Although after an 18 hour in vitro treatment AnaLPS stimulated BMG to release cytokines and chemokines involved in classical and alternative activation, AnaLPS was considerably less potent than EcLPS. Our laboratory continues to further characterize AnaLPS chemistry and immunotoxicology.

Acknowledgments and Support: Philip Williams, PhD, from the Department of Chemistry and Biochemistry, University of Hawaii at Manoa, Honolulu, for providing the Ana LPS, and the College of Health Sciences at Midwestern University for funding.

### **♦** B9

# Effect of Insulin on *Staphylococcus aureus* Growth and Biofilm Formation

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**Introduction:** The ability by *Staphylococcus aureus* to form biofilms is essential in its establishment and maintenance of infectious processes (eg, foot ulcers in individuals with type 2 diabetes mellitus). *S aureus* growth and biofilm formation during the course of infection in individuals with uncontrolled type 2 diabetes mellitus occurs in the presence of insulin and glucose. Biofilm formation by *S aureus* is modulated by physiological conditions present in the host including hormone levels and fermentable substrates. Insulin acts as an interkingdom quorum-signaling compound that affects biofilm formation of gram negative bacteria.

**Hypothesis:** Insulin is an interkingdom quorumsignaling compound that affects *S aureus* growth and biofilm formation.

**Methods:** *S aureus* ATCC25923 and global regulator deletion strains agr-, sarA- and agr-sarA-, as well as their parent strain 8325-4 were tested. Strains were grown in Luria-Broth (LB) with and without glucose (0.1% and 0.2%) or insulin ( $2 \mu U/mL$ ,  $20 \mu U/mL$ , and  $200 \mu U/mL$ ). Various media combinations, inoculated with  $10^5$  CFU/mL, were placed in 100 well honeycomb plates ( $200 \mu L/well$ ). Growth curves were measured using the Bioscreen C (GrowthCurves, Inc) (24 hours,  $37^{\circ}$ C, continuous shaking). Biofilm levels, after growth, were determined by measuring the amount of crystal violet staining (Abs580nm).

Results: Overall, addition of glucose (0.1% and 0.2%) resulted in monophasic growth as compared with biphasic growth in medium with and without insulin, regardless of strain tested. In addition, glucose, regardless of concentration tested, suppressed the second phase of growth so that the total levels of growth in the absence of glucose were approximately twice that of LB with glucose. Although insulin had no effect on rate of growth or total amount of growth, for the wild type ATCC isolate, the presence of  $20 \,\mu\text{U}$ / mL and 200 µU/mL insulin increased biofilm formation as compared with medium alone. A similar pattern was observed for strain 8325-4 with respect to insulin effects on growth. However, insulin enhanced biofilm formation in the presence and absence of glucose (0.2%) in a manner that was concentration specific (20 µU/mL and 200 µU/mL insulin, respectively). In the absence of sarA and agr, global regulator genes, there was a marked suppression of biofilm levels in the presence of insulin as compared with media with glucose alone, regardless of sugar and hormone concentrations tested.

**Conclusion:** These findings show that insulin modulates the response of *S aureus* to glucose. In addition, biofilm formation appears to be regulated, in part, by sarA and agr. Taken together, these findings indicate that insulin is an interkingdom quorum-signaling compound in *S aureus* that plays a role in modulating biofilm formation, an essential component in pathogenesis.

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#### ♦B10

# Effects of Urinary Catheter Composition on *Escherichia coli* Biofilm Formation in a Model for Type 2 Diabetes Mellitus

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**Introduction:** Biofilm formation is essential for the survival and growth of *Escherichia coli* in catheter-associated infections. Individuals with type 2 diabetes mellitus have an increased incidence of urinary tract infections. Prediabetic individuals and individuals with poorly controlled or undiagnosed diabetes can excrete insulin, a quorum-signaling compound that induces increased biofilm formation in the presence of glucose. The focus of this study was to determine if the composition of Foley catheter material, which would be used in this patient population, affects biofilm formation by *E coli* in a model system for diabetes.

**Hypothesis:** Urinary catheter composition affects catheter-associated biofilm formation in diabetic patients.

Methods: Rubber (lubricious-coated), siliconcoated, silver-coated, and nitrofurazone-coated catheter segments (5 mm; n=6) were tested. An overnight culture of E coli ATCC25922 (Muller Hinton) was added (104 CFU/mL, final concentration) to artificial urine alone, or with insulin (40  $\mu$ U/mL), glucose (0.1%), or both. After incubation (18 hours, 37°C, in air and anaerobically) the catheter segments were washed extensively in PBS, then stained with crystal violet (3 mL). After removal of excess stain by washing, biofilm-associated crystal violet was eluted with absolute ethanol (3 mL) and quantified spectrophotometrically (Abs550nm). Statistical analysis was done by ANOVA (InStat, GraphPad) with post-hoc analysis (Tukey).

**Results:** With the sole exception of silver-coated catheters grown under aerobic conditions, for all other catheters and growth conditions tested the presence of insulin and glucose significantly (P < .05) increased levels of catheter-associated biofilm by 11% to 30%. The highest level of biofilm was measured for rubber catheter incubated aerobically. Biofilm levels on growth aerobically or anaerobically in the presence of insulin alone or glucose alone were similar to that measured for the media alone control.

**Conclusion:** Regardless of catheter composition, with the sole exception of silver-coated catheters in the presence of oxygen, the combined presence of insulin and glucose enhanced biofilm formation by *E coli*. This finding indicates patient compliance regarding insulin use, and glycemic control should result in fewer complications associated with biofouling of urinary catheters

**Financial Disclosure:** Benny Rummani, OMS III, was supported by the Midwestern University Summer Fellowship Program. Balbina J. Plotkin, PhD, was supported by Midwestern University intramural funds through the Office of Research and Sponsored Programs.

### ♦B11

# Maturation Specific Sensitivity of Osteocytes to Calcium and Phosphate Ion Pair-Induced Apoptosis

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**Introduction:** Our previous work demonstrated the sensitivity of bone cells to microenvironmental apoptogens. As the lifespan of an osteocyte can extend into decades, osteocytic terminal differentiation must necessarily remove such sensitivity. **Hypothesis:** Therefore, the goal of this study was to probe the relationship between osteocyte differentiation state and sensitivity to apoptosis.

**Methods:** Murine Long bone-derived Osteocytes (MLO) A5 and Y4 cell lines, representative of early- and late-stage osteocytes, respectively, were seeded onto well plates, allowed to grow for 2, 7, or 14 days and subsequently exposed to increased levels of the calcium and phosphate ion pair for 24 hours. After exposure, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were performed to quantify cell death. Apoptosis was confirmed through caspase-3 staining. Osteocytic phenotypes were confirmed by alkaline phosphatase staining.

**Results:** Indications that when treated with increased levels of the calcium and phosphate ion pair, the MLO Y4 cell line, representative of late osteocytes, exhibited greater sensitivity to apoptosis than the MLO A5 cell line, representative of early osteocytes.

**Conclusion:** These data suggest that sensitivity to calcium and phosphate-induced apoptosis is a function of maturation level.

**Financial Disclosure:** This work was supported by a grant from the Center for Chronic Disorders of Aging at the Philadelphia College of Osteopathic Medicine.

### ♦B12

## Effects of Pregnancy on Voluntary Wheel-Running Activity in Rats

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**Introduction:** During pregnancy, blood flow to the uterus increases to supply oxygen and nutrients to the developing fetus. To understand how voluntary physical activity (PA) affects the control of uterine blood flow during normal

and compromised pregnancy states, it is necessary to establish the pattern (velocity, duration, and distance) of voluntary PA in rats allowed "24/7" access to a cage running wheel in nonpregnant (NP) state, during gestation, and after parturition.

**Hypothesis:** We hypothesized that voluntary PA would decrease as gestation advanced, be at least partially suppressed during nursing, and rebound after weaning of the pups.

**Methods:** Cage wheel PA was monitored daily with a bike computer (average velocity [m/min], distance [m], and duration [min]). Ten Sprague-Dawley female rats were studied. After a 4- to 6-week (week) run-in period, PA data were collected for 1 week in 10 nonpregnant (NP) rats. Subsequently, 5 rats were bred (pregnant, P), followed through gestation, and 5 rats were followed in parallel as time controls (TC) (3 weeks). In a subgroup of 4 age-matched rats, 2 P rats were followed through nursing (3 weeks), weaning (2 weeks) accompanied by 2 TC rats.

Results: Values are mean (standard deviation [SD]). One-way ANOVA with repeated measures was employed to evaluate group and time effects. NP rats (N=10) averaged 13,977 (8953) m/d, 226 (70) min/d and 58 (24) m/min. Pregnancy resulted in a considerable decrease in run distance (gestational week 3: 669 [96] m/d,  $\Delta 95\%$  [20]) compared with time controls (8568 [2718] m/d,  $\Delta 21\%$  [18], interaction term P < .01), which was primarily a result of a decrease in run time (gestational week 3: P, 17 [3] vs TC, 172 [65] min/d or Δ92% [3] vs  $\Delta 16\%$  [15], interaction term P<.001) with a trend toward a decrease in average velocity (interaction term, P=.07). Decreases in distance (P,  $\Delta 70\%$ [11] vs TC,  $\Delta 18\%$  [18]) and time (P,  $\Delta 68\%$  [11] vs TC,  $\Delta 13\%$  [15]) were evident (P<.001) during the first week of gestation. In a subgroup of 2 rats, suppression of wheel PA continued during 3-week nursing phase, but rebounded toward pregestation levels immediately after pup weaning.

**Conclusion:** We speculate that the immediate decline in voluntary PA with pregnancy in the rat reflects adoption of an energy conservation strategy. Peripheral feedback signals associated with hormonal response to implantation and early fetal development may initially signal the brain to reduce voluntary PA. Additional feedback signals may contribute to reduced PA during nursing.

### ♦B13

## Comparison of the Effects of Halothane and Isoflurane With Ion Channels

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**Introduction:** Volatile anesthetics (VAs) have been used for more than 160 years, yet their mechanism of action on the central nervous system is unknown. Recent evidence suggests that VA effects occur through multiple targets as opposed to a single common mechanism and that the action of VAs on specific ion channels produces general anesthesia.

**Hypothesis:** Because isoflurane and halothane have similar chemical structures with relatively similar pharmacodynamic effects in most animal models, these VAs act through the same ion channels.

**Methods:** We crossed Drosophila melanogaster stocks that possessed a transgenic construct that induces suppression of specific ion channel gene of interest via RNA interference (RNAi) with a stock that causes the expression of this transgenic RNAi construct specifically in the nervous system. Previously, we tested 359 individual ion channel genes within Drosophila melanogaster

in an RNAi screen to determine potential candidates for VA action with isoflurane. Using a previously validated airtight inebriometer and protocol, we placed a minimum of 100 flies into a column of the inebriometer to expose the flies with the suppressed ion channel to 1% isoflurane at a flow of 0.5 L/min. We determined the flies' resistance or sensitivity by evaluating their response to the VA in comparison to the 95% prediction intervals calculated from the results from separate control fly crosses. For this study, the screen was performed a second time using 340 of the original 359 RNAi stocks with 2% halothane as the VA.

**Results:** Of the 340 genes tested, almost 39% produced resistance in the flies, which is very similar to the 40% resistant observed with 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.

**Conclusion:** The expression of many specific ion channel genes being reduced produced resistance to the effects of both VAs. Many of these genes produced resistance in flies to both VAs. However, we rejected the null hypothesis because ion channels that, when silenced, conferred resistance to anesthesia depended on whether the fly was exposed to isoflurane or halothane. Therefore, it appears that similar anesthetics work through both shared and distinct mechanisms.

### ♦B14

PEGylated Liposomal Formulation of ET-B Receptor Agonist, IRL-1620, for the Management of Cerebral Ischemia Puneet Ralhan, DO; Anil Gulati, MD, PhD, FCP; Medha Joshi, PhD

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Introduction: Stroke remains to be a serious and debilitating neurologic disorder, making it the third leading cause of death in the United States. Statistical analysis demonstrates that 87% of all strokes are ischemic in nature, indicating the presence of thrombosis, embolism, or systemic hypoperfusion, which results in reduced oxygen and glucose delivered to the brain. Within minutes of interrupted blood flow, mitochondria are deprived of a substrate, which prevents adenosine triposphate (ATP) generation and results in membrane depolarization. This leads to increased intracellular calcium and sodium concentrations followed by generation of free radicals and initiation of apoptosis. Despite the severity of this condition, the only currently available pharmacological treatment approved by the US Food and Drug Administration for ischemic stroke is recombinant tissue plasminogen activator (rtPA), which dissolves the clot and restores blood flow to the brain. This treatment is complicated by the relatively short window of time between infarct and treatment (3-4 hours) and the increased risk for of subarachnoid hemorrhage. A neuroprotective agent IRL-1620, which is an endothelin receptor B (ETB) agonist has shown promise in previous research aimed at stopping or slowing the secondary damage associated with ischemic cascade after stroke (Leonard et al, 2011). Clinically however, there is a need to improve efficacy of delivery of IRL-1620 to the brain due to the characteristics of the blood brain barrier and the small window of opportunity available for its

responsiveness after a cerebral ischemic incident. Being water soluble, IRL-1620 has less chance of crossing the blood brain barrier.

**Methods:** Liposomal nanocarriers of IRL-1620 that can cross the blood-brain barrier were formulated in this study. PEGylated liposomal nanocarriers were prepared using the dry film evaporation method. The particle size and zeta potential were measured using Zetasizer Nano ZS via 25°C. Liposomes of about 50 nm with polydispersity index (PDI) of 0.14 were prepared with a zeta potential of -7.96 mV indicating stability.

**Results:** The encapsulation efficiency was analyzed with Agilent 1200 HPLC and was found to be 67%. Liposomal uptake into mouse brain endothelial bEND3 cell line was visualized using confocal microscopy with localization of the liposomes mostly in the nucleus. The stability profile of the liposomes showed complete degradation of the encapsulated liposomes by the seventh day. This may be attributed to the low stability of peptides in aqueous solutions. Stability was enhanced in this study by changing from the IRL-1620 made in 5% ammonia solution in water to the preparation in a water solution with neutral pH. Further measures such as freeze drying are being evaluated to improve the stability.

### ♦B15

### Receptor Physiology in the MDH Responsible for the Diving Reflex

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**Introduction:** The mammalian diving reflex is a response to underwater submersion that results in apnea, reduced heart rate, and altered blood flow to maintain perfusion of the heart and brain. This reflex is present in decerebrated ani-

mals, demonstrating that the neural mechanisms responsible for the reflex occur at the level of the brainstem and are not dependent on cortical output. Submergence of the nose stimulates free nerve endings supplied by the anterior ethmoidal nerve (AEN). The AEN then caries the afferent signal to the medullary dorsal horn (MDH). The main goal of our laboratory is to determine how the MDH relays this information to other brainstem locations to initiate the efferent aspects of diving. Prior studies have demonstrated that an intact glutamatergic pathway within the MDH is needed to generate the cardiac response induced by diving. Additionally, ammonia vapor stimulation of the nasal passages in anesthetized animals, which mimics the diving response, activates neurons in MDH. These MDH neurons express glutamate receptor subunits, specifically AMPA receptor subunits GluR2/3.

**Hypothesis:** We hypothesized that voluntary diving in rats would activate neurons within the superficial lamina of the MDH and that these neurons would express GluR2/3 subunits.

**Methods:** Rats were trained to repetitively dive through a 5-m long underwater maze every 5 minutes for 2 hours. Following pentobarbital overdose and left ventricular exsanguination, rats were perfused with 4% paraformaldehyde. Brainstem tissue was cut at 50  $\mu$ m, and serial sections were stained for Fos (as a measure of neuronal activation) and the GluR2/3 subunit.

**Results:** Our results demonstrate significantly greater Fos expression with co-localization of GluR2/3 in the MDH compared with adjacent brain structures (1-way ANOVA; tukey post-hoc, P < .05). Staining between the superficial and deep lamina within the MDH were not statistically different.

**Conclusion:** Our data demonstrate that a significant fraction of MDH neurons containing the AMPA subunit GluR2/3 are activated during diving. The increased activation of the deeper

MDH lamina in conscious voluntarily diving rats, as compared with ammonia stimulation in anesthetized animals, may be due to other stimuli (noise, grooming, tactile information) and additional trigeminal activation due to diving. Future studies will focus on selective receptor activation to elucidate how sensory information is processed and relayed in the diving reflex.

### ♦B16

## Effects of Diet Change on DDE-Induced Immune Alterations

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Introduction: Dichloro-diphenyl-trichloroethane (DDT) is an organopesticide that was used in the United States until 1972, when it was banned because of its toxic effects. Even though DDT was banned years ago, its long-term health effects may still be occurring today. In the body, DDT is broken down into its metabolite, DDE (dichlorodiphenyldichloroethylene), which is fatsoluble and is stored in high amounts in adipose tissue. How DDE affects the immune system is not well understood. This project was designed to better understand DDE-mediated effects on the immune system, and more specifically, how a change in diet may play a role in these changes. Hypothesis: Theoretically, in mice that are fed a high-fat diet, more DDE may be stored as a result of the higher amount of adipose tissue. If these mice then switch from a high-fat diet and start a diet much lower in fat, the amount of adipose tissue will decrease over time. We predict that when this happens, the DDE originally stored in fat will release into the blood, which may exacerbate DDE-mediated effects on the immune system.

 Abstract entered in the SOMA Student Poster Competition. **Research Design:** Mice were fed either a high-fat diet (60% kcal from fat) or a regular rodent diet (10% kcal from fat) for 3 months. The mice then

received either DDE or a cottonseed control at a dose of 200 mg/kg. After dosing, all of the mice returned to the regular rodent chow diet for 2 more months of feeding.

Methods: Mice were sacrificed and immune cell subpopulations were quantified using flow cytometry. CD3+, CD4+, and CD8+ populations in the spleen and the thymus were measured. Results: Regardless of treatment, mice fed the high-fat diet had a significantly lower number of CD3+ thymocytes, the most mature thymocytes, compared to mice fed the regular diet (P=.0326). Also, regardless of diet, treatment with DDE significantly decreased the number of T-Helper CD4+ splenocytes compared to the mice treated with cottonseed oil (P=.0167). Interestingly, in the mice that were fed the high-fat diet, DDE significantly increased the number of Cytotoxic CD8 + splenocytes (P = .040). Lastly, in the group of mice that received the cottonseed vehicle, the mice that were initially fed the high-fat diet displayed a trending decrease in the amount of CD8 + splenocytes (P = .061).

**Conclusion:** Dichlorodiphenyldichloroethylene does mediate effects on the immune system, specifically on CD3+, CD4+, and CD8+ populations, and these changes may be influenced by mobilization of DDE following weight loss.

### ♦B17

# Attachment of 2 Antibiotics to a Titanium Alloy Surface to Inhibit Colonization by Gram-negative and Gram-positive Bacteria

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**Introduction:** Despite immense improvements in anti-infective measures and implant technology, periprosthetic infection (PPI) remains a major complication for individuals with percutaneous prosthetic attachment. While various methods and materials have been developed to counter infection, these methods are often ineffectual. To combat PPI in orthopaedic applications, titanium (Ti) alloy implants tethered with antibiotics active against both gram-negative and grampositive organisms have been developed separately. However, as a percutaneous implant will be exposed to a highly variable soup of infective organisms, a broad-based surface must be created. Thus, it is the goal of this study to develop methods to bond 2 separate antibiotics (Vancomycin, active against gram-positive organisms, and Tetracycline, active against gram-negative organisms) to a single surface.

**Hypothesis:** Equal concentrations of Vancomycin and Tetracycline covalently bonded to a Ti surface are stable and active against *S aureus* and *E coli* colonization in vitro.

**Methods:** Vancomycin and Tetracycline were covalently bound to Ti separately (Van-Ti and Tet-Ti) and together (V/T-Ti) using a novel N-Hydroxysuccinimide (NHS) synthesis scheme. Distribution of the surface-bound antibiotics was detected by immunofluorescence and confocal microscopy. Stability and activity were evaluated after incubation in *S aureus* and *E coli* cultures for 1 day. The effects of Van-Ti, V/T-Ti, and Tet-Ti on bacterial adhesion and colonization were measured using scanning electron microscopy, fluorescently labeled bacteria evaluated by confocal microscopy and by direct colony counting of adherent bacteria.

**Results:** Immunofluorescence showed attachment of Vancomycin and Tetracycline to Ti. Scanning electron microscopy showed adherent bacterial colonies on control surfaces that were absent on Van-Ti. Live/dead staining of adherent bacteria showed that *S aureus* was inhibited from colonizing Van-Ti and V/T-Ti, while *E coli* were inhibited from colonizing Tet-Ti and V/T-Ti. These results were confirmed by colony counts. **Conclusions:** Both Vancomycin and Tetracycline can be covalently tethered to the surface of Ti. Vancomycin effectively prevents *S aureus* colonization while Tetracycline effectively prevents *E coli* colonization. The combined surface prevented colonization by both species.

### ♦B18

# Changes in the Blood Titers of Specific Autoantibodies Associated With Aging and Disease Are Consistent With Their Function as a Debris-Clearing Mechanism

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**Introduction:** Our previous studies have demonstrated the presence of a large number of autoantibodies in the blood.

**Hypothesis:** Here, we test the hypothesis that changes in the blood titers of individual autoantibodies seen in patients with a specific disease reflects the role of these autoantibodies in the clearance of cell and tissue debris generated by the disease.

**Methods:** To assess the complexity of autoantibody profiles, we used human protein microarrays, each containing 9486 human protein antigens. Arrays were probed with individual human serum samples and immediately scanned with a GenePix 4000B Fluorescence Scanner. For microarray analysis, fluorescence data were acquired by Genepix Pro analysis software and imported into Prospector 5. Analysis included background subtraction, outlier detection and calculation of z factor to define positive signals. Cross-group comparisons of the number of autoantibodies were done using the Student t test.

**Results:** To determine the relative abundance and titers of autoantibodies in the blood, protein microarrays were probed with human sera obtained from subjects of each gender from a variety of ages and health backgrounds. Thousands of autoantibodies were consistently detected in all sera, and increasing age was accompanied by a corresponding and nearly linear increase in the total number of detectable autoantibodies. By contrast, the presence of specific diseases (Alzheimer disease, Parkinson disease, and multiple sclerosis) was consistently associated with a reduction in the number of detectable autoantibodies compared with controls. The identities of some of the autoantibodies associated with these diseases are consistent with the concept that they are involved in the clearance of cell and tissue debris.

**Conclusion:** Here, we confirm that thousands of IgG autoantibodies are typically present in the blood, regardless of age, gender, or the presence of disease. This abundance and ubiquity of IgG autoantibodies in the blood imply that they perform a key physiological function. Data presented here on the identity of individual autoantibodies support their function as an adaptive mechanism to clear intercellular debris, a function that is naturally enhanced in the presence of disease. Supporting this concept, our previous studies have shown that it is possible to identify and make use of disease-induced changes in autoantibody profiles as diagnostic biomarkers. **Support:** Osteopathic Heritage Foundation.

#### B19

# Two-Hit Mechanism for Neurodegenerative Diseases: Brain-Reactive Autoantibodies and Blood-Brain Barrier Permeability

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**Introduction:** Our previous studies have consistently shown immunoglobulin G (IgG) bound selectively to certain types of neurons in regions of pathology in Alzheimer disease (AD) and Parkinson disease (PD) brains. Access of IgG to neurons implies loss of blood-brain barrier (BBB) integrity and an influx of plasma into the brain.

**Hypothesis:** Here, we propose a 2-hit hypothesis whereby brain-reactive autoantibodies in the blood and BBB breakdown are required as a common trigger for a number of different neurodegenerative diseases in the elderly.

Methods: Immunohistochemistry was used on AD and PD brains to determine the location and extent of BBB breakdown relative to sites of evolving pathology and to test for the presence and binding potency of brain-reactive autoantibodies in the blood. Western blot analysis was used to test for the presence of multiple brainreactive autoantibodies in sera from AD and PD patients and corresponding controls. Human protein microarrays were used to estimate the number of total autoantibodies in each serum sample.

**Results:** Immunohistochemistry revealed abundant IgG-positive neurons in brain regions targeted by AD and PD pathology. Most brain-reactive autoantibodies showed selective affinity for certain types of neurons, particularly pyramidal neurons in the cerebral cortex and hippocampus, brain regions that are particularly vulnerable to AD pathology. Roughly 1 in 10 sera also showed IgG binding to glia. Individual sera showed wide variations in potency in terms of the intensity of immunolabeling of neurons and glia in postmortem human brain. These same sera showed corresponding potencies with regard to their binding to living neurons in mouse brain hippocampal brain slice cultures. Western analysis confirmed the presence of brain-reactive autoantibodies in both sera from both AD and PD subjects.

**Conclusion:** Results suggest that chronic BBB breakdown and brain-reactive autoantibodies trigger a pathological cascade in AD and PD brains in which the latter leak into the brain tissue, bind to neurons and induce neuropathology. We propose that the location of this event and the specific type of autoantibody defines the type of neurodegenerative disease that will be manifested. These results point to the BBB as a key therapeutic target for prevention of the initiation and progression of neurodegenerative diseases such as AD and PD. Supported by the Osteopathic Heritage Foundation and the Michael J. Fox Foundation.

### ♦B20

# Extrachromosomal Circular DNA as the Basis for Human Aging

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**Introduction:** Chromosomal telomere-shortening and deficits in immune function are 2 well-established components linked to old-age morbidity and mortality. However, the ultimate cause for the inevitability of aging has yet to be identified. Nevertheless, a demanding growing older population in the United States has made it difficult to ignore the issue of whether biomedical interventions to postpone aging are scientifically feasible. Studies of yeast, nematodes, and flies have provided basic mechanistic principles of human aging. For instance, in saccharomyces cerevisiae (yeast), age and fecundity are linked to the nuclear accumulation of highly repetitive extrachromosomal circular ribosomal DNA (eccrDNA). Since much of basic biology is similar across species, we have studied this programmed phenomenon using human 5S and 45S eccrDNA cells under various experimental conditions.

Hypothesis: We hypothesize that aging in humans is fundamentally regulated by intrinsic mechanisms that involve chromosomal excision and replicative accumulation of eccrDNA. This working hypothesis could provide an important avenue for the identification of new drug targets and treatment strategies to increase human health and lifespan.

Methods: For an anti-aging effect,  $50 \mu$ M of resveratrol was applied to cultured human cells for 12 or 48 hours, respectively. Nuclear transport of eccrDNA was blocked using wheat germ agglutinin (WGA) at 0.1 mg/mL for 12 hours. Following treatment, cells were divided into cellular and nuclear fractions from which eccrDNA was purified according to well-validated protocols. Samples were assessed for 5S and 45S eccrDNA using appropriate DNA primers. One-way or 2-way ANOVA were used to determine differences among multiple means. Null hypotheses were rejected at the .05 level.

**Results:** We found that 5S, but not 45S eccrDNA, was able to toggle between nuclear and cytoplasmic subcellular compartments. Resveratrol treatment significantly increased cytoplasmic and concomitantly decreased nuclear 5S eccrDNA (n=20; P=.05). This effect was blocked with WGA.

**Conclusion:** Here, we successfully identified an eccrDNA-based mechanism that is evolutionary conserved across species as divergent as yeasts

and humans. This mechanism responds to antiaging treatment with resveratrol and it is therefore a regulatory pathway most likely involved in the cause of intrinsic aging.

### ♦B21

# Extrachromosomal Micro-DNA as Biomarkers for Human Disease

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Introduction: Maintenance of chromosomal integrity and stability is vital to the survival of an organism. The formation of extrachromosomal circular DNA (eccDNA) from repetitive genomic cycles appears to disrupt chromosomal integrity as they may give rise to genetic mosaicism and possibly cancer or apoptosis. Nevertheless, this mechanism is common to all eukaryotes, including humans. To better understand the role of eccDNAs and to determine their suitability as DNA biomarkers for pathologies, we have developed a screening technique allowing us to decipher genome-wide distribution of human eccDNA species.

**Hypothesis:** We hypothesize that human cells and tissues accumulate eccDNA reflective of a state of cellular health. This working hypothesis could provide an important avenue for the establishment of biological DNA biomarkers for the early detection of human pathologies.

**Methods:** All studies were carried out in human HEK-293 cells maintained under standard tissue culture conditions. Cells were collected and sequentially treated to remove linear DNA, RNA and protein before eccDNA purification. For pattern analysis, eccDNA samples were linearized with sonication, PCR amplified, cloned into a DNA vector, sequenced and analyzed with the repetitive sequence screening tool CENSOR (http://www.girinst.org/censor/index.php).

Results: The average insert size of 48 DNA clones analyzed was 636bp. Only 3 sequences were derived from tandemly arranged gene clusters which are known to be involved in eccDNA generation. Of the remaining 45 clones, the majority of 30 clones (66.7%) reflected intergenic DNA and 12 (26.7%) matched intron/regulatory region boundaries. These 42 clones incorporated various repetitive elements such as class-I retrotransposons or class-II nonautonomous DNA transposons. The remaining 3 sequences (6.7%) mapped to gene exons/intron boundaries and did not include any repetitive elements at all. These 3 eccDNA sequences resemble the recently discovered class of microDNAs known to give rise to genetic mosaicism.

**Conclusion:** Here, we have successfully demonstrated a novel technique facilitating genomewide analysis of eccDNA in human cells and tissues. Future applications of this technique will analyze the eccDNA content in certain pathological states and will test its feasibility as a biological biomarker for disease onset and risk.

### ♦ B22

## Stress-Induced Expression of Virulent Genes in *Salmonella* Typhimurium Prophages Fels1 and Gifsy2

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**Introduction:** Virulence of a pathogen is defined by the production of virulence factors involved in the disease process. These factors are products of expression of virulent genes. In *Salmonella* Typhimurium most of these genes are found in chromosomal pathogenicity islands and some are on mobile elements such as prophages. Pro-

phages are bacteriophage genomes inserted in the bacterial genome in a quiescent state. They are believed to play a major role in the evolution of infectious diseases by their ability to enhance bacterial virulence. Fels1 and Gifsy2 are both *Salmonella* prophages known to carry virulence genes.

**Hypothesis:** In this study, we measured the level of expression of prophages virulence genes to evaluate their role in the overall *Salmonella* virulence. Our hypothesis is that virulence is enhanced only when the prophage is induced and that each prophage will increase bacterial virulence independently to each other and only under certain conditions.

**Methods:** We compared expression of virulent vs viral genes during *S* Typhimurium growth under different conditions: temperature, media composition, competition, growth phase, pH, oxidative stress, and oxygenation. For each condition, gene expression was quantified after RNA extraction using Real Time PCR (polymerase chain reaction). The results are presented as fold difference compared with control growth conditions (37°C, rich media).

**Results:** Gifsy1 viral and virulent genes, STM1006 (excisionase) and STM1044 (superoxide dismutase), both show no or small changes in expression when exposed to the tested growth conditions with a 2-fold maximal increase during stationary phase and MitomycinC treatment. Fels1 viral and virulent genes, STM916 (tail protein) and STM924 (superoxide dismutase), both show significant increases in expression during MitomycinC and H2O2 treatment with 6.94- and 3.40-fold increase for STM 916, and 9.52- and 2.48-fold increase for STM924 respectively.

**Conclusion:** These data suggest that virulence genes carried on *Salmonella* prophages are under the same regulation mechanisms as the viral genes. Therefore, a prophage like Gifsy2 that shows spontaneous induction in control conditions and the same level in gene expression when exposed to stressors, will slightly but constantly contribute to *Salmonella* virulence. Conversely, a prophage-like Fels1 that shows no induction in control conditions but a strong response when exposed to specific environmental stressors can make the pathogen temporarily but significantly more virulent.

### ♦B23

## Maturation of Osteoblast-Like Cells Sensitizes Them to Calcium and Phosphate Ion Pair-Induced Apoptosis

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**Introduction:** Our previous work demonstrated the sensitivity of osteoblasts to the presence of microenvironmental apoptogens.

**Hypothesis:** As osteoblasts change their functional orientation with maturation, it follows that the sensitivity of these cells to apoptosis must change as well. It is the goal of this study to exam the relationship between the maturation of osteoblasts and the susceptibility of these cells to apoptosis.

**Methods:** For this study the Human Fetal Osteoblast (hFOB) cell line was used. These cells exhibit an early osteoblastic phenotype when cultured at 34°C while above 37°C, the cells mature. Cells were cultured for 2, 7, and 14 days. Phenotypic changes were confirmed. Cells cultured at both 34°C and 39.5°C were exposed to increasing levels of the calcium and phosphate ion pair. Percentages of viable cells were measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Apoptosis was confirmed by evaluating Caspase-3 activation.

**Results:** Results indicated that hFOB cells incubated at 39.5°C exhibited a greater sensitivity to the activation of apoptosis by the calcium and phosphate ion pair.

**Conclusion:** These results suggest that with maturation, osteoblasts become more sensitive to the activation of apoptosis.

**Financial Disclosure:** This work was supported by a grant from the Center for Chronic Disorders of Aging at the Philadelphia College of Osteopathic Medicine.

# Medical Education

### ♦ME1

# Portfolios in Osteopathic Medical Education: A Qualitative Pilot Study

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**Introduction:** Portfolios were used originally in art-based disciplines to collect examples of work for assessment purposes or to present to prospective employers. In medical education, portfolios are used as an assessment tool, as a record of achievements, and as evidence of continuous professional development.

**Hypothesis:** We are evaluating whether the portfolio folders we selected facilitate meaningful reflection, promote growth, and adequately reflect acquisition of new knowledge and skills. We want to assess feasibility and which of the folders require more attention and assistance for students to provide meaningful quality representative artifacts.

**Methods:** We recruited 23 second-year students at Touro University College of Osteopathic Medicine and provided each a portfolio with 10 labeled folders. Folder titles' were selected by student coordinators with guidance from the faculty research advisor. Participants were instructed to collect at least 2 artifacts in each folder representing related activities during the fall semester 2012. Participants completed a survey before the project started and another at the end and met with their assigned tutors at least twice during the semester. Portfolios were reviewed by the student coordinators and their faculty research advisor using a grading rubric to evaluate reflection, quality, and number of artifacts.

**Results:** Eighteen of our 23 participants completed the project. Folders that received the highest scores were Leadership/Service and Professional Development. Lowest scores were seen in Critical Thinking, Patient Presentations, Clinical Competencies, and Professionalism. Lack of meaningful reflection was the criteria most negatively impacting these folders and the criteria most commonly lacking in all areas.

**Conclusion:** In the development of a medical portfolio system, additional focus should be aimed at helping students gather meaningful artifacts in all areas. Lower scoring folders may indicate a particular lack of understanding of the folder or a difficulty in attaining meaningful artifacts that fulfill the folder criteria. To address these issues, future portfolio systems could ensure thorough training in identifying artifacts, provide templates for reviewing scientific articles, allow access to concrete faculty feedback on clinical skills, or provide a standard review on professionalism for each student.

# Health Policy

### ♦ HP1

# HIV/AIDS Care Delivery in the Dominican Republic— The Need for Capacity Building

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**Introduction:** The United Nations estimates that the human immunodeficiency virus (HIV) and AIDS prevalence in the Caribbean is the second highest in the world after Sub-Saharan Africa. Seventy-five percent of these cases reside in the Dominican Republic and Haiti. The epidemic seen in the country reflects a complex interplay of factors such as socioeconomic factors, health disparities, unsafe sexual practices, lack of health education, and geographic proximity to Haiti. The prevalence of HIV/AIDS in Haiti is almost 3 times that of the Dominican Republic.

**Hypothesis:** Identification of gaps and organizational capacity building would contribute to the impact of HIV care delivery implementation.

Methods: In this study, we designed questionnaires that were used for 52 HIV outpatients and 15 health care providers. The objectives of such questionnaires were to identify barriers and obstacles perceived by various healthcare professionals in the delivery of HIV/AIDS medical care. The study was conducted at the Boca Chica Clinic located in the Dominican Republic. Boca Chica is known in the country for having the highest prevalence of HIV/AIDS in the Dominican Republic primarily because of sex workers and drug abuse.

**Results:** Our results indicate that lack of transportation, the number of clinic service hours, and long waiting time were some of the urgent issues that patients were confronted with. Conversely, our data show that in the same clinic, only 43% of

providers were aware of national HIV programs aimed at collecting HIV data and 14% were not aware of the existence of these programs at all. Even though the remaining 43% of providers were aware, they lacked knowledge of how these programs worked and the services they provided. Conclusion: We concluded that geographic distances, lack of resources, and limited amount of hours of operation offered by the clinic were some of the key capacity-building issues. Furthermore, our observation indicates lack of effective implementation programs add to the complexity of HIV care services in the community of Boca Chica. Our study warrants a more robust national policy and implementation in the Dominican Republic to enhance capacitybuilding in HIV/AIDS care delivery.

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