



Editor's Note: Corrections to this article were published in the January 2011 issue of *JAOA—The Journal of the American Osteopathic Association* (2011;111[1]:3). The corrections have been incorporated in this online version of the article, which was posted January 2011. An explanation of these changes is available at <http://www.jaoa.org/cgi/content/full/111/1/3>.

54th Annual AOA Research Conference— 2010 Abstracts

This issue of JAOA—The Journal of the American Osteopathic Association features abstracts from the posters that will be presented at the 54th Annual 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 six groups:

- series F—fellowships (see below)
- series P—osteopathic manipulative medicine/osteopathic principles and practice (see page 447)
- series C—clinical studies (see page 452)
- series B—basic sciences (see page 459)
- series ME—medical education (see page 479)
- series HP—health policy (see page 483)

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 assume responsibility for the abstracts' content.

This year's AOA Research Conference, "Emerging Research Themes for Osteopathic Clinicians and Scientists in the Early 21st Century," will take place in San Francisco, California, from Sunday, October 24, to Tuesday, October 26, during the AOA's 115th Annual Osteopathic Medical Conference and Exposition (OMED 2010), "The Bridge to OpportUNITY."

For more information on the AOA Research Conference or other programs taking place during OMED 2010, access the conference's Web site at <http://www.omedconference.org>. The AOA Research Conference program can be accessed by selecting "Programs & Information" on the drop-down navigation bar and selecting "Non-specialty Affiliates." Information about the conference is also available through DO-Online (<http://www.do-online.org>).

AOA Research Fellowship

◆ F1

Assessment of Anterior Superior Iliac Spine Asymmetry by Osteopathic Predoctoral Fellows and Osteopathic Physicians

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Purpose: To evaluate accuracy and interexaminer reliability of anterior superior iliac spine (ASIS) positional asymmetry assessment in a pelvic model and to investigate the degree of accuracy seen with varying amounts of ASIS asymmetry when evaluated from different sides of the model.

Methods: Five osteopathic physicians and 5 osteopathic predoctoral fellows evaluated ASIS asymmetry of 13 randomized settings on a pelvic model first from the right side and then from the left on 2 separate days. Assessment occurred in pairs for physicians and a group of 5 for fellows. One minute was allowed for evaluation of each setting.

Results: Physicians and fellows were more accurate from the right side of the model ($\kappa=0.56$ and $\kappa=0.52$, respectively). Average interexaminer reliability was greatest from the left side of the model for physicians and right side for fellows (physicians $\kappa=0.46$; fellows $\kappa=0.37$, respectively).

Conclusions: The development of a novel, pelvic model allowed assessment of interexaminer reliability and accuracy of ASIS asymmetry assessment. As the amount of asymmetry increased, accuracy of assessment increased from each side of the model. Interexaminer reliability reached values that can be considered clinically significant in the physician group; however, the amounts of asymmetry in the methodology may be higher than amounts of asymmetry observed in clinical practice. Further experimentation is needed to explore the use of a model when developing skills of bony anatomic landmark asymmetry assessment.

(continued)

◆ Indicates posters entered in the AOA Council on Research's Student Poster Competition, a judged event that takes place during the poster session at the Research Conference.

◆ F2

Short-Term Starvation and Human Fibroblast Contractility

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Hypothesis: Fibroblasts, the chief cells found in connective tissue, respond to mechanical forces through changes in their shape and structure via unknown signaling cascades. Given that connective tissue is affected by mechanical forces, such as those used in osteopathic manipulative treatment (OMT), it is important to determine how the microenvironment of the fibroblast influences cellular biomechanical events. Short-term starvation is a well documented strategy for extending the lifespan and reducing the burden of age-related disease in a variety of species. Current research shows that short-term starvation also plays integral roles in the biophysical properties of cells, such as in the cytoskeletal system. Short-term starvation has been shown to modify these changes through a NAD-dependent protein deacetylase pathway, Sirtuin 1 (SIRT1). We hypothesize therefore that caloric restriction through SIRT1-signaling events would modify human fibroblast morphology, differentiation and contractility.

Materials and Methods: Human dermal fibroblasts (HDF) were exposed to a standard (4.5 g/L), a low glucose (1 g/L) or a very low glucose (0.5 g/L) Dulbecco's modified eagle's medium supplemented with 1% serum and a 1% antibiotic/antimycotic solution. Cells were exposed to an acute (3 days) or chronic (7 days) starvation period. Cells were counted at 48-hour intervals and expression analysis of gene activity was performed using quantitative PCR using specific human SIRT1 primers. All data were analyzed using Student's *t* tests, with a *P* value of <.05.

Results: HDFs exposed to a diet of low serum glucose (1 g/L or 0.5 g/L) showed a significant augmentation of SIRT1 levels (~2× increase; *P*<.05) when compared control (4.5 g/L). Further, we found that the total number of differentiated HDF cells was also highest in experimental groups fed a low serum glucose diet (0.5 g/L) than control groups (4.5 g/L and 1 g/L; *P*<.05).

Conclusion: These initial experiments support our working hypothesis that short-term starvation increases SIRT1 activity, as well as cellular differentiation in HDF cells. We are now prepared to assess whether short-term starvation can alter the biomechanical properties of fibroblast cells, a novel area of interest that could play an integral role in the development and treatment of somatic pathology. Nutritional status and fascial contractility could be a fresh perspective for appreciating the role of OMT at the cellular level.

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◆ F3

The Link Between Osteopathic Intervention, Endocannabinoids, and Developmental Epilepsy

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Background: Endocannabinoids have been shown to protect the brain from a variety of neuropathogenic insults possibly by reducing excitation. Elevated levels of endocannabinoids can attenuate seizure susceptibility and severity in mature animals. Moreover, seizures in adults cause redistribution of CB1 receptor expression within the hippocampus. Interestingly, osteopathic manipulative treatment (OMT) can elevate serum endocannabinoid levels and this may benefit individuals with seizures.

Hypothesis: Because the incidence of seizures is highest in the immature brain, we hypothesized that OMT may (I) raise the seizure threshold and decrease seizure severity in the immature brain, (ii) raise endocannabinoid levels of the immature hippocampus after a single episode of status epilepticus and (iii) alter CB1 receptor expression in the hippocampus after a single episode of status epilepticus.

Methods and Materials: Modified myofascial techniques were performed on male Sprague Dawley rats twice daily from postnatal day (P) 9 to P16 and once on P17 before sacrifice. Sham animals were handled for the same length and duration of time. The rat pups were injected with kainic acid (KA) on P13 to induce status epilepticus. A modified seizure scoring method was used to assess seizure severity. Synaptosomal uptake was used to measure endocannabinoid activity in the hippocampus post status epilepticus and OMT. Immunohistochemistry was used to determine CB1 receptor distribution and expression in the hippocampus.

Results: Animals that received OMT+KA proved to have significantly lower seizure scores compared to sham+KA and KA-only animals. Animals that received OMT+KA showed significantly higher levels of AEA in the hippocampus compared to the rest of the groups. All KA animals, (OMT+KA, sham+KA, and KA only) showed to have increased expression of CB1 receptor in the CA1 region of hippocampus and dorsal plate of the dentate gyrus compared to the control animals (OMT and sham-only groups).

Conclusion: The results from this study may be a stepping-stone in understanding the beneficial effects of OMT on the brain, particularly in young patients with seizures. We anticipate that OMT will eventually be used as a neuroprotective adjunctive treatment to decrease seizure susceptibility and severity by enhancing the endocannabinoid system and consequently reducing the need for pharmacological agents in epilepsy patients.

Osteopathic Manipulative Medicine/Osteopathic Principles and Practice

◆ P1

The Use of Osteopathic Manipulative Treatment in the Enhancement of Pulmonary Function in an Impoverished Urban Sector of Duran, Ecuador

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Background: A retrospective chart review approved by the Philadelphia College of Osteopathic Medicine was conducted in two Urban Clinics in Duran, Ecuador.

Hypothesis: Given the association of environmental risk factors and frequency of pulmonary illnesses and dysfunctions; we hypothesize that a single OMT treatment would improve pulmonary function tests (PFT), decrease secondary somatic changes, and enhance patient's assessment of breathing quality.

Materials and Methods: Patients received a history and physical with documentation of host and environmental risk factors. A predetermined grading system was used to risk-classify patient records based on quantifying somatic dysfunction (SD) and altered PFT. Patients received standardized osteopathic structural examinations, SD was recorded, and a standard OMT protocol focusing on pulmonary homeostatic support was implemented.

Results: A total of 21 charts met the inclusion criteria for this study. All patients with pulmonary complaints, including those with normal PFT, demonstrated some level of SD corresponding to pulmonary dysfunction (PD) including: pulmonary Chapman's points at intercostal spaces 2-4, facilitated segments (T1-T6), phrenic nerve-related sites (C3-C5) and disruptions in the Zink "respiratory-circulatory" alternating fascial pattern. The majority of these presented with a cough or shortness of breath. The highest-risk patients had the greatest burden of SD. Although the chart review did not indicate a direct relationship between restoration of the Zink pattern and improved PFT, the Zink pattern normalized in those with improved PFTs. Significant subjective symptom improvement was noted in 20 of 21 patients. Five of 16 patients demonstrated significant improvement in post-OMT PFT values.

Conclusion: Our results suggest that OMT involving removing lymphatic flow obstructions, in conjunction with autonomic balancing, biomechanical restoration and lymphatic treatments may have a role in improving environmentally related PD. It also appears that restrictive processes respond best to this OMT protocol as demonstrated by improved post OMT PFT values in this subset. An initial decrease in FEV₁ was noted when OMT was used for acute PD along with chronic obstructive processes, but more tests

are needed to determine if the drop in FEV₁ is temporary or due to fatigue rather than physiological change.

◆ P2

Chapman's Points: A Histological Analysis

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Hypothesis: A Chapman's Reflex Point is an area in tissue that possesses an increased density of neural, lymphatic and vascular tissue as compared to adjacent tissue.

Materials and Methods: Four Chapman's points (two anterior and two posterior) of the right and left lung were marked on eight cadavers by a board-certified practicing osteopathic physician. Anterior and posterior control points were also collected from adjacent tissue. The marked points were then reviewed and retrieved by a second Osteopathic Physician using a 3-mm width cork borer. The cork borer yielded core samples of skin down to the periosteum. The samples were then longitudinally sectioned and stained with hematoxylin and eosin according to standard histological techniques. S-100 immunohistochemistry was performed to identify neural tissue, CD 34 and Factor VIII were used to identify vasculature. The resulting slides were read by a board certified pathologist who noted the organization and quantity of neural, lymphatic, and vascular structures. Anterior and posterior samples were combined into 2 groups; control points (n=17) and test points (n=34) (Chapman's points). Statistical analysis was performed in 3 separate groups (lymphatics, nerves and vessels) using a 2-tailed, 2-sample *t* test with a confidence level set at 95%.

Results: At the specified sample size and level of confidence, no statistically significant anatomic difference was noted when comparing the two groups regarding vasculature ($P=.874$), lymphatics ($P=.769$), and nerves ($P=.947$). As a result of the lack of significant evidence, examiners in this study failed to reject the null hypothesis.

Conclusion: These new data not only limit the description of the points but also suggest that some of the existing theories attempting to explain the formation of these reflex points may be incomplete or invalid. However, these results do not disprove the existence of Chapman's points. Rather, they serve to highlight the need for further investigation into the pathophysiological mechanism of Chapman's reflex points.

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◆ P3

A Study of 80 Healthy Subjects: Can OMM Effects and Outcomes Be Measured Objectively Using Pulmonary Function Tests?

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Hypothesis: Osteopathic manipulative medicine (OMM) applied to the thoracic cage and diaphragm has empirically been used to improve chest excursion and breathing capability. However, research has been sparse in showing quantifiable measurements of therapeutic effect. The authors believe that using pulmonary function testing quantifiable measurements of therapeutic effect can be shown. This study investigates the effects of selected OMM techniques on the respiratory system using pulmonary function tests (PFTs).

Methods: Study subjects were involved in a randomized control, pretest, posttest crossover design requiring one sham visit and one treatment visit. During each visit, PFTs were performed by a blinded technician before and after either treatment or sham. The treatment visit included treatment with 5 OMM techniques: seated thoracic spine articular technique, seated rib articular technique, anterior abdominal diaphragm doming, thoracic outlet release via balanced ligamentous tension (BLT), and twelfth rib release via BLT. The PFT values measured were forced vital capacity (FVC), forced expiratory volume in 1 second (FEV₁), FEV₁/FVC, peak expiratory flow, and forced expiratory flow 25% to 75%.

Results: Eighty subjects met the inclusion criteria of the study. Twelve subjects were lost to follow up. For all PFT values measured, pretreatment was not significantly different from post treatment. Effect size was small ranging from 0.065 to 0.264. For all PFT values, posttreatment was not significantly different from post-sham. Effect size was small ranging from 0.008 to 0.202.

Conclusion: The 5 OMM techniques applied to healthy subjects did not result in an observable change in PFTs. This is an expected finding as the subjects had no known pathology. Yet, the techniques may offer some measurable change in PFTs of patients with respiratory disease. This study provides a basis for the exploration of the effect of OMM techniques on the PFTs of patients with known pathology. If there is an effect, the conclusions would be more powerful given that changes were non-existent in the healthy cohort. The authors intend to explore the new hypothesis that this study has generated: OMM techniques applied to the thoracic cage and

diaphragm of asthmatics provide a therapeutic effect that can be measured as changes in PFTs. Should this hypothesis prove true, it would provide a rationale for the use of OMM techniques as an adjunct to current asthma management.

◆ P4

Sympathetic Autonomic Exacerbation of Phantom Limb Pain: A Pilot Study

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Rationale: Somatic dysfunction exaggerates efferent sympathetic discharges by lowering action potential thresholds at the spinal cord via facilitated discs. Acute somatic dysfunction and exaggerated sympathetic efferents present with epidermal rubor, edema and increased limb temperature. Chronic somatic dysfunction and exaggerated sympathetic efferents present with paresthesia, contracture, epidermal pallor, and decreased temperature. Varied sympathetic nervous system regulation after amputation has been implicated in phantom limb pain. Somatic dysfunction exaggerating efferent sympathetic discharges may thus contribute to phantom limb pain.

Hypothesis: Signs of exaggerated sympathetic discharge will be higher in amputees with phantom limb pain vs amputees with no phantom limb pain.

Participants: Twenty-four amputees were included in the study. Thirteen subjects (54.2%) reported phantom limb pain. Eleven subjects (45.8%) with no phantom limb pain were controls. Amputation site distribution included: 7 right LE, 8 left LE, 1 right UE, 2 left UE, 4 bilateral LE, and 2 quadrilateral extremity. Average time since amputation was 13.6 years.

Materials and Methods: The study protocol was approved by the Northwestern University Medical School Institutional Review Board. A brief physical examination compared residual and whole limb tactile temperature, edema, skin color, sensation, and motor tone. Significance was determined by paired *t* tests.

Results: Phantom limb pain subjects had significantly greater residual limb epidermal rubor ($t=10$, $df=20$, $P=0$) and residual limb edema ($t=4.18$, $df=20$, $P=.0002$) versus controls. There was no significant difference between phantom limb pain subject incidence of epidermal pallor at the residual limb ($t=1$, $df=20$, $P=.16$); residual limb hypoesthesia ($t=1.26$, $df=20$, $P=.11$); cooler tactile skin temperature at the residual limb ($t=0.439$, $df=20$, $P=.332$); warmer tactile skin temperature at the residual limb, ($t=2$, $df=20$, $P=.0296$); or contracture ($t=0.597$, $df=20$, $P=.278$) in phantom limb pain subjects versus controls.

Conclusion: Signs of somatic dysfunction and exaggerated sympathetics were greater in phantom limb pain subjects

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versus controls in only two of seven criteria. This relative lack of difference suggests sympathetic drive is not directly related to phantom limb pain. While this study does not clearly validate a role for osteopathic manipulative medicine in treating phantom limb pain, a broader evaluation could improve upon this limited analysis.

P5

Retrospective Case Study of Jones' Iliacus Tenderpoints in the Ambulatory Setting

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Purpose: The purpose of this study was to determine the prevalence of the Jones' Iliacus tenderpoint (I-TP) in the adult population of a neuromusculoskeletal medicine practice.

Hypothesis: It was proposed that the Jones' I-TP would be present in 50% of the patient population; that it would be associated with low back pain in the past 7 days 50% of the time; that there would be no difference based on gender; and that activities of daily living would have an influence on its presence.

Research Design: Pilot, retrospective chart review.

Materials and Methods: Upon receiving IRB approval from the VCOM, 48 patient encounters from October 14 through October 30, 2009, were reviewed. Men and women aged 18 to 89 years were included in the data analysis. Charts were reviewed for the following demographic data: height, weight, patient age, gender, as well as: amount of time spent sitting during a 7-day period, type of exercise performed and number of days per week, whether the patient had low back pain in the past 7 days, the presence of a Jones' I-TP on the right or the left. Statistical analysis included: one sample proportion upper tail *t* test; Fisher's Exact test; and the odds ratio.

Results: Statistical significance supporting the hypothesis that the I-TP would be found in at least 50% of the sample population was found. In comparing prevalence of the I-TP of 2 population (men and women) proportions with independent samples, the result reflected that there was no significant difference in tenderpoint presence based on gender. Analysis of the data for correlations between activities of daily living and I-TP presence revealed trends supporting that individuals who sit for a prolonged period of time will have a greater chance of developing an I-TP; as well as those who engage in running or biking more than 3 days per week. There was statistical significance in evaluating the association between the complaint of low back pain in the previous 7 days and I-TP presence.

Conclusions: Iliacus tenderpoints are relatively common in the adult population. Analysis of the data shows a significant

correlation between the presence of an I-TP and the symptom of low back pain. This data will support future research assessing the effectiveness of treating the iliopsoas muscle with osteopathic manipulation as a conservative approach to low back pain.

◆ P6

The Cervical-Thoracic Connection

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Hypothesis: There is a statistically significant correlation between occipitoatlantal (OA) somatic dysfunction and upper thoracic (T1-T4) somatic dysfunction.

Materials and Methods: A retrospective analysis of the incidence of cervical and thoracic somatic dysfunction diagnosed in a normal population of second year osteopathic medical students (N=338) was conducted. Somatic dysfunction was defined as a vertebral unit possessing any of the following palpatory characteristics: tissue texture changes, asymmetry of motion and relative position, restriction of motion, and tenderness (T.A.R.t). Somatic dysfunction diagnosed in each subject was recorded, on a segmental level, in the regions of the cervical spine (OA, atlantoaxial [AA], C2-C7) and the thoracic spine (T1-T12). Descriptive and regression analyses, and tests of correlation based on a chi-squared test were performed.

Results: The five vertebral segments with the highest incidence of somatic dysfunction were found to be OA (76%), C3 (76%), T3 (73%), T5 (67%), and T4 (66%). A chi-square test of correlation between the OA and the following segments were found to be statistically significant: AA ($P < .024$), C2 ($P < .032$), and T4 ($P < .045$). Regression analysis revealed statistical significance between the incidence of upper-cervical (OA, AA, C2) somatic dysfunction and the incidence of upper-thoracic (T1-T4) ($P < .000$) and mid-thoracic (T5-T9) ($P < .000$) somatic dysfunction; the incidence of lower-thoracic (T9-T12) ($P < .084$) somatic dysfunction was not found to be significant.

Conclusions: The present study was conducted to statistically confirm the commonly described clinical correlation between cervical somatic dysfunction and thoracic somatic dysfunction. The results obtained revealed significant segmental correlation between the cervical and thoracic spine, specifically between the OA and T4. The results also suggest that the incidence of dysfunctional segments found in the

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upper- and mid-thoracic spine (T1-T9) is directly proportional to the incidence of dysfunctional segments found in the upper cervical spine (OA, AA, C2).

◆ P7

The Effect of OMT on Blood Pressure in Nonhypertensive Subjects

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Hypothesis: Osteopathic manipulative treatment (OMT) has been theorized to have beneficial effects on the management of hypertension. However, there is a scarcity of research studies proving OMT to be an effective treatment for hypertension. A study by Morgan et al (1985) showed no beneficial effect on blood pressure when soft tissue OMT was performed. Nevertheless, OMT is believed to normalize sympathetic tone, thereby decreasing the vascular constriction associated with a hypersympathetic state. We hypothesize that using an osteopathic treatment targeting the sympathetic nervous system will reduce blood pressure in prehypertensive and normotensive patients. We furthermore hypothesize that patients with hypertension who are not on sympathetic antagonists will have lower blood pressure after OMT due to the normalization of sympathetic tone.

Background: Hypertension is one of the most prevalent diseases of nonpregnant adults. NHANES data from 1999-2000, showed there were approximately 58 to 65 million adults with hypertensive diseases. The JNC-7 guidelines define hypertension as the average of two or more readings of a systolic blood pressure (SBP) of greater than 140 mm Hg, a diastolic blood pressure (DBP) of greater than 90 mm Hg, or both on each of 2 or more office visits. While there is a vast amount of research concerning the pharmacologic treatment and management of hypertension, there is very little in the literature about the use of OMT in this population.

Methods: We conducted a sham versus OMT single-blinded pilot study. We recruited patients aged 18 to 65 years that had a blood pressure no greater than 140/90 mm Hg. The study was carried out over a 6-week period in which subjects received 2 weeks of OMT versus a benign sham procedure. We measured heart rate and blood pressure prior to and following every visit.

Results: Preliminary *t* test results and a 95% CI error plot, (n=10), showed that there was no significant difference (*P*

value for Δ SBP=.696 and *P* value for Δ DBP=.207) in the use of OMT (n₁=6) versus sham treatments (n₂=4) in normotensive or prehypertensive subjects.

Conclusions: Based on these preliminary data, we conclude that patients without significant blood pressure dysfunction are able to maintain normal homeostasis of their autonomic system with and without OMT.

P8

Differential Effects of Suboccipital Inhibition and Fourth Ventricle Compression On Heart Rate Variability: Evidence For Bi-Directional Modulation Of Autonomic Tone

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Hypothesis: It is a long held belief that some of the effects of osteopathic manipulative treatment (OMT) techniques are mediated by the regulation of autonomic function. We hypothesized that OMT would produce measurable changes in autonomic balance which could be detected by spectral analysis of heart rate variability (HRV).

Methods: To test this hypothesis, we examined the effects of three cervical manipulative techniques, high-velocity, low-amplitude (HVLA), suboccipital inhibition (SOI) and fourth ventricle compression (CV4), and two control procedures, laser sham (LS) and no treatment (NT), in a crossover study of 35 healthy adult subjects with a minimum washout period of 1 week. HRV was based on 5-minute electrocardiograms taken immediately before and after the experimental procedure using Biocom Heart Rhythm Scanner 3.0 software with a Biocom 3000 ECG recorder.

Results: Analysis of the data using the Wilcoxon signed ranks test revealed a significant increase in normalized high frequency HRV (HFnorm) following SOI (mean [SD]; pretreatment 39.03 [18.00] nu to posttreatment 43.27 [30.04] nu; *P*=.049). Following CV4, normalized low frequency HRV (LFnorm) increased (mean [SD]; pretreatment 54.68 [20.22] nu to post-treatment 60.14 [20.91] nu; *P*=.005) as did the ratio of low to high frequency (LF/HF; mean [SD]; pretreatment 1.89 [1.85] to post-treatment 2.37 [1.95]; *P*=.02). HVLA, LS and NT did not produce a significant change in these parameters. None of the experimental procedures produced a significant change in total HRV as evaluated by the standard deviation in the normalized inter-beat interval (SDNN).

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Conclusions: The increase in HFnorm following SOI is indicative of enhanced parasympathetic (vagal) tone while the increase in LFnorm and LF/HF likely reflects a shift in balance toward sympathetic dominance. These results support the belief that specific manipulative techniques are capable of modifying the balance of autonomic tone.

Acknowledgments: ATSU IRB approval #080617-001. This study was funded by ATSU's Strategic Research Fund.

◆ P9

Somatic Dysfunction Among the Homeless Clients of Suitcase Clinic in Berkeley, California—A Retrospective Chart Review

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Introduction: Since February 2008, first- and second-year osteopathic medical students, supervised by DO faculty from Touro University College of Osteopathic Medicine—California (TUCOM-CA), have provided free osteopathic manipulative treatment (OMT) to the homeless clients of Suitcase Clinic (SC) in Berkeley, California. Most of the clients had multiple body regions with symptomatic somatic dysfunction (SD). The students, in various stages of OMT training, found the task of diagnosing, treating, charting, and providing stretching exercises in the allotted time challenging. If there were chief complaints (CC), patterns of SD common to this population, or both, students could be trained to be more effective and efficient; tailored self-care exercise/stretching pamphlets could be developed for these high-yield regions.

Hypothesis: There are CC, patterns of SD, or both common to the homeless population served by SC.

Materials and Methods: Retrospective chart review of 203 AAO SOAP Notes from 86 clients February 26, 2008, to June 23, 2009. Students' findings were validated by the supervising DO. Frequency distribution of CC and ICD-9 body region with SD calculated using Epi Info 3.5.1. Sacrum (739.4)+Innomminates (739.5) were combined into Pelvis in order to compare our data with previous studies (Licciardone et al, 2005; Sleszynski et al, 2005).

Results: From all encounters (n=203), regardless of CC (new or recurring, single or multiple), the most prevalent SD diagnosed was pelvis. For encounters with new CC (n=132) or single CC (n=70), the most prevalent CC in descending order were pain in the following regions: lower extremities (knee, foot/ankle), low back, neck, and shoulder. The most prevalent SD by body region in descending order was: pelvis, thoracic, cervical, and lumbar.

Conclusions: The areas of SD with the highest prevalence in the clients were: pelvis, thoracic, cervical, and lumbar regions. This contrasts with two studies performed in private practice clinic settings in which the areas of SD with the highest preva-

lence were: thoracic, cervical, lumbar, pelvis (Licciardone et al, 2005 [n=1331]) and cervical, thoracic (Sleszynski et al, 2005 [n=3908]). Further studies are underway to determine if this difference represents SD prevalence unique to the urban homeless or is due to sample size or diagnostic error. Suitcase training sessions will be revised to improve proficiency in diagnosis and treatment of, as well as demonstrating exercises for, these high-yield regions. Exercise pamphlets are being developed for them as well.

P10

Establishment of DO-Touch.NET, an OMM Practice-Based Research Network

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Objective: The osteopathic manipulative medicine (OMM) practice-based research network, DO-Touch.NET, was established at the A.T. Still Research Institute to compile practice-based data for conducting observational studies evaluating the utilization, safety, effectiveness, and costs associated with osteopathic manipulative treatment (OMT). Its objectives are to identify conditions that appear to be responsive to OMT, determine if certain patient characteristics are present in those who are more responsive to OMT, and identify and accurately describe techniques that are most beneficial in treating conditions responsive to OMT.

Materials and Methods: Data are collected directly from patients and physicians regarding history of complaints, severity levels of symptoms, current and past treatments, physical examination findings, diagnoses, OMT treatment performed, and patient immediate response to OMT. Patient symptoms are further evaluated on a daily basis for one week after treatment and quality of life data is collected at the office visit and 7 days after treatment.

Results: Based on 173 adult patient encounters (aged 18-92 years) by 12 DOs, a vast majority of conditions treated with OMT involve musculoskeletal pain. Both the most severe and average level of pain significantly diminishes over the week following OMT ($P=.004$). While 90% of patients report feeling better or much better immediately after OMT, this percentage drops to 74% after one week. There is also a significant decrease in the patients' symptoms interfering in their enjoyment of life ($P=.0004$), mood ($P<.0001$), sleep ($P<.0001$), and usual or general activities ($P<.0001$).

Conclusions: Preliminary results from DO-Touch.NET

◆ Indicates posters entered in the AOA Council on Research's Student Poster Competition, a judged event that takes place during the poster session at the AOA Research Conference.

demonstrate the importance of collecting practice-based OMM data. As the number of clinicians and subsequent encounters increase within the network, the value of this data for government and insurance agencies as well as training and research programs will clearly increase. Outcomes will also provide evidence supporting future research programs and developing evidence-based educational programming.

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P11

Characterizing Osteopathic Manipulative Techniques Used in Treating Pneumonia During the MOPSE Study

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Purpose: The purpose of the training program for the Multicenter Osteopathic Pneumonia Study in the Elderly (MOPSE) was to standardize osteopathic manipulative treatment techniques commonly used to treat patients with pneumonia. For three of the techniques, rib raising, suboccipital inhibition, and thoracic lymphatic pump, a pressure mapping system was used to assist in the training of the treatment providers at the sites, to objectively evaluate the characteristics of the techniques, and to better standardize the performance of the techniques.

Materials and Methods: Depending on the technique, 66 to 73 physician treatment providers were evaluated and trained using the pressure sensing instrument (XSensor, Sensor Products LLC, East Hanover, NJ) on 1 to 3 occasions over a 3-year period. A calibrated flexible sensor pad (4.6 cm x 4.6 cm with 4096 sensors) was placed between the patient and one of the clinicians' hands for the thoracic lymphatic pump or both hands in the other techniques. The maximum and average pressure, frequency and consistency of force applied, and contact area were measured while physicians performed these techniques primarily on one standardized patient.

Results: For rib raising, the mean (95% CI) maximum and minimum forces applied were 284 N (177-391) and 181 N (93-268). The mean interval between cycles was 2.7 seconds (2.3-3.1). For suboccipital inhibition, the mean force applied was 209 N (182-236). For the thoracic lymphatic pump, the mean initial force was 957 N (732-1182) and the peak force was 1914 N (1696-2132). The change in force per second was 41 N. (39-42).

Conclusion: Results are the first attempt to objectively quantify manipulative techniques performed by a large number of osteopathic physicians. Findings demonstrate that there was significant variability in the performance of the techniques. Using this methodology, it may be possible with further

research to more accurately determine what technique characteristics are most therapeutic.

Acknowledgment: MOPSE was funded by a group of osteopathic medicine supportive foundations lead by the Osteopathic Heritage Foundation and the Foundation for Osteopathic Health Services.

Clinical C1

Adverse Reactions and the Effect on Intensive Care Unit Time Period and Hospital Length of Stay in Carotid Endarterectomy Under General Anesthesia vs Regional Cervical Plexus Block

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Introduction: Debate has existed concerning the comparative virtues of performing carotid endarterectomy under general or regional anesthesia. This operation poses significant risks to the patient for cerebral vascular ischemia and risks inherent in imposing major anesthetic and surgical stress on patients with serious cardiovascular disease.

Hypothesis: Carotid endarterectomy performed under regional cervical plexus block will result in less adverse reactions and a decreased length of stay (LOS).

Materials and Methods: A random retrospective analysis was performed on a total of 101 ASA 3 and ASA 4 patients during a 6-year period. Regional cervical plexus anesthesia (59 patients) consisted of a 3-part local anesthetic block with intravenous sedation titrated to alleviate anxiety. General anesthesia (39 patients) consisted of a balanced endotracheal technique.

Results: Intensive Care Unit (ICU) LOS was determined by the amount of hours between Post Anesthesia Care Unit (PACU) discharge and ICU discharge. Likewise, postoperative LOS was determined as the total amount of hours between PACU and hospital discharge times. Adverse reactions (in the form of hypertension, hypotension, and neurologic or cardiovascular insults) were noted. ANOVA *P* value statistics were utilized to determine significance.

Conclusion: Regional cervical plexus block resulted in less adverse reactions and a decreased total LOS on the hospital floor as demonstrated by an ANOVA *P* value of .029. Significance was not noted in the ICU LOS (approximately 24 hours). Surgeon training and the conservative habit of admitting the patient to the ICU for a 24-hour period may have contributed to this insignificance. General anesthesia did significantly increase adverse reactions and hospital floor LOS as demonstrated by an ANOVA *P* value of <.001.

C2

Serum Aspirin Esterase Activity Increases After Hemodialysis in Patients With End-Stage Renal Disease

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Background: Research on variations of serum aspirin esterase (AE) in specific disease entities is scarce. End-stage renal disease (ESRD) patients undergoing hemodialysis have a cycle of clearance and accumulation of uremic toxins.

Aims: We evaluated AE status in renal failure in a case-controlled study with 39 ESRD patients on hemodialysis who were recruited from the Nephrology Unit, Department of Internal Medicine, Showa University Northern Yokohama Hospital, Japan. Age and gender-matched control subjects (n=30) were selected from hospital personnel with no history of renal disease. We also evaluated the changes in AE after dialysis.

Materials and Methods: Creatinine, urea; glycemia, cholesterol; triglycerides; high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, albumin were measured by standard methods. Aspirin esterase activity was measured kinetically at 300 nm, 37°C for 15 minutes. Data were expressed in nmol of acetylsalicylic acid hydrolyzed per minute and per milliliter (nmol/mL/min). The runs were blanked against reagent (to control for spontaneous hydrolysis of acetylsalicylic acid). The intra-assay CV is 4% and the inter-assay 5%, respectively.

Results: Levels of AE are lower in our series of ESRD patients (30.9±8.9 nmol/ml/min vs 39.9±9.1 for control subjects; $P<.0001$). Levels of AE increase dramatically by 33% to control levels after each dialysis ($P<.0001$).

Conclusions: In conclusion, our data suggest that AE activity is 33% lower in ESRD patients, hence, their serum metabolism of aspirin is slower and the doses more efficient. However, after dialysis, the situation reverses, they have more activity and then less efficiency for each dose. Changes in AE activity in these patients are shown here for the first time and suggest mechanisms such as removal of inhibitors by dialysis or modulation of lipid parameters as significant pathways to explore in further studies. Sponsored by Touro University-CA, Showa University School of Medicine.

C4

Serum Aspirin Esterase Activity in Type 2 Diabetic Patients and Control Subjects: Modulation by Lipid Metabolism

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Background: Increased hydrolysis of circulating aspirin is thought to participate as a causal factor in aspirin-resistance. However, despite the universal use of aspirin, the underpinnings of the etiology of aspirin resistance remain unclear and research on variations of serum aspirin esterase (AE) in diabetic patients is scarce.

Aims: We evaluated AE status in diabetes in a case-controlled study with 27 subjects with type 2 diabetes mellitus (18 men and 9 women), ages 60±14 years, who were recruited from an outpatient diabetic population consulting the Diabetes Unit, Department of Clinical Pathology, Showa University Northern Yokohama Hospital, Japan. Age and gender-matched control subjects (n=30) were selected from hospital personnel with no history of diabetes. Exclusion criteria were complications and dyslipoproteinemia.

Materials and Methods: Fasting glycated hemoglobin was measured by HPLC; glycemia, cholesterol; triglycerides; high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine, albumin and cholinesterase were measured by standard methods. Aspirin esterase activity was measured kinetically at 300 nm, 37°C for 15 minutes. Data were expressed in nmol of acetylsalicylic acid hydrolyzed per minute and per milliliter (nmol/mL/min). The runs were blanked against reagent (to control for spontaneous hydrolysis of acetylsalicylic acid). The intra-assay CV is 4% and the inter-assay 5%, respectively.

Results: Levels of AE are not significantly different in our series of diabetic patients 41.6 (range, 37.1-46.5) vs 39.2 (range, 32.5-48.7) nmol/ml/min for control subjects. This is in disagreement with a recently published observation that included patients with dyslipoproteinemia and obesity. We found a strong, significant positive correlation between AE and both cholesterol ($r=0.58$, $P<.007$) and triglycerides ($r=0.85$, $P<.0001$) in the control subjects, that is lost in the diabetic population.

Conclusions: In conclusion, our data suggest that AE is unchanged in normolipidemic type 2 diabetic subjects and that this may not be a factor explaining aspirin resistance in this subset of patients. We show that AE activity is strongly modulated by lipid levels, only in control subjects, suggesting that qualitative changes in lipoproteins in diabetes may play a role. Sponsored by Touro University-CA, Jichi Medical University, and Showa University School of Medicine.

C5

Changes of Serum Paraoxonase 1 and Ischemia Modified Albumin Levels in Acute Cerebrovascular Damage, a Pilot Study

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Background: Paraoxonase 1 (PON1) is an esterase enzyme which exerts protective effects against oxidative damage of low density lipoprotein and cells. Ischemia modified albumin (IMA) is produced by reactive oxygen species during ischemic states. Since reports of serum PON1 and IMA levels in stroke are limited, we studied their behavior and putative biomarker prognostic value in cerebrovascular disease (CVD).

Materials and Methods: Thirteen subjects (7 ischemic, 6 hemorrhagic CVD; 8 men, 5 women; ages 63.1±17.2 years) were enrolled. Sera were obtained on admission within 24 hours after the onset, and compared to those obtained the second day after the admission. Activity of PON1 was determined using paraoxon as a substrate and IMA was measured by the albumin-cobalt-binding assay.

Results: When the first and second samples were compared, PON1 activity was decreased in 92.3% of our patients by 20.8±16.0%, $P<.05$. On the other hand, IMA increased in 84.6% of our patients by 16.8±21.9%, $P<.05$. The delta IMA/delta PON1 increased to 1.21 in mild cases to 2.3 in severe cases. The most severe case (vegetative state) recorded a 21% decrease of PON1 and an 83% increase of IMA.

Conclusions: Even if the data are preliminary, these results indicate PON1 and IMA could be useful markers in CVD severity and prognosis, since PON1 behaves like a negative acute phase reactant and IMA indicates ischemia. Because those assays require simple, quick, methods they could be automated and inexpensively applied to bedside diagnosis.

C6

Intermediate-Density Lipoprotein Is Associated With Ischemia-Modified Albumin and in Patients With End-Stage Renal Disease

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Hypothesis: Ischemia-modified albumin (IMA), a cardiac ischemia biomarker is associated with lipid/lipoprotein profiles changes such as increase in intermediate-density lipoprotein (IDL), an atherogenic particle related to oxidative stress, in patients with end-stage renal disease (ESRD).

Subjects and Methods: Serum IMA and lipid/lipoproteins were analyzed in fasting blood samples in ESRD patients (n=15; mean age 60 years) on long-term hemodialysis.

Results: IMA mean levels were 0.56±0.11 arbitrary units and were not different from control subjects. Subfraction levels of IDL were 10.6±6.8%. A close correlation between IDL and IMA levels was observed ($r=0.6$, $P=.02$).

Conclusion: In summary, this study found a significant positive relationship between IDL and IMA in ESRD patients. Albeit this being a preliminary study it provides a proof of principle suggesting a link between the characteristic dyslipidemia found in renal failure and IMA levels and, if confirmed in larger series should pave the way for further work on pathogenic mechanisms and exploratory studies on the potential of the IDL-IMA ratio as prognostic biomarkers. Further research is warranted to confirm the present results. A mechanism to explain the observed data.

C8

The Clinical Characteristics of Methicillin Resistant Staphylococcus Aureus (MRSA) Infections in Complicated Pediatric Patients

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Hypothesis: Complicated pediatric patients are at significant risk from infection by methicillin-resistant *Staphylococcus aureus* (MRSA)

Materials and Methods: We performed a retrospective chart review of MRSA infections in children aged 0 to 18 years with confirmed MRSA admitted to the Children's Hospital at St. Francis in Tulsa, Oklahoma, from March 2003 through April 2009. The objective of this study was to investigate the epidemiologic and clinical aspects of MRSA among pediatric patients; and to characterize the types of infection, treatment, and resource utilization. The study population was limited to patients with complicated medical conditions such as cardiac, renal, genetic, immunologic and neurologic problems.

Results: There were 123 hospital visits identified; 63% were male; 46% white and 24% black; and 70% were on Medicaid. The mean length of stay was 12.5 days (range, 1-101); the mean length of ICU days was 13.1 (range, 1-94). Forty-six percent of these encounters resulted in a Pediatric Intensive Care Unit (PICU) stay, with the most common type of infection being soft tissue 41%, closely followed by pneumonia

39%. The most frequently reported resources used were tissue (93%) and serum (72%) microbiological cultures, vancomycin troughs (73%), and extended electrolyte panel (68%). The majority of patients were discharged to their home (89%) with 37% receiving home health services.

Conclusion: Methicillin-resistant *Staphylococcus aureus* is a major cause of soft tissue infection and pneumonia in this population. Enhanced understanding of MRSA in pediatrics can identify at-risk patient groups, thus leading to improved therapies, resource utilization and patient outcomes in pediatric patients with MRSA infection.

C9

Incidence of Fractures and Respective Causes in an Aging Population in El Salvador

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Background: Fractures and the mechanism of injury seen in the ageing have been studied extensively in the developed world. However, there is little data available on the incidence of fractures in developing countries, such as El Salvador. This information is pertinent because fractures will also increase as life expectancy continues to rise in developing countries. Research will enable prevention methods to reduce fractures among the aging in El Salvador.

Methods: This study used a researcher-designed survey to measure knowledge of bone health and fracture risk factors among aging patients with fractures. The 38 survey questions included 6 questions on demographic information, 7 questions regarding medical history and risk factors, 9 questions regarding dietary risk factors, 8 questions about physical activity, and 11 questions regarding knowledge of osteoporosis and risk factors. Every patient older than 45 years presenting to Hospital Zacamil with a fracture during the data collection period, January to May 2008, was given the opportunity to participate voluntarily in the study. Institutional review board approval was obtained for the study.

Results: One hundred and fifty-five patients met the criteria. The location of fractures presenting during this time were 25 ankle, 25 forearm, 73 hip, 1 tibia, and 31 that fell into the other category including those that occurred in the hand, clavicle, and ribs. One-hundred ten fractures (71%) resulted from a fall from standing height while 45 (29%) resulted from trauma.

Conclusion: This research shows that the trends in location of fractures and the mechanism of injury seen in the aging population of El Salvador are similar to those recorded in the developed world. This research will aid in creating educational tools focusing on prevention in this specific population. Future research should target prevention of population-specific risk factors for osteoporosis and evaluate the effectiveness of prevention tools aimed at reducing the overall risk.

C10

Combination 5% Acyclovir and 1% Hydrocortisone Cream (XERESE) Is Safe in Adolescents With Recurrent Herpes Simplex Labialis

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Hypothesis: Approximately 62% of adolescents have been infected with the herpes simplex virus type-1 (HSV-1). Treatment should always be considered as oro-facial herpes can affect quality of life in adolescents in a negative way. Monotherapy with an anti-viral drug alone reduces virus replication; but might not alter the symptoms caused by the immune-mediated response to the virus. It is hypothesized that improved clinical benefit could be achieved with the administration of combination anti-viral and corticosteroid therapy by reducing the skin reaction caused by the immune-mediated response to the infection.

Materials and Methods: A phase 3, one-arm, open-label, multicenter, subject-initiated study designed to detect any possible safety concern following administration of combination 5% acyclovir and 1% hydrocortisone cream (ME-609, Xerese) in adolescents with recurrent herpes simplex labialis. Eligible subjects were aged 12 to 17 years with a history of recurrent labial herpes with 2 or more episodes during the last 12 months. Subjects were instructed to initiate treatment at the first signs or symptoms of a herpes recurrence, at the earliest prodromal phase and preferably before the presence of papule or vesicle. Subjects applied the cream topically 5 times per day for 5 days. Categorization of recurrence (ulcerative or non-ulcerative), maximum lesion area (maximum area of an ulcerative lesion), and adverse events were assessed.

Results: One hundred thirty-four subjects were analyzed for safety. Of the 131 who had data for categorization of recurrence at the post-treatment visit 3±1 week after the last dose, 78 (59.5%) had non-ulcerative recurrences and 53 (40.5%) had ulcerative recurrences. All 131 subjects reached the stage of normal skin/no signs or symptoms at the follow-up visit. The mean (standard deviation [SD]) maximum lesion areas in the 53 subjects with ulcerative herpes lesion was 39 (40.8) mm² (range, 6-260 mm²). There were 5 adverse events reported by 5 subjects (secondary herpes labialis recurrences, n=2; infectious rhinitis, n=1; application site inflammation, n=1; bronchial asthma, n=1). There were no serious adverse events; all adverse events were of mild-to-moderate intensity.

Conclusions: The combination of 5% acyclovir and 1% hydrocortisone cream was safe and well tolerated in the treatment of recurrent herpes simplex labialis in adolescents.

(continued)

◆ C11

Outpatient Colonoscopy by Family Physician

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Background: Colorectal cancer (CRC) is the third most common cancer in the United States. The key to successful CRC treatment is screening and early detection. Colonoscopy examinations are commonly used for screening and diagnosing CRC. Currently, there is a well-documented shortage of physicians capable of providing colonoscopy examinations. Recent studies have demonstrated the efficacy and quality of colonoscopies performed by rural family physicians. There is an interest among suburban family physicians to explore colonoscopies as a means of CRC detection. The primary objective of this study is to demonstrate the outcomes of suburban family medicine outpatient colonoscopies in a clinical practice.

Methods: A retrospective chart review of 2815 colonoscopy procedures performed by a family physician between January 1999 and June 2008 was completed. All procedures were performed in a suburban hospital-based outpatient endoscopy suite. Specific data points were tabulated and analyzed. Data points included length of procedure, pathologic findings, types and amounts of anesthetic, cecal intubation rates, and complication rates.

Results: The average patient age was 57 years. There were 1722 male patient colonoscopies and 1093 female patient colonoscopies. Adenomas were detected in 31% of the cases. Fourteen adenocarcinomas, 320 tubular adenomas, 308 villous adenomas and 1399 adenomatous polyps were detected. More than 35% of the adenomas detected were beyond the reach of a 60 cm flexible sigmoidoscope. A complete examination to the cecum was possible in 99.8% of the cases. The average length of procedure was 16.7 minutes. The complication rate was 0.1%.

Conclusion: The results of this study indicate that outpatient colonoscopies in a suburban hospital setting can be performed efficiently, safely, and competently by a trained family physician. The results are comparable to currently reported benchmarks from other endoscopists. The data support the idea that family medicine and primary care endoscopists can emerge as large contributing factors in the early detection of colorectal cancer, given that opportunities for well-qualified training and credentialing are readily available.

◆ C12

TAp63 May Play an Important Role in Osteoblast Differentiation

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A recently discovered homolog of p53, p63, may play an important role in development and some cancers. p53 research has shown that p53 is an important tumor suppressor gene and the loss or mutation of p53 has been implicated in several cancers. p53 has been shown to have a role in the late stages of osteoblast differentiation and especially in the regulation of bone specific genes. Our laboratory has shown p53 to be important transactivator in the regulation of the osteocalcin gene a bone specific differentiation related gene. Structurally, p63 is very similar to p53 in many ways. However, p63 has 6 isoforms, TAp63alpha, TAp63beta, TAp63gamma, deltaNp63alpha, deltaNp63beta, and deltaNp63gamma. The TA isoforms have the same transcription activating region at their N-terminal end as p53 and the nucleotide sequence recognized by TAp63 is very similar but distinct from p53. Given the structural similarities between the two proteins, functional similarities may be present also. Recent studies have suggested a differentiation specific role for TA63 in epithelial differentiation. Several lines of evidence suggest a role for p63 isoform in mesenchymal cell differentiation. We attempted to determine if p63 does play a role in osteocalcin expression and osteogenesis. Cells from a rodent osteosarcoma line, ROS 17/2.8, were used in our experiments. Experiments performed included RT-PCR, Real Time PCR, and CAAT assays. PCR was performed using cells that were exposed to differentiation promoting (DP) media. RNA was then isolated from these cells from 0, 2, 4, 6, and 8 days of exposure to DP media and PCR was performed using primer specific for all isoforms of p63. The TA subsets showed increased expression during differentiation. The deltaN subsets showed undetectable expression in osteoblasts. Our results generally showed that the TAp63 isoforms are the predominant form expressed during differentiation. The TAp63 forms also was able to transactivate the osteocalcin promoter in transient transfection assays suggesting a more direct role for this protein in the regulation of bone specific gene expression. In other assays we compared the effect of p53 to p63 isoforms and found TAp63 to be 20-fold more potent than p53 in the regulation of the osteocalcin gene. Our studies generally point to an important role for TAp63 in osteogenesis.

◆ C13

Persons With Evidence of Non-Alcoholic Fatty Liver Have Increased Gluconeogenic Flux Towards Hepatic Glycogen

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Morris Schambelan, MD³; Nathalie Bergeron, PhD¹; Sue

◆ Indicates posters entered in the AOA Council on Research's Student Poster Competition, a judged event that takes place during the poster session at the AOA Research Conference.

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Background and Hypothesis: The liver plays a critical role in glucose homeostasis by modulating hepatic glucose production (GP) and uptake. During hyperglycemia, GP is suppressed and glycogen stores can be replenished directly by glucose (direct pathway) or indirectly by converting gluconeogenic (GNG) substrates to glucose (indirect pathway). Previous studies have shown that persons with insulin resistance (IR) have an increased supply of GNG precursors. Since GNG precursors can stimulate both GNG and hepatic de novo lipogenesis (DNL), we assessed GNG flux to hepatic glycogen in subjects with and without fatty liver.

Methods: Seven subjects with evidence of fatty liver (high hepatic lipid to water ratios by proton magnetic resonance spectroscopy) and 11 controls were admitted to the Clinical Research Center at San Francisco General Hospital. Following an overnight fast, subjects underwent tracer studies to measure hepatic uridine diphosphate (UDP)-glucose flux in the fasted state and during a hyperinsulinemic-euglycemic clamp. D-galactose-1d was used to label UDP-glucose, which in turn was “sampled” by acetaminophen, serving as a “pharmacological probe.” Acetaminophen is conjugated with UDP-glucose in the liver to form acetaminophen glucuronide (GlcUA), which is subsequently excreted in the urine. Labeled urinary GlcUA was isolated by HPLC, and derivatized for GC/MS analysis. The flux of UDP-glucose was calculated by tracer dilution and GNG by mass isotopomer distribution analysis (Hellerstein et al. Measurement of hepatic Ra UDP-glucose in vivo in rats. *AJP*. 1997;272;155-162).

Results: The average liver lipid to water ratio was $20.0 \pm 3.0\%$ in subjects with fatty liver vs. $3.7 \pm 1.0\%$ in controls ($P \leq .0001$). Comparing the change of UDP-glucose flux from fasting to hyperinsulinemia (clamp), a significantly greater increase of flux in the indirect pathway was observed in subjects with fatty liver compared to controls (0.15 ± 0.02 vs 0.07 ± 0.03 mg/kg*min, 68 vs 28%; $P = .031$).

Conclusion: Our results suggest that GNG substrates are taken up by the liver and channeled by the indirect pathway to hepatic glycogen. This GNG flux is increased significantly from fasting to hyperinsulinemia in subjects with fatty liver compared to controls. This increased flux augments hepatic glycogen stores and may also stimulate hepatic DNL, a pathway that can exacerbate NAFLD.

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C14

Evaluating Muscle Activation Asymmetry in Women with Low Back Pain Syndromes

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Purpose: The purpose of this prospective, single-blind study was to investigate whether muscle activity differed among women with chronic low back pain (LBP), chronic sacroiliac joint pain (SIP), and control (CTL) groups.

Hypothesis: Women with LBP or SIP have different muscle activation patterns compared to the CTL group.

Materials and Methods: Women were classified into the LBP (n=8), SIP (n=5), and CTL groups (n=6), based on their history and structural palpatory examination. Knee angle electrogoniometry and muscle activity for the erector spinae, rectus abdominus, gluteus medius, rectus femoris, and biceps femoris were measured at 1000 Hz with a Noraxon Telemetry system. Standardization tasks were performed for each muscle for normalization of muscle activity. In random order, each subject completed sit-to-stand, squat, tandem walk, and step-over functional tasks on a NeuroCom Balance Master System. After calculating the relative activation of each muscle, absolute differences between the right and left sides of the body were used to evaluate muscle activation asymmetry. Differences between groups were statistically evaluated with Kruskal-Wallis tests. Because of the small sample size, $\alpha = .10$ was used.

Results: During the sit-to-stand task, the erector spinae asymmetry was slightly higher for the CTL than LBP and SIP ($P = .09$). For the squat task, the erector spinae activity was more asymmetrical for CTL than for SIP at 30 degrees of knee flexion ($P = .09$). The biceps femoris had greater asymmetry for CTL and SIP than LBP at 60 degrees of knee flexion ($P = .01$). Both the erector spinae and biceps femoris had greater asymmetry for SIP than LBP at 90 degrees of knee flexion ($P = .04$ and $.02$). For the step-over task, the gluteus medius had greater asymmetry for CTL than LBP during both upward and downward motions (both $P = .07$).

Conclusions: Based on these outcomes, muscle activation asymmetry may differentiate functional characteristics between the groups. If these results are supported in a larger study, these methodologies and variations in muscle activity will provide functional measurements that may be used to help differentiate origins of low back pain syndromes. Further, these functional measurements may be useful for determining the impact of interventions like osteopathic manipulative treatment.

(continued)

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C15

Mental Health and Physical Function Responses to Opioid Therapy for Chronic Noncancer Pain Over 1 Year: Data From the Opioid Utilization Study

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Hypothesis: Most patients treated with opioids for chronic noncancer pain (CNCP) respond favorably with respect to measures of mental health and physical function.

Methods: The Opioid Utilization Study (OPUS) was a 1-year, multicenter, observational study of opioid therapy for patients with CNCP. OPUS enrolled adults (≥ 18 years) with a history of CNCP (≥ 3 months) and currently taking or initiating opioid therapy. Exclusions were a history of or risk for substance abuse, an active workers' compensation claim, or cancer pain. Participating physicians followed their routine practice, with no direction from the sponsor. Patients completed the Depression, Anxiety, and Positive Outlook Scale (DAPOS) and the Short Form-12 General Health Survey (SF-12: mental and physical functioning components) at time 0 (baseline), 6, and 12 months. The frequency distribution of DAPOS scores was analyzed to quantify the proportions of the population with mild (1–<2), moderate (2–<4), or severe (4–5) depression and anxiety and mild (4–5), moderate (2–<4), or severe (1–<2) impairment of positive outlook. Similarly, the frequency distribution of SF-12 scores quantified the proportions of the population with mild (50–80), moderate (30–<50), or severe (10–<30) impairment of mental and physical functioning.

Results: At baseline, 2003 patients enrolled; 59.8% were women, 88.4% were white, and 78.1% had used opioids for longer than 1 year. At 0, 6, and 12 months, respectively, the percentage of patients reporting depression scores as mild was 57%, 61%, 61%; as moderate, 37%, 35%, 35%; and as severe, 6%, 4%, 4%. For anxiety, the corresponding percentages were 56%, 58%, 63% for mild; 35%, 36%, 30% for moderate; and 9%, 7%, 7% for severe. Impairment of positive outlook at 0, 6, and 12 months was mild in 37%, 39%, 40% of patients; moderate in 55%, 52%, 54%; and severe in 8%, 9%, 6%. The percentage of patients with mildly impaired SF-12 mental functioning scores was 33%, 40%, 36%; moderately impaired 52%, 48%, 51%; and severely impaired 15%, 13%, 13%. For the SF-12 physical function domain, the corresponding percentages were 2%, 2%, 4% for mild impairment; 39%, 44%, 43% for moderate impairment; and 59%, 54%, 53% for severe impairment.

Conclusion: Measures of mental health and physical function remained stable for up to 12 months in a population of patients prescribed opioids for CNCP.

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C16

Bedside Ultrasound in the Surgical Assessment of Acute Biliary Disease

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Introduction: General surgeons are often called upon to evaluate patients in the emergency department (ED) with acute biliary diseases. Right upper quadrant (RUQ) ultrasound imaging may be useful in the management and disposition of these patients. Traditionally, the radiology department has performed RUQ ultrasounds. However, with the increased training of emergency physicians in use of bedside ultrasound, it is becoming more common for RUQ ultrasounds to be initially performed in the ED with formal RUQ ultrasounds performed later by the radiology department. We are interested in finding out if ED bedside RUQ ultrasound is accurate enough to be used alone in surgical decision making. As a screening tool for acute biliary disease, ED bedside RUQ ultrasound has the potential to shorten the time to treatment and to decrease costs.

Methods: A retrospective chart review was done on 88 patients who presented to our 456-bed hospital from April 2009 to January 2010 with RUQ pain. These patients received bedside ultrasounds performed by ED providers as well as formal RUQ ultrasounds by the radiology department. The objective was to evaluate the sensitivity and specificity of ED bedside ultrasounds for detection of cholelithiasis, gallbladder wall thickening, and common bile duct dilatation. Emergency department bedside ultrasound findings were compared directly to those of the radiology department formal ultrasounds.

Results: Emergency department bedside RUQ ultrasound sensitivity for cholelithiasis was 90.4% (95% confidence interval [CI], 79.8–96.1%), with a positive predictive value of 93.4% (95% CI, 83.3–97.9%). Emergency department bedside RUQ ultrasound findings negative for cholelithiasis had a specificity of 84.0% (95% CI, 63.1–94.7%) and a negative predictive value of 77.8% (95% CI, 57.3–90.6%). Bedside ultrasound was neither specific nor sensitive compared to radiology department ultrasound in determining gallbladder wall thickness or common bile duct dilatation.

Conclusion: Overall, this study demonstrates both the utility and the limitations of bedside ultrasound for the evaluation of RUQ abdominal pain. Although not as sensitive as radiology department RUQ ultrasounds, when combined with clinical and laboratory assessment, bedside RUQ ultrasounds that are positive for cholelithiasis could potentially help expedite the surgical management of biliary disease. With additional training and experience, ED providers could potentially improve their diagnostic skills with RUQ ultrasound. This would lessen the need for repeated formal RUQ ultrasounds and thus will shorten time to treatment and decrease cost.

C17

Safety and Tolerability of Topical Diclofenac Sodium 1% Gel for Hand Osteoarthritis Pain in Patients With Comorbidities

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Hypothesis: Current American Geriatrics Society guidelines recommend topical nonsteroidal anti-inflammatory drugs (NSAIDs) for pain relief in the elderly, noting that low systemic NSAID exposure with topical formulations may mitigate the risk of dose-related adverse events (AEs) associated with NSAID use (*J Am Geriatr Soc.* 2009;57[8]:1331-1346 and *Pain Med.* 2009;10[6]:1062-1083). To test the hypothesis that topical NSAIDs are well tolerated in older patients with certain comorbidities, we evaluated topical diclofenac sodium 1% gel (DSG) in patients aged 40 years or older who also had cerebrovascular or cardiovascular disease (CCVD, such as stroke or myocardial infarction), hypertension, or diabetes mellitus.

Materials and Methods: The safety and efficacy of DSG for relief of hand osteoarthritis (OA) pain has been demonstrated in 2 randomized, double-blinded, placebo-controlled 8-week trials (Altman RD et al. *J Rheumatol.* 2009;36[9]:1991-9). Pooled data from the 2-hand OA studies were used in the current posthoc subanalysis. The population included 400 DSG-treated patients aged 40 years or older (≥ 65 years: 45%, n=185), and AE rates were compared between patients with a history of CCVD n=66), hypertension (n=182), or diabetes (n=25) vs patients without these comorbidities.

Results: The frequency of reporting 1 or more AEs was similar in patients with hypertension (39.6%) and without hypertension (41.7%), lower in patients with diabetes (28.0%) than without diabetes (41.6%), and higher in patients with CCVD (48.5%) than without CCVD (39.2%). No cardiac AE or renal AE was reported in any DSG-treated patient. Elevated blood pressure was reported by 1 patient with hypertension and 1 patient with no comorbidities. No DSG-treated patient reported a serious treatment-related AE. Most AEs (97%) were mild or moderate in severity.

Conclusions: The similar and low rates of AEs in DSG-treated patients with and without preexisting CCVD, hypertension, and diabetes suggest that DSG is a generally well-tolerated option when used as directed in patients with hand OA and either comorbid CCVD, hypertension, or diabetes.

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Basic Science B1

Inferior Vena Caval Filters Use in Palliative/Hospice Patients: Rethinking Its Use

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Hypothesis: We suspect inferior vena cava (IVC) filters are being placed in patients whose overall outcome would not change or benefit with this invasive procedure and incurring additional cost and morbidity. The decision to place an IVC filter does not always account for the overall prognosis of patients, and it is being done independent of the palliative care consultation.

Methods: A retrospective chart review was performed on all patients who had pulmonary embolism acute, chronic DVT, and/or DVT and placement of an IVC from 2007 to 2009 at our institution. We specifically looked at patients who died during their admission who had both an IVC filter placement and a formal palliative consultation. VTE sites, use of anticoagulation before and after IVC filter placement, hypercoagulable states, and final diagnosis at time of death were evaluated. Patients were also assessed if they were under the care of a medical teaching or nonteaching service.

Results: A total of 1503 patients had a VTE diagnosis. There were 423 patients who received an IVC filter. Of the total of 124 deaths (8.3%), 32 patients received an IVC filter of which 11 patients had a palliative consult. The median age of these patients was 75 years. Medical teaching patients were more likely to receive palliative care consultation. The mean time between the placement of IVC filters and palliative consult was 3.75 days. Two patients had an IVC placed after the palliative consultation was completed. The average time between the IVC placement and death among the palliative care patients was 6.5 days. Seven of the 11 patients (64%) were subsequently transferred to the inpatient palliative care unit where they subsequently died. The average time of death from the IVC filter placement among these patients was 7.5 days. Compared to patients who were not transferred to the palliative care unit, the mean length between IVC placement and death was 4 days. Only 6 of the 11 patients had an absolute contraindication to anticoagulation use preceding or following IVC filter placement. However, the remaining patients either never had anticoagulation resumed following their IVC filter or

when resumed, the anticoagulation dose and regiment was inappropriate.

Conclusion: Inferior vena cava filter use should be reassessed and carefully selected in palliative care patients whose overall outcome would not be changed by this additional invasive procedure.

B2

Nebivolol Attenuates Redox-Sensitive Glomerular and Tubular Mediated Proteinuria in Obese Rats

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Obesity and insulin resistance related proteinuria is associated with oxidative stress and impaired tissue bioavailable nitric oxide (NO). Recent data suggest that NADPH mediated oxidative injury to the proximal tubule, like that seen in the glomerulus, contributes to proteinuria in insulin resistant states. The vasodilator b-blocker nebivolol reduces NADPH oxidase activity, increases bioavailable NO, and improves insulin sensitivity in overweight/obese animals and man. To test the hypothesis that a treatment strategy that reduces oxidative attenuates obesity-associated increases in glomerular and proximal tubule derived protein, we treated young Zucker obese (ZO) and age-matched Zucker lean (ZL) male rats with nebivolol (10 mg•kg⁻¹•day⁻¹) for 21 days. Compared to ZL, ZO controls exhibited increased proteinuria and gamma-glutamyl transpeptidase (GGT), reductions in systemic insulin sensitivity in association with increased renal renin, angiotensin II, aldosterone immunostaining, oxidative stress and tubulo-glomerular structural abnormalities that were substantially improved with in vivo nebivolol treatment. Nebivolol treatment also lead to improvements in glomerular podocyte foot-process effacement and improvement in podocyte-specific proteins (nephrin and synaptopodin), as well as proximal tubule-specific proteins (megalin and LAMP-2) and proximal tubule ultrastructural remodeling in the ZO kidney. Our findings support the notion that obesity and insulin resistance leads to increased glomerulo-tubular oxidative stress and resultant glomerular and tubular sources of excess urine protein. Further, the results of this study suggest the beneficial effect of nebivolol on proteinuria were derived from improvements in insulin sensitivity and reductions in renal oxidative stress in a state of obesity and insulin resistance.

◆ Indicates posters entered in the AOA Council on Research's Student Poster Competition, a judged event that takes place during the poster session at the AOA Research Conference.

◆ B3

Osteoarthritis and Obesity: Using Cadavers to Test the Associations and Pathophysiology of OA

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Background: Osteoarthritis (OA) is the most common cause of disability among the elderly in the United States. Epidemiologic analysis has shown a clear association between OA and obesity but the pathophysiological link between the two is still debatable. One possibility is that increased mechanical load on a joint can initiate and exacerbate OA. An alternative hypothesis suggests that some systemic biochemical dysfunction in obesity is responsible.

Hypothesis: This study tests the hypothesis that increased loading is responsible for increased OA in the obese by comparing joints that bear high weight (hip and knee) with a differently loaded joint in the hand, the fifth distal interphalangeal joint (DIP). These results were then compared to abdominal fat thickness measurements.

Materials and Methods: We dissected 54 specimens of each of the three joints and macroscopically graded the severity of OA on a scale of 0 to 3; 0 representing no signs of OA and 3 representing a joint surface where more than 50% of the subchondral bone is exposed. These ranked results were then compared to measurements of abdominal subcutaneous fat thickness taken at the level of the umbilicus.

Results: Regression analyses indicate a statistically significant association between abdominal subcutaneous fat thickness and the presence and severity of OA in all three joints examined.

Conclusion: These results corroborate the well-established association between obesity and OA, but suggest that the connection is not limited to joints that typically bear high mechanical loads. The hypothesis that increased loading increases the severity of OA does not explain the association we found between abdominal fat thickness and macroscopic OA lesions at the DIP joint. This finding suggests that another, more systemic, mechanism is at work, contributing to OA progression throughout the skeleton. Future studies should focus on correlating clinical OA with the histological finding presented here in order to elucidate the pathophysiology behind the seemingly systemic OA-obesity connection.

B4

Aspirin Inhibits Serum Paraoxonase 1 Physiological Activity: Preliminary Report

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Background: The antioxidant activity of high-density lipoprotein resides largely on its paraoxonase 1 (PON1) content. Experiments with transgenic knock-out mice indicate the potential for this enzyme to protect against atherogenesis. This relationship has been further strengthened by the publication of the first prospective study showing that low serum PON-1 activity is an independent predictor of new CHD events. Paraoxonase 1 is a promiscuous esterase and lactonase associated with HDL and linked to atheroprotection.

Hypothesis: Given the ester nature of aspirin and its similarity with some of the PON-1 substrates we hypothesized that aspirin inhibits PON1.

Materials and Methods: We set up dose response curves for PON1 activities in the presence of 0 to 2 mmol/L aspirin. We run the experiments both in purified HDL and in 3 human volunteer serum samples. We isolated HDL by sequential flotation ultracentrifugation ($d=1.21$ g/mL). paraoxonase 1 monoesterase activity was measured using phenylacetate as substrate and following product formation at 270 nm. PON1 triesterase activity was determined using paraoxon as a substrate, from the initial velocity of p-nitrophenol production at 37°C and recorded at 405 nm. PON1 lactonase activity was kinetically determined at 37°C using 5 (thiobutyl) butyrolactone (TBBL) and recorded at 405 nm.

Results: We found a dose-dependent inhibition by aspirin of both lactonase and arylesterase activities of PON1 that reaches 30% at 2 mmol/L ($P<.001$). The results are consistent for pure HDL and total serum in all samples studied. The effect on PON1 triesterase activity (paraoxon) is minimal.

Conclusion: This is the first report to show aspirin inhibits paraoxonase activity. Results also show the complexity of PON1 active site, since one of the activities changes little. The lactonase activity, which is considered to be the physiologically relevant, is impacted. After a 500 mg dose, aspirin levels in plasma peak at 0.15 mmol/L, and we show inhibition of 8% to 10% of PON1 activity at those concentration, thus these results may be physiologically relevant. Low dose (preventive) aspirin would have no deleterious effect on PON1, while anti-inflammatory full doses may well have. Lineweaver kinetic studies are ongoing in our lab. Sponsored by Touro University.

B5

The Antioxidant Enzyme Serum Paraoxonase 1 Is Not a Serum Acetyl-Salicylic Esterase

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Background: Serum aspirin esterase (AE) activity may be one of the factors responsible for the aspirin resistance syndrome which complicates prevention of stroke and myocardial infarction. This activity has been linked to pseudo-

cholinesterase and albumin, however, recent work has shown correlations indicating that more than 50% of the AE activity cannot be accounted for by the former two proteins. Paraoxonase 1 (PON1) is a promiscuous esterase and lactonase associated with HDL and linked to atheroprotection.

Hypothesis: We hypothesized that PON1 displays AE activity.

Materials and Methods: We determined AE and PON1 activities in 20 human volunteer serum samples. Serum AE activity was evaluated at 37°C with 1 mM aspirin as substrate, kinetically following salicylate absorption at 300 nm and PON1 activity was measured using phenylacetate as substrate and following product formation at 270 nm. We isolated HDL by sequential flotation ultracentrifugation ($d=1.21$ g/mL) and determined its aspirin esterase and PON1 activities.

Results: Serum AE activity in our volunteers was 101.2 ± 23.3 nmol/mL/min, which is in agreement with previous reports. No significant aspirin esterase activity was found in purified HDL, which displayed high arylesterase activity. No correlation was found between serum aspirin esterase and PON1 activities.

Conclusion: In spite of the typical promiscuity of PON1 in HDL our work failed to demonstrate any significant role in aspirin metabolism. The identity of all the enzymes responsible for hydrolysis of aspirin in serum, which may explain, in part, the aspirin resistance syndrome, remains an open field. Sponsored by Touro University-California.

◆ B6

Zebrafish Encoded 3OST3 Receptor Allows Herpes Simplex Virus Infection

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Hypothesis: Cell surface heparan sulfate modified by an enzyme 3-O-sulfotransferase-3 (3OST3) generates unique herpes simplex virus type-1 (HSV-1) entry receptor in human target ocular and neuronal cells. However the role of zebrafish (ZF) encoded 3OST3 has not been examined. The working hypothesis was to investigate ZF encoded 3OST3 receptor for their ability to mediate HSV-1 infection because both ZF and human encoded 3OST-3 isoforms share more than 70% similarity in DNA sequences.

Material and Methods: Zebrafish-encoded 3OST-3 was cloned into pDream2.1 mammalian expression plasmid. The expression plasmid of human and ZF encoded 3OST3 was expressed in HSV-1 resistant CHO-K1 cells by liposome mediated DNA

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transfection. Herpes simplex virus type-1 entry assay was conducted by using Lac Z reporter HSV-1 (HSV-1 gL86). In addition, a visual evidence of HSV-1 infection in ZF encoded 3OST-3 was demonstrated by using fluorescent microscopy. The HSV-1 spread from ZF encoded 3OST3 cells to the neighboring cell was demonstrated by HSV-1 induced cell fusion luciferase assay.

Results: We demonstrated that expression of ZF encoded 3OST-3 isoform renders Chinese hamster ovary (CHO-K1) cells to become susceptible to HSV-1 entry and spread. The green fluorescent tagged HSV-1 (HSV-1 K26GFP) virus was clearly able to infect cells expressing ZF encoded 3OST3 receptor. The ability of target cells expressing ZF encoded 3OST3 to fuse with HSV-1 glycoprotein expressing effector cells was significantly higher than human isoform of 3OST3. This result was quantified by using luciferase assay along with visual polykaryocytes formation.

Conclusion: Our data provide novel insight of ZF encoded 3OST3 receptor as an HSV-1 entry and fusion receptor and future development of HSV-1 infected model of ZF.

◆ B7

Src Family Kinases in Renal Development

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Background: The adult mammalian kidney begins to develop when an outgrowth of the primary nephric duct, termed the ureteric bud (UB), extends into the surrounding metanephric mesenchyme (MM). The UB epithelium invades the MM and, in response to factors secreted by the MM, undergoes multiple rounds of bifurcation (branching morphogenesis) leading to formation of the collecting duct system. The UB, in turn, induces conversion of the MM cells to epithelial cells and formation of the renal nephrons. Growth factor/receptor pairs have been implicated in renal development but the downstream signaling pathways regulating renal development are not well understood. Src family of kinases (SFKs) is a family of nonreceptor tyrosine kinases that are activated by and mediate the downstream effects of many receptors.

Hypothesis: We hypothesize that SFKs regulate one or more aspect of fetal renal development. This hypothesis was tested by examining the effect of an SFK inhibitor, PP1, on fetal renal development using a fetal kidney organ culture system.

Materials and Methods: Mouse fetuses were harvested from pregnant CD1 mice at approximately 12 days gestation, a point early in UB branching morphogenesis. Fetal mouse kidneys were isolated by microdissection and cultured in growth medium. Fetal kidneys were cultured in either control growth medium or growth medium containing 5 to 10 μM PP1. After 3 days, kidneys were fixed with paraformaldehyde, stained with a ureteric bud-specific lectin, fluorescein-Dolichos biflorus agglutinin, and imaged by fluorescence microscopy. Numbers of branch points for each branch stage and length of branch segments were measured.

Results: Under control conditions, fetal mouse kidney in culture undergo a developmental process analogous to that occurring in vivo, including multiple rounds of UB bifurcation and extension. Inhibition of SFKs produced: (1) a decrease in the number of UB branching points; and (2) an increase in the length of UB branch segments. The total cross-sectional area of control and PP1-treated kidneys was similar suggesting that SFK inhibition did not inhibit overall cell growth.

Conclusion: These results suggest that SFK activity promotes specifically branching of UB segments. They raise the possibility that disruption of SFK function during fetal renal development could influence UB branching morphogenesis and nephron number and could contribute to disease pathology.

◆ B8

Inhibition of Activated Rat Microglia Thromboxane B₂ Release by the Marine Sponge *Hymeniacidon* sp Metabolites

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Introduction: Neuroinflammation has been shown to be associated with activated brain microglia and the concomitant release of thromboxane B₂ (TXB₂) and superoxide anion (O₂⁻). Inflammation of cerebral tissue is recognized as a significant component of brain pathologies caused by infectious diseases, trauma, tumors, ischemia, Alzheimer disease, Parkinson disease, Down syndrome, multiple sclerosis, and AIDS.

Hypothesis: The purpose of this investigation was to determine the effect of five marine sponge *Hymeniacidon* sp-derived amphilectane metabolites and two semi-synthetic analogs on TXB₂ and O₂⁻ generation from *E coli* LPS-activated rat brain microglia (BMG).

Materials and Methods: Short and long term viability of BMG was assessed by lactate dehydrogenase (LDH) release (1.5 hours), and mitochondrial dehydrogenase (MTH) activity (2.5-18 hours), respectively. O₂⁻ levels were determined via superoxide dismutase-inhibitable reduction of ferricytochrome

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C and TXB₂ by enzyme-linked immunosorbent assay (ELISA) per manufacturer's instructions.

Results: (n=3-4) *Hymeniacidon* sp diterpenes and derivatives did not appear to affect BMG O₂⁻ release but in contrast potently inhibited TXB₂ (IC₅₀=0.20-5.69 mM) generation with concomitant low toxicity. Comparison of the IC₅₀ of the related amphilectane diterpenes (1) (TXB₂ IC₅₀=0.20 μM) and (2) (TXB₂ IC₅₀=0.23 μM) supports the notion that TXB₂ inhibition was associated with an isocyanide functionality at C-15, with a second isocyanide moiety within the same amphilectane core further increasing the activity. However, the amphilectane diterpenoid skeleton played a significant role, as suggested by comparison of IC₅₀ values of these two compounds and (6) (TXB₂ IC₅₀~3.14 μM), where the original isonitriles have been replaced by formamide groups.

Conclusion: Among the 7 *Hymeniacidon* sp metabolites and derivatives tested, metabolite (2) displayed the greatest anti-inflammatory potential against LPS-activated rat neonatal microglia as evidenced by potent TXB₂ inhibition, and minimization of short-term and long-term in vitro cytotoxicity. In conclusion, via their inhibition of TXB₂, the marine *Hymeniacidon* sp metabolites and derivatives show promise as novel chemical leads for the preclinical pharmaceutical development of novel anti-neuroinflammatory agents. Supported by Midwestern University, and the RISE and SCORE Programs, University of Puerto Rico at the Río Piedras Campus.

B9

Evidence for 13-Hydroxyoctadecadienoic Acid Dehydrogenase (13-HD) Activity in Human Placenta

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Background: Oxidized metabolites of linoleic acid such as 13-hydroxyoctadecadienoic acid (13-HODE) and 13-oxooctadecadienoic acid (13-Oxo-ODE) are endogenous ligands of peroxisome proliferator activated receptor gamma (PPARγ). PPARγ is expressed in human placenta and believed to be involved in gestational diseases such as preeclampsia and gestational diabetes. 13-HD converts 13-HODE into 13-Oxo-ODE using NAD⁺ as a cofactor and was previously characterized in rat liver. 13-HD activity has also been shown in human colon and prostate tissue as well as in several cultured human cell lines. Based on substrate specificity, 13-HD was shown to be distinct from prostaglandin metabolizing enzymes; however, it has not yet been cloned.

Hypothesis: We hypothesize that 13-HD activity is present in human full term placenta as this enzyme acts upon two known endogenous ligands for PPARγ.

Materials and Methods: After obtaining IRB approval, term placenta was obtained from women with no history of tobacco, alcohol, or drug use and with unremarkable past medical history. Villous tissue was dissected, homogenized, and used for all subsequent assays. 13-HD activity was measured spectrophotometrically based on the increase in absorbance at 285 nm representing the formation of the 2, 4-dienone moiety of 13-Oxo-ODE. Reaction rates were determined by converting the change in A₂₈₅/min into nM/min using a molar absorptivity of 28000 M⁻¹ cm⁻¹ for 13-Oxo-ODE. Reactions were conducted in 1 ml aliquots at 37°C for 10 minutes in 20 mM sodium phosphate buffer (pH 7) containing 0.2 mM 13-HODE, 0.4 mM NAD⁺, and 200 μg of placental homogenate.

Results: 13-HD activity was measured in placental tissue at a rate of 100±33 nM/min/mg protein (n=3). No appreciable activity was observed in the absence of NAD⁺ or after boiling the sample. Activity in homogenate samples appeared stable for at least 4 weeks when stored at -80°C. Comparable rates were obtained from separate dissections on the same placenta.

Conclusion: We present preliminary evidence of 13-HD activity in human placenta. Further purification of this placental form of 13-HD is underway. Our results raise the possibility that 13-HD plays a role in placental dysfunction by modulating the activity of PPARγ through its effects on the levels of 13-HODE and 13-Oxo-ODE. 13-HD may represent a diagnostic or therapeutic target for gestational disease.

◆ B10

Evaluation of Site-Specific Nephrotoxic Injury Using Ethidium Homodimer and Novel Urinary Biomarkers

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Overview: A difficult methodologic problem in the field of renal toxicology involves the quantification of cytotoxic injury along the nephron. In a series of recent studies, we have shown that in situ perfusion of the kidney with the nuclear fluorochrome ethidium homodimer provides a sensitive means of labeling necrotic and apoptotic cells in various models of proximal tubular injury in the rat (Edwards et al. *BMC Physiology*. 2007;7:1).

◆ Indicates posters entered in the AOA Council on Research's Student Poster Competition, a judged event that takes place during the poster session at the AOA Research Conference.

Hypothesis: The objectives of the present study were to determine if this technique could be used to label injured cells in other segments of the nephron and to correlate ethidium labeling with changes in the urinary excretion of several emerging markers of nephrotoxic injury.

Methods and Materials: To accomplish this, rats were treated with the site-specific nephrotoxicants gentamicin (proximal tubule; 100 mg/kg i.p. per day for 8 consecutive days), amphotericin B (distal tubule; 15mg/kg i.p. per day for 5 days), and indomethacin (papillus; 20 mg/kg oral gavage, single dose). The treatment protocols were all approved by the Animal Care and Use Committee of Midwestern University. Urine was collected and assayed for the biomarkers alpha-GST, mu-GST, and RPA-1. Animals were anesthetized and ethidium homodimer (5 µM) was perfused through the left kidney (Edwards et al, 2007). The kidney was then removed and cryosectioned at a thickness of 5 µm. The ethidium-labeled cells were viewed and counted using fluorescent microscopy.

Results: Gentamicin treatment resulted in an increase in labeling primarily in the proximal tubules and an increase in alpha-GST in the urine. Amphotericin treatment caused an increase in primarily distal tubule epithelial cell labeling and an increase in urinary mu-GST. Indomethacin increased papillary cell death.

Conclusion: These findings indicate that ethidium labeling provides an accurate and effective method for labeling necrotic cells in different portions of the nephron. Supported by Grant R01-E5006478.

◆ B11

Osteoarthritic Lesioning and Increased Subchondral Bone Density at the Proximal Articular Surface of the Human Medial Cuneiform

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Background: Osteoarthritis (OA) is a disease of synovial joints involving both cartilage and subchondral bone. The inciting event and pathogenesis of cartilage lesioning, however, remain unknown. Joint tissues accrue quantifiable indicators of pathology, such as chondrocyte clustering and changes in relative apparent subchondral bone density.

Hypothesis: This study tests the hypothesis that subchondral bone density changes are associated with (and may

locally predispose) the initiation of OA lesions. We also test the hypothesis that micropathological indicators of OA occur well in advance of the appearance of such lesions.

Materials and Methods: We collected 12 right medial cuneiforms from human cadavers, photographed their distal articular surfaces, and graded each on a scale of 0 to 3 based on OA lesion severity. We used CT scans of the specimens to generate maximum intensity projection maps of apparent bone density. We made histological sections through the highest and lowest relative density regions (HRDR, LRDR) and through the most severe articular cartilage lesions. Sections were viewed microscopically to measure cartilage thickness and identify other OA markers. We compared densities of different regions and chondrocyte clustering within regions using paired *t* tests and Wilcoxon signed-rank tests.

Results: Apparent density of most severe sections differed significantly from LRDRs, but were statistically indistinguishable from those of HRDRs. Chondrocyte clustering was observed in a majority of sections, including 92% of HRDRs and 67% of LRDRs. Within LRDRs, density was similar regardless of whether chondrocyte clustering was observed. Specimens with grade 2 lesions had significantly thinner cartilage than those with grade 0. We found a negative but nonsignificant correlation between articular cartilage thickness and apparent density.

Conclusions: Results support the hypothesis that a histologic cascade of changes, beginning with chondrocyte clustering, is initiated before changes in apparent density and appearance of macroscopic lesions. Results also indicate a local coupling of chondrocyte clustering and increase in relative density during the progression of lesions. Increased subchondral bone density may therefore be a necessary component of OA progression, but ultimately insufficient alone to cause cartilage lesions. Information about the pathogenesis of OA will ultimately inform physicians about proper treatment and management of this disease.

◆ B12

Essential Tremor: Candidate Gene Identification Using Microarray Comparative Genomic Hybridization

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Hypothesis: It is hypothesized that essential tremor (ET) may be caused by previously unrecognized genomic copy number variations (CNVs). The aim of this study was to identify and catalogue pathologic CNVs in a cohort of ET subjects in order to identify novel ET candidate genes, provide a more accurate means for diagnosis and risk stratification, and allow rational

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therapeutic development. This was accomplished by genome wide and ET pathway focused CNV analysis, using microarray comparative genomic hybridization (aCGH).

Overview of Research Design: This was a pilot study designed to capture CNV distribution in a cohort of subjects with clinically confirmed ET. Copy number variations identified in the ET cohort was compared to normal cohorts to delineate statistically significant differences in CNV distribution and enable candidate gene identification.

Materials and Methods: After obtaining IRB approval to perform aCGH on DNA samples from 40 subjects with ET using standard fluorescence based protocols on a customized oligonucleotide aCGH platform (Agilent), 3 μ m resolution scanning and Agilent feature extraction software generated data files. Agilent DNA analytics software was then used to identify significant CNVs. Significant CNVs were ranked on a 6-point scoring system to prioritize validation and further study. Points were assigned to CNVs in two categories based on significance of genes to ET pathogenesis and CNV frequency within the normal population.

Results: A number of significant CNVs have been identified in ET subjects, receiving priority scores of 5 or greater, including CNVs containing the following ET candidate genes: potassium channel, calcium-activated, intermediate/small conductance, subfamily N, member 2 (KCNN2), and PARK2. CNV analysis and validation is ongoing and identification of a number of additional high-priority CNVs is anticipated based on the degree of already identified genetic heterogeneity.

Conclusions: A large number of previously unrecognized significant CNVs exist within the ET population, many harboring promising candidate genes. Further characterization and elucidation of mutation frequencies within these genes is necessary to determine their ultimate significance to ET. Downstream studies must include analysis of candidate genes in large numbers of normal controls and validation in independent ET cohorts along with assessment of pathways to determine realistic targets for future interventions.

◆ B13

SRC Family Kinases Regulate Renal Epithelial Cell Paracellular Permeability

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Hypothesis: Tight junctions join adjacent epithelial cells at the apical surface to form continuous epithelial cell sheets allowing for tissue compartmentalization. These epithelial cell sheets play a role in body homeostatic control of solutes and water and limits the paracellular entry of toxins, antigens, and microbes.

Tight junctions are composed of continuous adhesive strands of transmembrane proteins (eg. claudins and occludins), which interact between adjacent cells to seal the paracellular space. The signaling pathways involved in regulation of tight junction function are a current area of intensive investigation. Src family protein tyrosine kinases (SFKs) interface with an array of signaling pathways that relay information from the external environment and influence amongst other things, cell division, differentiation, survival, motility and adhesion.

Materials and Methods: Previous studies have yielded conflicting results regarding the role of SFKs in regulation of tight junction permeability. The role of SFKs in regulation of tight junction function in two renal epithelia, mIMCD3 (collecting duct) and LLC-PK1 (proximal tubule) was investigated by monitoring the effect of SFK inhibitors, PP1 and PP2, on the transepithelial movement of a nontransported compound, calcein.

Results: The rate of calcein movement was dramatically decreased by the presence of lateral cell junctions and was linearly proportional to calcein concentration, consistent with calcein movement through a strictly paracellular pathway. Treatment of either mIMCD3 or LLC-PK1 cell monolayers with PP1 or PP2, increased the rate of paracellular calcein movement. The effects of PP1 and PP2 were concentration-dependent and were not replicated by treatment of cell monolayers with a chemically-related compound that is not an SFK inhibitor, PP3. Activation of several signaling pathways implicated in tight junction regulation, ERK 1/2, PI3 kinase/Akt, and PKC, did not mediate the ability of PP 1/2 to increase paracellular calcein movement. Overexpression of fyn, but not c-src, in LLC-PK1 cells produced a marked increase (2-4-fold) in paracellular permeability, consistent with observations in several carcinomas correlating increased SFK expression/activity with decreased tight junction integrity.

Conclusion: These results suggest that individual SFKs may differentially regulate tight junction permeability.

◆ B14

And Then There Were Four: Anatomical Observations on the Pollical Palmar Interosseous Muscle

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Background: An understanding of intrinsic hand musculature is important for many clinicians, particularly orthopedic surgeons and osteopathic physicians. Despite some evidence to the contrary, classic and modern textbooks assert that the

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human hand possesses four dorsal and three palmar interosseous muscles. The presence or absence of a pollical palmar interosseous muscle (PPIM) remains a topic of debate among anatomists.

Hypothesis: We tested the hypotheses that (1) the PPIM is present in the majority of human hands, and (2) the PPIM has a high muscle spindle density relative to adjacent muscles.

Materials and Methods: We dissected 45 hands from 23 human cadavers. In each hand, the first dorsal interosseous muscle was removed from its bipennate origin, exposing the more palmar muscular layer. The PPIM was identified as present if it fulfilled specific criteria of origin, insertion, and anatomical relationship to other structures. Samples of the PPIM and adjacent musculature were resected, histologically sectioned, stained with trichrome, and observed using light microscopy.

Results: We identified the PPIM in 41 (91%) of 45 hands, including instances of unilateral and bilateral expression. Size and anatomical relationship of the PPIM to the princeps pollicis artery were variable. Preliminary observations of relative muscle spindle density were equivocal when comparing sections of PPIM with those of adductor pollicis.

Conclusion: This study presents the strongest evidence to date of the existence and prevalence of four palmar interossei in the human hand. Accordingly, modern textbooks of anatomy and surgery should consider the presence of a PPIM as typical anatomy, and not an obscure variant. This study further establishes the range of morphological expression of the PPIM, including size, insertion, and relationship to the princeps pollicis artery. Further histological investigation is necessary to establish the PPIM as a component of a “parallel muscle combination.” Given its relatively small size and close anatomical relationship to the adductor pollicis, it is possible that the PPIM acts as a kinesiologic modulator of the adductor pollicis for contraction. This role for the PPIM could be corroborated by a greater muscle spindle density in the PPIM compared to the adductor pollicis. Future studies should focus on the normal functioning of the PPIM as well as its possible implication in thumb injuries such as Gamekeeper’s thumb and Bennett’s fracture.

◆ B15

Effects of pH, Citrate, and Phosphate on Bacterial Growth to Model Skin Infection

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Background and Aims: Bacterial infections of the skin are associated with many contributing factors, including fecal contamination. There are many physiological factors that may influence bacterial growth at the site of infection including pH, temperature, and substrate availability. We hypothesize that maintaining skin pH at approximately 4 to 5 will inhibit and/or attenuate the growth of potentially pathogenic bacteria and thereby reduce the incidence and severity of infection. The purpose of this study was to investigate the effects of pH and citrate or phosphate buffers on the growth rate of selected bacterial pathogens in vitro.

Materials and Methods: Luria broth (LB) was adjusted to pH 4, 5, 6, and 7 using either 100 mM citrate or phosphate buffers and NaOH or HCl as necessary. Triplicate samples of unadjusted LB and each buffered solution were inoculated with *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, or *Staphylococcus epidermidis*. Samples were then placed into a 37°C shaker bath and bacterial growth at various times (up to 24 hrs) was determined by measuring optical density at 600 nm.

Results: No growth was observed for any bacterial species grown in LB plus citrate at pH 4. There were various degrees of reduced growth rate observed in the citrate-buffered LB at pH 5 and 6. Across all species, citrate-buffered LB reduced growth rate more than phosphate-buffered LB at the same pH. Growth rates for all study bacteria were not affected by phosphate buffering of LB at pH 6 or 7.

Conclusions: LB buffered with citrate at pH 4 inhibited the growth of all test bacteria. Citrate buffer was more effective in reducing bacterial growth than phosphate buffer at all investigational pHs. The reason for this is not clear, but may be related to the ability of citrate to chelate divalent cations. These results provide evidence that preservation of the skin’s ‘acid mantle’ with citrate may be an effective way to prevent skin infections and promote skin health.

B16

Optimization and Reliability of Experimental Techniques to Assess Cortical and Stretch Reflex Excitability of the Erector Spinae Muscles

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Introduction and Hypothesis: Our understanding of the neurophysiologic properties of the human trunk muscles has historically been limited to studies examining voluntary muscle activation patterns. In this study, we describe our recently developed techniques for quantifying cortical and stretch-reflex excitability of the erector spinae muscles. We also report

the intrasession reliability and tolerability of these techniques.

Materials and Methods: We used transcranial magnetic stimulation (TMS) to measure intracortical facilitation (ICF), short-interval intracortical inhibition (SICI), and motor evoked potential (MEP) amplitude of the erector spinae muscles in healthy adults ($n=5$, 27.2 ± 8.1 years). Electromechanical tapping applied to the erector spinae muscles was used to assess stretch reflex excitability. All measurements were obtained twice on the same day to examine intrasession reliability while subjects were positioned in an upright, seated posture requiring low-intensity trunk muscle contraction.

Results: No differences were observed between the left- and right-sided erector spinae muscles for any of the outcome variables. The MEP amplitude was 216.5 ± 139.9 μ V, and the coefficient of variation (CV) as measured on two occasions was 7.6%. When a subthreshold conditioning pulse was delivered 15 milliseconds prior to the test pulse, classical ICF was observed ($149.5\pm 43.1\%$ of unconditioned pulse). Similarly, when a subthreshold conditioning pulse was delivered 3 milliseconds prior to the test pulse, classical SICI was observed ($80.9\pm 7.1\%$ of unconditioned pulse). The intrasession CV for ICF and SICI were 6.4% and 16.1%, respectively. The tolerability of the TMS protocol as rated on a scale of 1 to 10 with 10 being intolerable was 8.0 ± 1.8 . A short-latency muscle stretch reflex was observed 7.5 ± 1.1 milliseconds after the delivery of a rapid mechanical tap (net force: 90 N). The stretch reflex amplitude was 474.5 ± 317.9 μ V, and the coefficient of variation (CV) as measured on two occasions was $7.5\pm 1.2\%$.

Conclusions: This work indicates that it is feasible to assess ICF and SICI using appropriately designed paired-pulse TMS protocols, and that short-latency stretch reflexes can be successfully elicited from the erector spinae muscles in a reliable manner. This work is significant, as the development of these innovative techniques will permit the study of cortical and spinal properties of the erector spinae muscles to be evaluated in individuals with low back pain and following interventions.

◆ B17

Inhibition of the 26S Proteasome Induces Apoptosis in Gastric Carcinoma Cells

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Protein degradation is an important part of cellular metabolism, influencing a wide variety of cellular processes. Inhibitors of the proteasome have long been studied, but are beginning to gain ground as potential chemotherapeutic agents. In order to further understand the general characteristics of proteasome inhibitors on intact gastric carcinoma cells, we have conducted a series of experiments comparing the irreversible proteasome inhibitor NLVS with MG-132, a

reversible inhibitor of the proteasome. We have demonstrated that the human gastric carcinoma cell line AGS exhibits significant activity against proteasomal substrates corresponding to the 3 proposed proteasomal peptidase activities, and that each of these activities are specifically inhibited by NLVS and MG-132. Buildup of ubiquitylated proteins in inhibitor-treated cells confirmed inhibition of proteasome activity. Based on the mechanistic differences in activity of NLVS and MG-132, we next asked whether the different proteasome inhibitors were capable of inducing differential cellular effects on AGS cells. Using fluorescence based caspase activity assays we found that each of the inhibitor compounds was capable of inducing apoptosis in AGS cells. Western blot analysis of inhibitor treated cells was utilized to visualize the presence of cleaved poly ADP-ribose polymerase, a hallmark for the terminal stages of apoptosis. In order to understand the mechanism of apoptosis induction, we investigated the relative amount of the cyclin-dependent kinase inhibitor p27/Kip1 in treated versus untreated AGS cells. Following inhibition by either MG-132 or NLVS, we observed a significant accumulation of p27/Kip1, suggesting that apoptosis in AGS cells was induced by both inhibitors following a blockage of the cell cycle at the G1/S border. Although there are mechanistic differences by which each of the inhibitor compounds function to inhibit the proteasome, our results suggest that the downstream effects of proteasome inhibition are similar following inhibition of the AGS proteasome.

◆ B18

Involvement of BK Channels in Inflammatory Responses of BV-2 Microglial Cells

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Background: Microglia are the immunocompetent, macrophage-like cells that play a crucial role in neuroinflammatory processes in the central nervous system (CNS). Activated microglia produce multiple mediators of inflammation, among them nitric oxide (NO). Excessive production of inflammatory mediators can exacerbate neuronal cell death and demyelination as a result of stroke or traumatic brain injury. Moreover, increasing evidence indicates that neuroinflammation plays an important role in the development of many neurodegenerative conditions such as Alzheimer or Parkinson diseases. Large conductance Ca^{2+} -activated K^{+} (BK) channels are present in many cell types, including neurons. Importantly, BK channels are targeted in treatment of acute ischemic stroke. These channels are also activated during inflammatory responses of macrophages.

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Hypothesis: The purpose of this study was to examine whether BK channels are involved in inflammatory responses of microglia.

Materials and Methods: BV-2 mouse microglial cells were stimulated with LPS (100 ng/mL) in the presence or absence of interferon- γ (IFN γ , 5 U/mL), and their inflammatory response was assessed by measurement of NO production. Involvement of K⁺ channels was examined by including tetraethylammonium (TEA), a non-selective K⁺ channel blocker, and iberiotoxin, which specifically targets BK channels.

Results: Nitric Oxide was produced by BV-2 cells stimulated with LPS, whereas it was not detected in unstimulated cells. The addition of either TEA or iberiotoxin significantly lowered NO production in LPS-stimulated cells. The rate of inhibition depended on the concentration of channel blockers (17% and 38% with 5 mM and 10 mM TEA, and 25% and 40% with 300 nM and 500 nM iberiotoxin, respectively). Nitric Oxide production was also induced by IFN γ alone. The presence of IFN γ nearly doubled the level of LPS-induced NO. However, addition of IFN γ significantly lessened the inhibitory effects of TEA and iberiotoxin.

Conclusion: These results indicate that K⁺ channels, in particular BK channels are activated during LPS-stimulated NO production by BV-2 microglial cells. This inhibitory effect was nearly eliminated when the cells were additionally activated with IFN γ , indicating the inflammatory effects of IFN γ may be independent of BK channels.

◆ B19

Manzamine A Decreases Monocyte Migration by Decreasing Chemokine Secretion From Various Rheumatoid Arthritis Cell Types

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Purpose: Many of the pro-inflammatory mediators produced in rheumatoid arthritis (RA) are made by monocyte/macrophages and RA fibroblast-like synoviocytes (FLS). Manzamine A (MZA), a compound isolated from an ocean sponge, has previously been shown to have anti-inflammatory properties through rat neonatal microglia.

Hypothesis: Our hypothesis was that MZA would decrease: (1) levels of pro-inflammatory cytokines secreted by RA synovial tissue (ST) explants, (2) cytokines secreted by the human monocyte/macrophage THP-1 cell line, and (3) THP-1 migration to RA FLS conditioned media (CM).

Methods: THP-1 cells were treated with either vehicle control or MZA for 24, 48, or 72 hours using undifferentiated and 25 nM phorbol 12-myristate 13-acetate (PMA)-differentiated THP-1 cells. Viability was assessed by trypan blue exclusion. Human cytokine antibody arrays and enzyme-linked immunoabsorbent assays (ELISAs) were used to assess cytokine levels. Amount of THP-1 chemotaxis induced by MZA-treated RA FLS CM was also determined. All tissues were collected with IRB approval.

Results: Manzamine A significantly decreases migration of THP-1 cells to RA FLS CM (n=6, *P*<.05). ELISAs suggest no alterations in growth related oncogene (GRO)- α or monocyte chemoattractant protein (MCP)-1 secretion from RA ST explants. THP-1 cells stimulated with PMA increase GRO, GRO- α , interleukin (IL)-1 β , IL-8, MCP-1, MCP-2, and RANTES secretion. Treatment with 0.1 μ M MZA for 48 hours decreases secretion of MCP-1, MCP-2, and IP-10 from PMA-differentiated THP-1 cells. ELISAs confirmed significant decreases in IP-10 as well as GRO- α following treatment with MZA while MCP-1 and MCP-2 showed no significant differences (n=6, *P*<.05). Significant decreases in viability were observed in both vehicle-treated and 0.1 μ M MZA PMA differentiated THP-1 cells at 48 hours (n=3, *P*<.05).

Conclusions: Treatment of RA FLS with MZA caused a significant decrease in migration of THP-1 cells to CM compared to vehicle-treated CM. This is consistent with previous results demonstrating that treating RA FLS with MZA leads to a decrease in pro-inflammatory cytokines. This suggests that MZA acts in an anti-inflammatory manner on RA FLS. In contrast, we did not demonstrate that MZA altered GRO- α or MCP-1 secretion by RA ST explants. In PMA-differentiated THP-1 cells treated with MZA, significant decreases in GRO- α and IP-10 were noted. The latter finding may, in part, be caused by a reduction in viability of THP-1 cells by PMA.

◆ B20

Magnitude and Timing of Neuroinflammation in a Forceps Lateral Compression Model of Spinal Cord Injury

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We sought to evaluate the neuroinflammatory response in a compression model of spinal cord injury (SCI). Because compression is a common mechanism of injury in human spinal cord trauma, such a model has clinical relevance. In this study we used forceps to induce lateral compression injury to the spinal cord of female Sprague-Dawley rats, producing a moderate degree of injury as previously described in the contusion model (Basso et al, 1996). Postinjury inflammation has been implicated in secondary degeneration but has not been char-

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acterized in this model. We evaluated the inflammatory response in the injured spinal cord using immunohistochemistry. Consistent with other injury models, neutrophils were abundant at the injury site within 1 day postinjury (dpi), but by 3 dpi few scattered cells remained in the tissue parenchyma. Activation of macrophages was evident at the lesion site by 1 dpi and reached a peak at 14 dpi. At later time points, activated macrophages were predominantly localized to areas undergoing Wallerian degeneration (ie, the dorsal columns). This response could be observed as far as 4 mm rostral and caudal to the impact site. T cells were present by 1 dpi; at this time they were mainly restricted to the gray matter at the epicenter. At later time points, they could be observed throughout the lesioned tissue. T cells peaked in number at 3 dpi, decreased from 7 to 28 dpi, but remained elevated at 42 dpi. Overall, we confirm that lateral forceps compression of the spinal cord produces a neuroinflammatory response similar to what has been described in human spinal cord trauma and in contusion models of experimental spinal cord trauma. Thus, forceps compression represents a clinically relevant experimental model for evaluating secondary injury.

◆ B21

The Effects of Cranial Osteopathy on Neuronal Damage After Juvenile Seizures

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Rationale: Cranial osteopathic treatment (COMT) may benefit individuals with epilepsy by attenuating stress responses associated with seizures. The effect of COMT on cell damage after juvenile seizures is unknown. Elevated corticosteroid (CORT) levels occur after seizures and are associated with neuronal cell death.

Hypothesis: We hypothesized that COMT may protect the hippocampus from injury by decreasing CORT levels that accompany epileptogenic seizures.

Materials and Methods: Modified COMT was performed daily on the sub-occipital region of juvenile rats from postnatal (P) day 15 to P20. Two or five additional treatments were performed after induction of status epilepticus (on P20) with kainic acid (KA) (2 mg/kg). Age-matched controls and shams were used simultaneously. All animals were sacrificed after 72 hours. We examined morphological changes and cell death of pyramidal neurons of the CA1 region of the hippocampus in response to COMT using Nissl, silver and Golgi staining methods. Cell body injury, spine density and number of dendrites of CA1 hippocampal pyramidal cells were quantified to assess the level of damage of these neurons relative to control groups. In addition, circulating plasma CORT levels were

measured using radioimmunoassay (RIA) by collecting the animal's blood 72 hours post seizure.

Results: Following COMT in the absence of seizures, CA1 neurons appeared healthy and spine density and dendritic branching were indistinguishable from the controls. In the presence of seizures, COMT did not prevent typical CA1 injury, such as silver stained somata, spine density loss and accompanying swellings and shortening of dendritic segments. Although elevation of CORT plasma levels were not attenuated in animals who received COMT pre and post seizure compared to KA only treated animals, healthy animals that received COMT showed a reduction of CORT levels compared to the control group.

Conclusion: Findings indicate that COMT may attenuate the stress response by reducing CORT levels in healthy animals. Lack of morphological protection and CORT attenuation was likely due to failure of reducing the total stress response associated with sustained status epilepticus suggesting that either increasing the number of treatments or provoking shorter seizure episodes may allow COMT to be more effective. Additional treatment utilizing different osteopathic approaches or using another seizure model (eg, febrile) may lead to desired protective effects.

◆ B22

The Elderly Brain: A Chemical Environment Conducive to Alzheimer Disease

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Insoluble aggregates of misfolded proteins characterize Alzheimer disease (AD) as well as other neurodegenerative diseases that typically afflict the elderly. Patients with AD experience changes in personality and progressive loss of cognitive function. Autopsied AD brains exhibit extracellular plaques and intracellular protein deposits that primarily consist of amyloid-beta peptide (ABP) and tau protein, respectively. Amyloid-beta peptide is a proteolytic fragment of a protein normally produced in neurons. It was recently proposed that ABP may exhibit beneficial anti-microbial properties. Conversely, ABP may form oligomers that are also toxic to neurons because misfolding stress exceeds the tissue's ability to clear amyloidogenic proteins. The process by which ABP forms these neurotoxic oligomers is still not completely understood. We hypothesize that the tissue's chemical environment, unique to the elderly, contributes to the downstream

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events that lead to ABP toxicity. The elderly brain exhibits characteristic chemical alterations. Brain levels of acrolein, a small molecular weight lipid peroxidation byproduct, increase with age. The current study examined whether acrolein-ABP complexes alter the physio-chemical properties of ABP. With commercially available ABP (human isoform 1-42), we examined the effects of acrolein on the properties of ABP using biophysical techniques. Incubation conditions were carefully chosen, as they are crucial determinants of protein folding. Amyloid-beta peptide was incubated with acrolein under high agitation to mimic a condition where contact with hydrophobic surface is maximized. Agitation promotes air/water surface area with the air providing a hydrophobic contact. Additionally agitation increases the shear forces exerted on proteins. We tested the resulting solution for changes in hydrodynamic properties using a KSV Theta tensiometer and digital refractometer. ABP together with acrolein affects the surface tension of water and contributed to changes in refraction of light. These changes in hydrodynamic properties may alter the fluid mechanics of the interstitium that may affect neuronal surfaces. The working model is that ABP, which is coproduced with acrolein (a marker for the elderly brain) at the surface of axonal processes, leads to asymmetric lipid bilayer loss that impairs axonal function, initiating downstream synaptic degeneration.

◆ B23

Beta-Catenin Coordinates Signaling by Cadherin and Wnt Factors to Regulate Optic Axon Pathfinding in Vivo

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Hypothesis: β -catenin signals downstream of Cadherin and Wnt, major axon guidance cues, through α -catenin and GSK-3 β . Here, we propose that optic axon pathfinding requires β -catenin- α -catenin interaction in the Cadherin pathway, with functional co-regulation by Wnt component GSK-3 β . This will establish novel mechanisms for β -catenin in coordination of Cadherin and Wnt signaling during pathfinding of optic axons in vivo.

Methods: Our approach is to induce loss- and gain-of-function for β -catenin and associated factors in single optic axons in living *Xenopus* tadpoles. We then image these optic axons and their growth cones pathfinding in vivo.

Results: Perturbation of interaction of β -catenin with α -catenin in the Cadherin pathway, and with GSK-3 β in the Wnt

pathway oppositely regulated the amount of defasciculation of optic axons that facilitates their innervation of specific target regions, the level of fine-scale scanning of optic axons for guidance cues, as well as the formation growth cone protrusions that guide and drive optic axonal pathfinding in vivo.

Conclusions: These results suggest that β -catenin coordinates signaling by α -catenin in the Cadherin pathway, and GSK-3 β in the Wnt pathway to regulate several fundamental pathfinding behaviors of optic axons in vivo.

◆ B24

Interaction of Human Insulin and Essential Amino Acids in *Escherichia Coli* Adherence

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Overview: Insulin affects both glucose and amino acid transport in mammalian cells. We have reported that insulin affects *Escherichia coli* adherence to latex, a catheter component, in response to various sugars.

Hypothesis: We hypothesize that insulin also affects *E coli* adherence to latex in response to amino acids.

Materials and Methods: *E coli* ATCC 25923 was grown in peptone (1%) yeast nitrogen base broth with homologous amino acid to either logarithmic (log) or stationary (stat) growth phase. Adherence to latex was determined using 7-mm latex squares (n=8) placed in a suspension of washed cells (10³ CFU/mL; 30 min; 37°C) in buffer containing insulin (20 and 200 μ U/mL) with and without each of the amino acids (10⁻¹ to 10⁻³ M). Adherence levels to latex were determined by the press plate method. Controls were buffer alone, insulin alone, and amino acid alone. InStat (GraphPad) was used to determine data significance.

Results: Overall, adherence of stat phase cells was greater than that of log phase cells regardless of conditions tested. Insulin enhanced adherence as compared to amino acid control for log and stat *E coli* grown in Tyr (3x and 2.8x, respectively) or Phe (2.9x and 2.0x, respectively). Adherence of stat phase cells only was affected by Iso, Cys, Meth, and Thr (1.5x, 1.6x, 1.4x, 1.6x, respectively) in a suspension with 200 μ U/mL insulin, whereas no significant effect was seen with 20 μ U/mL insulin. Conversely, 20 μ U/mL insulin only affected adherence of log phase cells in the presence of Met (1.8x) and Glu (1.08X), whereas 200 μ U/mL insulin had a significant effect on adherence in the presence of Pro (2.74x) and Glu (1.2X). Interestingly, while insulin inhibited adherence of log phase *E coli* in the presence of Thr (0.48x), stat phase cell adherence was enhanced (1.7x). The reverse pattern was observed for Leu and Pro where log phase cell binding was increased (1.2x and 2.8x, respectively) and stat phase cell adherence was

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inhibited (0.71x and 0.63x, respectively) at 200 μ U/mL insulin. Insulin had no effect on adherence in the presence of Ala, Val, Asp, Gln, Lys.

Conclusion: Insulin at physiologic levels (normal and hyperinsulinemic) can affect adherence of *E coli* to catheter-associated material in both a positive and negative manner. However, no global pattern based on amino acid structure was evident. These findings may provide insight into catheter composition modifications that would have clinical applications.

B25

Topical Application of 5% Acyclovir and 1% Hydrocortisone Cream (XERESE) Does Not Cause Phototoxicity in Healthy Volunteers

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Hypothesis: The combination of 5% acyclovir and 1% hydrocortisone cream (AHC, ME 609, Xerese) is approved for the topical treatment of recurrent herpes labialis. AHC has an absorption spectrum in the UVB region (290-320 nm). A phototoxic material will produce either a wheal-and-flare response immediately after exposure, or erythema and edema 24, 48, or 72 hours later. The objective of this study was to determine whether the administration of AHC when applied to skin that is exposed to sunlight, would cause a phototoxicity reaction.

Materials and Methods: A single-center, double-blinded, randomized, vehicle controlled study. Healthy men and women aged 18 to 65 years with skin types I, II, or III (Fitzpatrick classification) were eligible for inclusion in the study. Following the determination of the minimal erythema dose (MED), 5 test sites measuring 2 \times 2 cm were outlined in a horizontal fashion on the lower aspect of the mid-back region of each subject. A single application of AHC 40 mg and the vehicle were applied in duplicate to designated test sites. All test sites were covered with a semi-occlusive tape for 24 hours. One site served as an irradiated control. After removal of the patches, the test sites were exposed to 20 J/cm² of long-wave ultraviolet (UVA) 320 to 400 nm plus 0.5 MED of full spectrum solar-stimulated radiation. Test sites were examined for reactions at the end of UV-exposures and at 24, 48, and 72 hours post-exposure. Dermal reactions were graded on a scale of 0 to 4 (0=normal skin, 4=vesicular or blistering reaction). Adverse events (AEs) were assessed.

Results: Thirty subjects were analyzed for skin reactions and safety. No subject had a phototoxicity reaction following UVR exposure to the test site. At 24 hours post-exposure, 9 of 30 subjects developed mild erythema (Grade 1) that was of equal intensity and considered a normal sunburn response to the UVR exposure. No reactions were observed in the remaining 21 subjects. Faint erythema (Grade 1) was still present in some of the exposed test sites in 4 of 9 subjects at 48 hours post-expo-

sure. The erythema resolved in the remaining 5 subjects. No residual erythema was detectable in any subject at 72 hours post-exposure. There were no AEs and no withdrawals.

Conclusions: Topical application of the combination of 5% acyclovir and 1% hydrocortisone cream and its vehicle were safe and well tolerated and did not exhibit detectable phototoxicity in this study.

◆ B26

Brain-Reactive Autoantibodies Are Nearly Ubiquitous in Human Sera and May Be Linked to Pathology in the Context of Blood-Brain Barrier Breakdown

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Background: Previous studies have reported antibodies bound to cells in postmortem Alzheimer disease (AD) brains, which are only rarely observed in the brains of healthy, age-matched control.

Hypothesis: Brain-reactive autoantibodies exist in the sera of AD individuals and can gain access to the brain interstitium.

Materials and Methods: To investigate this possibility, we determined the prevalence of brain-reactive antibodies in sera from AD patients, patients with other neurodegenerative diseases, age-matched, non-demented controls and healthy younger individuals via immunohistochemistry and western blot analysis.

Results: Western analyses revealed that 92% of all human sera tested contain brain-reactive autoantibodies. When sera were used to probe western blots of human, pig, or rat brain membrane proteins, a number of comparably sized protein targets were detected, suggesting cross-species reactivity. While the presence of brain-reactive autoantibodies was nearly ubiquitous in human sera, some autoantibodies appeared to be age- and disease-specific. Furthermore, the intensity of antibody binding to brain tissue elements, especially the surfaces of neurons, correlated more closely to the serum's autoantibody profile than to age or the presence of neurodegenerative disease. However, while the blood-brain barrier (BBB) in control brains remained intact, BBB breakdown was common in AD brains.

Conclusion: Our results suggest a high prevalence of brain-reactive antibodies in human sera which, in the common context of BBB compromise, leads us to propose that these anti-

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bodies may contribute to the initiation, pathogenesis, or both of AD and other neurodegenerative diseases.

B27**Involvement of Endothelin in Cardiovascular Actions of Beta-Amyloid**

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Introduction: Studies indicate that vascular factors play a significant role in the pathophysiology of Alzheimer disease (AD). ET and its receptors play an important role in regulation of the cerebral vasculature. The most prominent feature of AD is the extracellular plaques having β -amyloid ($A\beta$).

Objective: It is possible that $A\beta$ produces cerebrovascular effects mediated through ET which causes cerebral vasoconstriction and oxidative damage leading to degeneration of neurons. The present study was conducted to investigate the effect of $A\beta$ on systemic hemodynamics, regional brain circulation, expression of ET-1 mRNA, expression of ETA receptors, and oxidative stress markers.

Methods: Male rats were treated with vehicle or $A\beta$ (1-40) (20 μ g icv in 3 equally divided doses). Cardiovascular effects were studied using a radioactive microsphere technique in urethane (1.5 g.kg⁻¹ i.p.) anaesthetized rats. Contractions of aortic ring were measured using Radnoti 4 unit tissue bath (Radnoti Glass Technology, Monrovia, CA) and the force transducer coupled with a Grass P7D polygraph. Expressions of ET-1 mRNA and ETA receptors were determined using PCR and immunoblotting techniques.

Results: Rats treated with $A\beta$ had significantly higher mean arterial pressure compared to vehicle group. A decrease in cardiac output and stroke volume but an increase in total vascular resistance was observed in $A\beta$ treated rats. Rats treated with $A\beta$ showed significantly greater response to contractile effect of epinephrine, norepinephrine and phenylephrine in the isolated aortic ring preparation. The contractile response was augmented by treatment with ET-1 which was significantly greater in aortic rings of $A\beta$ treated rats. A significant decrease in blood flow was observed in the brain of $A\beta$ treated rats compared to vehicle. The vasoconstrictor effect of ET-1 on cerebral cortical blood flow was increased in rats treated with $A\beta$ compared to vehicle treated rats. The hippocampus and brain stem showed an increase in expression of ET-1 mRNA and ETA receptors. In rats treated with $A\beta$ there was development of oxidative stress in the brain, indicated by significant elevation in malondialdehyde levels and decrease in superoxide dismutase and glutathione levels.

Conclusion: Results indicate that ET-1 plays an important role in mediating the vasoactive effect of $A\beta$. It is speculated that ET receptor antagonists may have a role to play in AD by preventing $A\beta$ induced cardiovascular effects.

B28**Catecholaminergic Neural Elements in the Human Hypothalamus: Distribution and Morphology**

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Hypothesis: Previous studies have demonstrated that catecholaminergic, tyrosine hydroxylase (TH)-immunoreactive (IR) perikarya and fibers are widely distributed in the human hypothalamus. Since TH is the key and rate-limiting enzyme for catecholaminergic synthesis, these immunoreactive neurons may represent dopaminergic, noradrenergic or adrenergic neural elements. However, the distribution and morphology of these neurotransmitter systems in the human hypothalamus is not entirely known.

Materials and Methods: Since the different catecholaminergic systems can be detected by identifying the neurons containing the specific key enzymes of catecholaminergic synthesis, in the present study we mapped the different catecholaminergic elements in the human hypothalamus using immunohistochemistry against the catecholaminergic enzymes, TH, dopamine beta-hydroxylase (DBH), and phenylethanolamine-N-methyltransferase (PNMT). Dopaminergic elements were detected by utilizing double label immunohistochemistry. First, the DBH-IR elements were visualized; then the TH-IR structures, that lack DBH, were detected with a different chromogen.

Results: Only a few, PNMT-IR, adrenergic fiber varicosities were found mainly in the infundibulum and the periventricular zone. DBH-IR fibers were more widely distributed in the human hypothalamus occupying chiefly the infundibulum/infundibular nucleus, periventricular area, supraoptic and paraventricular nuclei. TH-IR neuronal elements were represented by both fibers and perikarya, located mainly in the infundibular zone and in the periventricular area.

Conclusion: In our study, we conclude that all of the catecholaminergic perikarya and the majority of the catecholaminergic fibers represent dopaminergic neurons in the human hypothalamus. Because of the extremely small number of PNMT-IR, adrenergic structures in the human hypothalamus, the DBH-IR fibers represent almost exclusively noradrenergic neuronal processes. These findings suggest that the juxtapositions between the TH-IR and numerous peptidergic systems revealed by previous reports indicate mostly dopaminergic synapses.

◆ B29

Reduced Antiarrhythmic Efficacy of Verapamil in Isolated Rat Hearts in the Presence of Elevated Extracellular Calcium

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Rationale: Effective and safe antiarrhythmic drugs are needed for the prevention of sudden cardiac death due to ventricular fibrillation. Determination of the mechanisms of action of novel antiarrhythmic compounds is necessary for establishing drug safety but can be difficult if the compounds affect multiple targets. The purpose of this study was to establish a whole heart method for determining the extent to which Ca channel blockade contributes to the antiarrhythmic effects of novel compounds.

Hypothesis: The antiarrhythmic effect of L-type Ca channel blockade could be surmounted in isolated hearts by increasing the Ca content of the perfusion solution. Verapamil, a phenylalkylamine L-type Ca channel blocker, was used for this proof-of-principle experiment.

Materials and Methods: In an experimental study, isolated rat hearts (n=10 per group) were perfused in a randomized and blinded manner with one of three solutions: normal Ca (1.4 mM Ca) Krebs solution containing drug vehicle only; normal Ca Krebs solution containing 300 nM verapamil; or a high Ca (2.8 mM Ca) Krebs solution containing 300 nM verapamil. After 10 minutes, the left main coronary artery was occluded, and the occurrence of ventricular fibrillation in the first 30 minutes of ischemia monitored from the ECG. The Ca concentration of the high Ca Krebs solution was established in a separate group of isolated rat hearts by determining the Ca concentration that, in the presence of 300 nM verapamil, normalized left ventricular developed pressure relative to verapamil-free controls.

Results: The incidence of ventricular fibrillation was significantly reduced from 80% in controls to 20% by perfusion with verapamil in normal Ca Krebs solution ($P < .05$). Perfusion with the high Ca Krebs solution increased the incidence of ventricular fibrillation in the presence of verapamil to 40% ($P < .05$ versus controls).

Conclusions: The antiarrhythmic effect of verapamil in isolated hearts can be surmounted by increasing the Ca content of the perfusion solutions. This method can be used to determine the extent to which Ca channel blockade contributes to the antiarrhythmic effects of novel compounds in whole hearts.

◆ B30

Juxtapositions Between the Catecholaminergic and Growth Hormone-Releasing Hormone-Immunoreactive Neurons in the Human Hypothalamus: The Possible Morphological Substrate of the Impact of Stress on Growth

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Hypothesis: Previous data indicate that stress is a pivotal factor in the regulation of growth. Psychological harassment often results in psychosocial dwarfism with short stature, delayed puberty and depression. It has been shown that growth hormone (GH) secretion is suppressed by stress, possibly via the attenuation of growth hormone-releasing hormone (GHRH) secretion. However, the exact mechanism of the impact of stress on growth is not entirely understood. Our previous studies revealed intimate associations between NPY-immunoreactive (IR) axonal varicosities and GHRH-IR perikarya in the human hypothalamus. Since NPY is considered to be a stress molecule, NPY-GHRH juxtapositions may represent an important factor of stress-suppressed GHRH release. In addition to NPY, catecholamines are among the major markers of stress. Thus, in the present study we examined the putative juxtapositions between the catecholaminergic, tyrosine hydroxylase (TH)-IR and GHRH-IR systems in the human hypothalamus.

Materials and Methods: In order to reveal these juxtapositions, double-label immunohistochemistry was utilized.

Results: Our findings revealed that the majority of the GHRH-IR perikarya formed intimate associations with TH-IR fiber varicosities. The majority of these juxtapositions were found in the infundibular nucleus/median eminence.

Conclusion: The density of the abutting TH-IR fibers on the surface of the GHRH perikarya suggest that these juxtapositions may be functional synapses and thus, in addition to the NPY, catecholamines may regulate GHRH secretion via direct synaptic mechanisms.

B31

Hypothalamic Galaninergic Neurons Are Innervated by Neuropeptide Y System in Human

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Hypothesis: Galanin and neuropeptide Y (NPY) are among the most abundant neuropeptides in the hypothalamus. The role of NPY and galanin in the regulation of the secretory

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activity of the anterior pituitary has been well established. In addition, the two peptides interact with a number of neurons synthesizing the releasing and inhibiting hormones and a large number of other neuropeptides. The aim of the present studies was to explore if, as in rodents, NPY innervates galanin-immunoreactive (IR) neurons in the human diencephalon.

Materials and Methods: As a result of the long postmortem period and subsequent lack of optimal preservation of the cell membranes in the brain, electron microscopy could not be employed to show the presence of NPY-IR synapses on galanin-IR neurons. Therefore, we used light microscopic double label immunocytochemistry and high magnification microscopy with oil immersion to identify putative juxtapositions between NPY and galanin.

Results: Our studies show that similarly to rats, numerous NPY-IR nerve terminals surrounded galanin-IR neurons in the human hypothalamus. Among the hypothalamic regions, the infundibulum (infundibular or arcuate nucleus) contained the largest number of galanin-IR neurons heavily surrounded with NPY-IR nerve terminals.

Conclusion: These en passant-type intimate associations between NPY-IR and galanin-IR neuronal elements may be by functional synapses and may provide the morphological basis for the NPY-mediated galanin release. Consequently, NPY-galanin communication may mediate effects of NPY on neuronal systems innervated by galanin, and therefore may play a pivotal role in the regulation of reproduction, growth, energy and metabolism.

◆ B32

Immune Effects of DDE in a Murine Diet-Induced Obesity Model

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Background: DDE (1,1-dichloro-2,2 bis(p-chlorophenyl)ethylene) is a metabolic breakdown product of the pesticide DDT (dichlorodiphenyltrichloroethane). DDE acts as an androgen receptor antagonist. Androgens protect against autoimmunity and DDE may therefore impact autoimmune development. Previously we found DDE-induced changes in immune populations and kidney expression of monocyte chemoattractant protein-1 (MCP-1) and osteopontin (OPN), which are also increased in murine lupus. Since DDE is lipophilic, we now use a model of diet-induced obesity. Our hypothesis predicts that immune effects of DDE will be greater in mice with more adipose tissue.

Materials and Methods: We used male C57BL/6J mice, fed either a high-fat (60 kcal% fat) or control low-fat (10 kcal% fat) diet from weaning. At 8 weeks of age, mice were divided into DDE and vehicle groups. Body weights were measured and proteinuria and glucosuria were monitored by urine dipstick. Treatment with DDE (200 mg/kg body weight) or equal volume of vehicle was by oral gavage twice, 2 days apart. Mice were sacrificed at 10 weeks or 6 months of age and kidneys, thymuses and spleens were removed. MCP-1 and OPN expression in kidney sections was assessed by immunohistochemistry. Flow cytometry was used to measure immune cell populations in spleens and thymuses. One-way analysis of variance with Tukey's posttest was used to determine statistical significance.

Results: Body weights at least 50% greater were seen in mice on the high fat diet. Trace proteinuria was seen in all mice but glucosuria was not observed. Total spleen and thymus cell numbers were not altered by diet or DDE. In thymuses we found fewer mature T cells (CD3+) in the DDE high fat group compared with the DDE low fat group (25.22 + 2.76% vs 47.55 + 3.69%, $P < .05$) at 10 weeks. High fat diet, independent of DDE treatment resulted in increased T cytotoxic (CD8+) thymocytes in high fat controls compared with low fat controls (2.04 + 0.9% vs 1.13 + 0.13%, $P < .05$) at 10 weeks and increased T helper (CD4+) splenocytes in high fat compared with low fat controls (25.63 + 2.67% vs 16.30 + 0.79%, $P < .05$) at 6 months. DDE caused a 10-15% increase in lymphocytic infiltrates in kidney glomeruli regardless of diet. High fat diet and DDE treatment increased kidney expression of MCP-1 while DDE treatment increased OPN expression.

Conclusion: In conclusion, diet and obesity influence some effects of DDE on the immune system. Study of additional immune parameters is warranted.

◆ B33

The Effects of Repeated Lymphatic Pump Treatment on Thoracic Duct Lymph Flux and Its Clinical Application

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Background: Lymphatic pump techniques (LPTs) are used by osteopathic practitioners for the treatment of edema and infection; however, the mechanisms by which LPTs enhances the lymphatic and immune systems are poorly understood. Previous studies from our laboratory demonstrate that lymphatic pump treatment (LPT) enhances thoracic duct lymph flow and leukocyte numbers in both dogs and rats. Further, there are inconsistent reports in the literature discussing the optimal duration and repeated application of LPT. This presentation will show a novel scientifically backed approach to LPT. The

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purpose of this study was to measure the effects of repeated LPT on thoracic duct lymph flow and leukocyte concentrations.

Hypothesis: Repeated application of LPT would enhance thoracic duct lymph flow and leukocyte concentrations.

Methods: Under anesthesia, the thoracic ducts of 6 dogs were cannulated, lymph flow was measured by timed collection and lymph samples was collected at (1) 30-minute baseline (pre-LPT), (2) during 4 minutes of LPT, (3) continuously for 2 hours following LPT (resting), (4) during 4 minutes of a second application of LPT, and (5) for 30 minutes following the second application of LPT (resting). Leukocytes in samples of thoracic duct lymph were enumerated using the Hemavet 950 (Drew Scientific). Leukocyte flux in the thoracic duct was computed from the product of lymph flow and leukocyte concentration.

Results: The baseline leukocyte flux in thoracic duct lymph was $4 \pm 1.5 \times 10^6$ cells/min and LPT significantly increased their concentration to $14 \pm 50 \times 10^6$ cells/min. During the 2-hour resting period between LPT treatments, leukocyte numbers were similar to baseline ($2.8 \pm 0.66 \times 10^6$ cells/min). A second application of LPT again increased thoracic duct leukocyte concentrations to $137 \pm 17 \times 10^6$ cells/min. During the 30 minutes following the second LPT, the thoracic duct leukocyte flux was $5.8 \pm 1.7 \times 10^6$ cells/min.

Conclusions: These results indicate that repeated applications of LPT will transiently enhance thoracic duct leukocyte flux. The information gained from this study provides a rationale for the clinical use of LPT to enhance immunity.

B34

Repetitive Motion Strain (RMS) Induces Human Fibroblast (HF) Sensitivity to Nitric Oxide (NO) In Vitro: Potential Roles of Myofascial Release (MFR) in Wound Healing

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Objective: We have previously investigated how biomechanical strains affect fibroblast wound closure (WC) rates in vitro. Results indicate that RMS impairs fibroblast WC and treatment via MFR post-RMS significantly improves WC rates. In this study we examined myofibroblast differentiation and the potential roles of NO and IL-1 β as signaling molecules in mediating these observed wound closure differences.

Methods: A modeled scratch-wound approximately 2 mm wide was applied to sub-confluent monolayers of cultured HF. Cells were treated with cyclic strain for 8 hours (RMS), acyclically strained for 60 seconds (MFR) or combined RMS+MFR treatment. NO secretion was determined by measuring total nitrate/nitrite accumulation in conditioned media 48 hours post strain. We also tested the effects of Na nitroprusside

(SNP; NO donor: 5, 10 and 25 μ M) and recombinant IL-1 β (5 ng/mL) on fibroblast WC, measured microscopically at times 0, 24, and 48 hours posttreatment. Detection of myofibroblasts in response to strain was assessed by staining for α -smooth muscle action.

Results: RMS and MFR treatments alone did not alter NO secretion when compared to non-strain (NS) control. However, when MFR followed RMS we observed an 11.7% ($P < .05$, $n=7$) increase in NO secretion. In NS cultures, IL-1 β induced a 108% increase in NO secretion ($P \leq .05$, $n=3$) compared to vehicle; however WC was unaffected by NO levels in both IL-1 β (vehicle vs. IL-1 β : $P = .33$, $n=3$) and NO donor-treated wound cultures (5, 10, 25 mM vs vehicle; $P \geq .05$, $n=3$). RMS-treated fibroblast appears to have a biphasic response to changes in NO level; low level (5 mM: $P \leq .05$, $n=6$) promoted WC and high concentrations (10-25 mM: $P = .9$, $n=6$ and $P = .2$, $n=6$, respectively) expressed inhibitory effects compared to vehicle. No differences were observed in myofibroblast expression among the different strain treatment groups.

Conclusions: RMS-impaired fibroblast WC does not correlate with strain-induced NO secretion. However, we found delayed WC induced by RMS is modified by varying NO donor concentrations, whereas NS had no effect, indicating that RMS may sensitize fibroblasts to NO. This suggests that the biomechanical environment may have regulatory roles in determining fibroblast response to changes in NO levels; potential roles for P53 and PKG are under investigation. MFR post-RMS may reverse impaired WC via low-level NO induction. This in vitro model, if clinically translatable, may support manual therapy such as MFR in enhancing wound repair.

B35

Fibroblast-Derived Mediators Improve Skeletal Muscle Differentiation in a Strain-Dependent Manner

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Objective: Myoblast fusion and differentiation to multinucleated muscle fibers are essential for myotube hyperplasia and muscle repair. Fibroblasts composing the fascia and surrounding the skeletal muscle system may facilitate differentiation through the release of cytokines, growth factors, and inflammatory mediators. We have previously reported that secretion of several of these molecules, such as interleukin 6, is modified in strain-dependent manners. To investigate the roles of strain and fibroblasts in muscle differentiation, we optimized an in vitro coculture using human fibroblasts seeded onto flexible collagen coated plates and mouse myoblasts (C₂C₁₂) seeded on to nondeformable coverslips situated two millimeters apart. The coculture thus allows for diffusion of potential differentiation mediators and selective fibroblast biomechanical manipulation.

Methods: Using the Flexercell apparatus, we strained fibroblasts using repetitive motion strain (RMS; 8 hours 1.3 Hz, 110% L0), a single sustained slow-loading strain modeling myofascial release (MFR; 60 seconds 106% L0), or combined MFR 3 hours post-RMS. No strain (NS) coculture and myoblast uniculture served as controls. Myoblasts were fixed and hematoxylin/eosin stained 88 hours post-strain. Eight individual wells and 6 images per coverslip were analyzed per treatment by blinded observer. Assessments included number of myotubes (>3 nuclei/cell), nuclei per myotube, and myoblast fusion efficiency measured as percent of nuclei population contained within myotubes.

Results:

	Uniculture Myoblast	Coculture			
		NS	MFR	RMS	RMS+MFR
No. myotubes/mm ²	1.5±0.2*	7.3±1.2	6.8±0.7	6.0±0.9	11.7±1.4†‡
Nuclei per myotube	3.1±0.0*	4.1±0.2	3.4±0.0†	3.6±0.1†	4.0±0.2
Fusion efficiency (%)	1.4±0.3*	11.5±1.9	7.8±0.4	8.4±0.9	17.0±1.6†‡

Significance indicated by P<.05. *Versus all coculture groups; † vs no strain; ‡ vs MFR; ¶ vs RMS

Conclusion: We conclude that fibroblast-derived cytokines have profound effects on several measures of myoblast differentiation. Data further show that biophysical strain modifies these outcomes. MFR-treated and RMS-treated fibroblasts resulted in fewer nuclei/myotube vs NS. Combined RMS+MFR resulted in greater myotube number and fusion efficiency when compared with all other treatments. These data show that MFR following RMS improves muscle differentiation in vitro, and suggests that fascia-directed treatment after repetitive strain may increase myoblast differentiation and improve muscle repair posttrauma.

B36

Acetazolamide (Diamox) Inhibits the Growth of Human Prostatic Adenocarcinoma Cells in Culture

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Introduction: Prostatic adenocarcinoma is the most common cancer in males. Almost 250,000 new cases are diagnosed annually in the United States. Therapy for prostate cancer depends on the stage of the tumor. Currently it is most commonly treated by surgery, radiation therapy and hormonal manipulation. Carbonic anhydrase (CA), a zinc enzyme that is expressed in virtually all tissue types including prostate, catalyzes the hydration of CO₂ to bicarbonate and a proton. It has been implicated in the growth of cancer cells both through the

provision of bicarbonate, which is an essential prerequisite for the synthesis of nucleotides and other cell components, and through elimination of the excess acid produced in cancer cells by their characteristically hypoxic metabolism.

Hypothesis: We tested the hypothesis that specific inhibitors of carbonic anhydrase also inhibit the growth of human prostate adenocarcinoma cells.

Materials and Methods: We measured the effects of several specific inhibitors of carbonic anhydrase on the growth, in culture, of a cell line derived from a bone metastasis of a grade IV prostatic adenocarcinoma. PC-3 prostate adenocarcinoma cells (obtained from ATTC) were propagated in F-12K medium. Cell growth, at a starting concentration of 1 × 10⁵ cells/mL, was measured in the presence and absence of acetazolamide (diamox; Sigma Chemical Co) over a 1-day period by staining with crystal violet (0.1%) and measuring absorbance at 570 nm. All assays were performed in replicates of 24, and standard deviation values were calculated.

Results: Acetazolamide (diamox) strongly inhibited the growth of human prostatic adenocarcinoma cells in culture, with a GI50 value of approximately 0.15 mM. Preliminary data demonstrated even higher levels of inhibition with the CA inhibitors neptazane and cardrase.

Conclusion: Our data suggest that specific inhibitors of carbonic anhydrase may be of value in therapy for adenocarcinoma of the prostate.

◆ B37

Affect of Genistein on CFTR Localization in Murine Intestine

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Background and Hypothesis: The major route for chloride entry into the small intestinal lumen across the apical membrane of the epithelia, is via the cystic fibrosis transmembrane conductance regulatory protein, CFTR. We have previously shown that the naturally occurring phytoestrogen, genistein (600 mg/kg body weight/day) administered via daily subcutaneous injections, significantly increases basal and cAMP-dependent transepithelial chloride secretion (Isc) across freshly isolated jejunum epithelia of both female and male mice, after 1- and 2-weeks treatment respectively. We hypothesized that genistein-stimulated increases in Isc were likely mediated via an increase in CFTR localization to the epithelial plasma membrane.

Materials and Methods: Standard immunohistochemical techniques were used to detect CFTR protein. Localization of CFTR was quantified using Image J with a modified EdgeRatio macro (NIH) at both apical and sub-apical regions of

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randomly assigned cells. Sub-apical regions were defined as 1.5 μm below the cell membrane surface. Data were collected from ~250 cells/animal, 4 to 5 animals per group.

Results: The apical/sub-apical ratio of CFTR localization was unchanged in the 1-week genistein-treated females (1.173 ± 0.006) compared to controls (1.173 ± 0.009). The apical/sub-apical ratio of CFTR localization was significantly increased in the 2-week genistein-treated males (1.168 ± 0.006) compared to controls (1.105 ± 0.008 , $P < .0001$). Furthermore, CFTR apical/sub-apical ratios were significantly increased in the female 1-week control group (1.173 ± 0.009) compared to the male 2-week control group (1.105 ± 0.008 , $P < .0001$), suggesting sex-dependent differences in basal levels of plasma membrane CFTR expression in intestinal epithelia.

Conclusion: Genistein-stimulated increases in intestinal chloride secretion appear to be mediated via an increase in CFTR localization to the epithelial plasma membrane in males but not females.

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◆ B38

Sensing Mechanical Manipulations: Calcium Signaling Pathways in the Epidermis

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Introduction: An essential first step in elucidating the mechanism of action of osteopathic manipulative medicine is to understand how mechanical forces are sensed at the cellular level and converted into biochemical signals. It is known that mechanically gated calcium channels are present in cells of the epidermis which allow calcium ions to rush into individual keratinocytes when mechanical deformation occurs in the epidermis. How the resulting change in calcium ion concentration is converted into biochemical signals in keratinocytes is a focus of active research. Our laboratory is actively investigating one signaling system, present in neurons, which converts fluxes in calcium concentrations into changes in cyclic GMP. This system is known as the membrane-bound Guanylate Cyclase (mGC) transduction system. This study set out to investigate the following hypothesis: Components of the mGC transduction system are present in keratinocytes and are poised to convert mechanical forces into biochemical signals.

Materials and Methods: Human cadaveric skin tissue was obtained for this study according to guidelines approved by the UMDNJ-SOM institutional review board. Biopsies were

taken that included the epidermis, dermis, and hypodermis. For identification and characterization of the components of the mGC transduction system within the skin, RNA and protein were isolated according to standard protocols. RT-PCR and Western Blot analysis was then performed. To localize the components of the mGC transduction system within the three layers of the skin, immunohistochemistry was performed. Briefly, tissue samples were fixed in 4% paraformaldehyde, cryoprotected in 30% sucrose overnight and sectioned at 30 μm . Immunohistochemistry was performed with primary antibodies specific for each component of the mGC transduction system.

Results: All necessary signaling components of the mGC transduction system are present in human skin tissue based on RT-PCR and Western Blot analysis. Based on immunohistochemistry, the components of the mGC transduction system are located primarily in the basal layer of the epidermis, in addition to being located in nerve fibers coursing through the adjacent hypodermis. Further, the mGC transduction system components co-localize with mechanically-gated calcium channels in basal keratinocytes.

Conclusions: The mGC transduction system is present in the basal layer of the epidermis and is positioned to convert mechanical forces into biochemical signals.

B39

Patient Physician Communication in the Twitter Age: Patient Preferences

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Hypothesis: The essential role that information and communication technologies play in healthcare cannot be understated. The use of communication technologies between patients and doctors may increase efficiency and improve effective communication by providing flexibility especially in non-emergency situations hence transforming healthcare delivery. This study assessed the use of communication technologies such as e-mail and Facebook between diabetes patients and their doctors in a rural socioeconomic disadvantaged area in Southeast Ohio.

Materials and Methods: A descriptive cross-sectional survey was conducted. A sample of 300 diabetes patients from Southeast Ohio, using convenience sampling at the diabetes center in Athens, were issued Ohio University institutional review board-approved questionnaires from February 2010 to April 2010. Descriptive statistics were analyzed using SPSS software 17.0.

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Results: Of the 300 patients surveyed, 46% were men and 53% women. Sixty two percent of the sample had type 2 diabetes and 33% were on Medicare/Medicaid insurance. The mean age of the sample ranged between 51 to 60 years, 45% completed 12th grade or GED and 61% earned less than \$35,000 annually. Ninety one percent of the sample owned a phone at home and 75 % owned a computer, but only 69% had internet at home. Forty two percent of the sample surveyed communicated with their doctors using phones outside scheduled face-to-face appointments and 13% used e-mail. Facebook and Twitter communication were not used in this area. When asked whether they were willing to pay for communication services with their doctors outside scheduled face-to-face appointments, 62% were not willing to pay for the services.

Conclusion: The use of communication technologies has attracted a small audience among patients in socioeconomic disadvantaged rural areas. Data suggest that accessibility to new communication technologies, especially internet, should be addressed so that patients can benefit from the growing healthcare delivery methods and possible inclusion in the patient-centered medical home.

B41

Lymphatic Pump Treatment Increases Leukocyte Trafficking and Inhibits Tumor Formation in the Lungs of Rats

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Introduction: Immune responses play a vital role in the development and metastasis of tumors. Studies from our laboratory demonstrate that lymphatic pump treatment (LPT) increases lymph flow and mobilizes leukocytes into lymphatic circulation in both dogs and rats. In addition to the direct effects of increased numbers of circulating leukocytes produced by LPT, it seems likely that these cells improve immune surveillance, which may enhance protection against tumor development and metastasis.

Hypothesis: Lymphatic pump treatment enhances antitumor immunity and reduces solid tumors in the lungs of rats.

Methods: F344 rats were injected intravenously with MADB106, a commonly used cancer cell line that forms solid tumors in the lungs of rats. Twenty-four hours following tumor injection, rats received no treatment (control), 4 minutes of light touch under anesthesia (sham), or 4 minutes of LPT under anesthesia, for 7 consecutive days. Eight days after tumor injection, tissues were removed and measured for solid tumors, leukocyte populations, immune cell activity, and lysis of tumors. Serum and lung fluid was measured for vascular endothelial growth factor C (VEGF-C), a biomarker for lymphangiogenesis.

Results: Lymphatic pump treatment significantly ($P<.05$) reduced solid tumors in the lungs compared to control and sham treatment. In addition, LPT significantly ($P<.05$) increased the numbers of B cells, CD4+ T cells, CD8+ T cells, NK cells, and macrophages in the lungs. There were no differences in the levels of VEGF-C between treatment groups, suggesting that LPT does not promote lymphangiogenesis during the early stages of tumor development. Finally, to measure antitumor immune responses, leukocytes were isolated from the lungs and spleen and cultured with tumors in vitro. There were no differences in tumor specific cytokine production or tumor lysis between treatment groups, suggesting that LPT does not enhance leukocyte activities in vitro.

Conclusion: Our data suggest LPT inhibits pulmonary tumor formation by enhancing the numbers of leukocytes with anti-tumor activities that migrate into the lungs. The results from this study provide scientific support for the clinical use of LPT to enhance antitumor immunity.

B42

The Effect of Microcystis Aeruginosa Lipopolysaccharide (LPS) on Neonatal Rat Brain Microglia Cytokine and Chemokine Generation

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Hypothesis: Microcystis aeruginosa (Ma) is a cyanobacterium that may contaminate freshwater. Cytokines and chemokines are glycoproteins hypothesized to cause neuroinflammation when released by LPS-stimulated microglia. We hypothesized that Microcystis aeruginosa (Ma) LPS would cause a dose-dependent release of both cytokines and chemokines from rat microglia in vitro.

Materials and Methods: Primary rat neonatal microglia were treated in vitro with MaLPS (0.1-100,000 ng/mL) and *Escherichia coli* (Ec) LPS (0.1-100 ng/mL) for 17 hours at 35.9°C. Thereafter protein expression in supernates from control and MaLPS (1×10^5 ng/mL)-treated microglia was investigated by RayBio biotin label-based rat antibody array technology (RayBiotech, Norcross, Georgia). Up-regulated cytokines and chemokines were assayed using rat-specific enzyme-linked immunoabsorbent assays (ELISAs).

Results: The RayBio antibody array revealed that MaLPS caused greater than two-fold up-regulation of several proteins: cytokine TGF- β 2 (7.4), chemokines MIP-1 α (6.58) and MCP-1 (3.09), and MMP-inhibitor TIMP-1 (2.58). ELISA results demonstrated that MaLPS triggered a concentration-depen-

dent release of MIP-1 α and MCP-1 with maximal release observed with 100,000 ng/mL MaLPS: MIP-1 α : 58,153 \pm 24,074.5 pg/mL, n=3, P <.05, and MCP-1: 539.7 \pm 174.7 pg/mL, n=3. In contrast, no significant TGF- β 2 or TIMP-1 release was observed with either MaLPS or EcLPS stimulation.

Conclusions: Our data provide partial support for our working hypothesis. Although antibody array technology appeared to determine up-regulation of TGF- β 2, MIP-1 α , MCP-1, and TIMP-1 after MaLPS treatment, ELISAs only confirmed enhanced release of the chemokines MIP-1 α and MCP-1, but not the cytokine TGF- β 2 nor the matrix metalloproteinase inhibitor TIMP-1. Furthermore, MaLPS appeared less potent than EcLPS in activating rat microglia in vitro. Current studies in our laboratory are extending our investigation to other proteins released by MaLPS-treated rat microglia.

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Medical Education

◆ ME1

Dermatology Education and the Internet: Traditional and Cutting-Edge Resources

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Background and Hypothesis: The number and variety of dermatological online medical resources has grown exponentially over the past decade. Internet-based resources allow for immediate and easy access to information for both reference and learning purposes in medical education. Although clinicians continue to turn to the internet for clinical information and still images, tech-savvy medical students are currently accessing a variety of exciting new resources, including discussion boards, wikis, video, podcasts, journal clubs, online communities, and interactive diagnostic experiences to augment their medical education. We hypothesized that a group of 6 medical students in our university dermatology research lab would rank cutting-edge resources, including audio, video, and interactive Web sites, as favorably as traditional resources, such as image banks, peer-review sites, treatment-focused sites, and general information sites geared toward the public.

Materials and Methods: Resources were drawn from literature searches for "online dermatology resources" as well as from hand searching compiled resource sites. These resources were ranked by 6 medical students. Students used a matrix derived from the Silberg Criteria, which evaluated authorship, attribution, disclosure, currency, and content. Authorship, attribution, disclosure, and currency were ranked "yes/no," with a point assigned per "yes" answer. Content was rated on a 1 to 5 scale, 5 indicating superior content compared to other Web sites in its Web site class. Scores were compiled and ranked for comparison, with the noncontent total score being weighted by content score.

Results: There was comparable ranking and approval of cutting-edge resources and traditional online sources. Dermatology podcasts, peer-review sites, and treatment-oriented sites received the most favorable reviews by students in this survey. Interactive online sources, including online communities, journal clubs, and virtual grand rounds, received the lowest average scores. The highest-ranked dermatology resources in each category with URL address are provided for readers' use.

Conclusion: The cutting-edge online dermatology resources examined in this study represent excellent sources for continuing education for medical students and clinicians alike. Resources such as these likely represent the future of medical education, as they allow for self-directed and supplementary education as well as remote access.

ME2

Standardized Patients and Osteopathic Manipulative Treatment During COMLEX-USA Level 2-PE

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Objective: This study reports the use of a safety phrase (the "stop" phrase) by standardized patients (SPs) during the performance of osteopathic manipulation treatment (OMT) in the Comprehensive Osteopathic Medical Licensing Examination-USA Level 2-Performance Evaluation (COMLEX-USA Level 2-PE).

Methods: The performance of OMT on SPs is unique to this examination. The "stop" phrase is used for SPs to employ when they feel discomfort during the physical examination or OMT on the COMLEX-USA Level 2-PE. Standardized patients are told to use the phrase, "That's a bit rough, Doctor" in this situation. Candidates are reminded of the stop phrase during orientation. High-velocity, low-amplitude or articulatory-type thrust techniques are not permitted because of the repetitive nature and potential exposure to multiple treatments in

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one examination session. These encounters were viewed by physicians to document and verify circumstance of use.

Results: In the 2007-2008 and 2008-2009 test cycles, 8452 candidates were tested (101,416 encounters, as 1 candidate left after the fourth encounter). The stop phrase was used 69 times (0.07%). The most common use of the stop phrase was during the head, eyes, ears, nose and throat examination (n=24, 34.8%) OMT accounted for only 17.4% of all indicated uses (n=12), or 0.01% of all encounters. The pass rate for all candidates during the 2008-2009 testing cycle was 94.7%. The pass rate for candidates who heard the stop phrase was 91.3%. No candidate failed after the stop phrase was employed during the performance of OMT. The pass rate for the candidates who received the stop phrase was slightly lower than all candidates tested during the 2008-2009 cycle, but there were no significant differences between the 2 groups (assuming equal variances and correcting for unequal sample sizes) for mean scores of GPA ($t[4191]=1.051, \alpha=.05$); Data Gathering ($t[4191]=-0.157, \alpha=.05$); SOAP Note ($t[4191]=0.171, \alpha=.05$); and OMT ($t[4191]=0.336, \alpha=.05$).

Conclusions: The performance of OMT did not significantly increase the use of the stop phrase. Standardized patients were twice as likely to use the stop phrase for HEENT examination. The pass rate was slightly lower with candidates in this cohort than with all candidates, though not statistically significant. Perhaps candidates who are aggressive in their physical examination skills leading to the SP using the stop phrase have other deficiencies in overall clinical skills. This serves as evidence OMT can be performed and evaluated safely during a standardized patient examination without adversely affecting SPs or candidates.

ME3

COMLEX Level 2-PE Performance Differences between Left-handed and Right-handed Candidates.

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Background: The assessment of written communication is an important component of the Comprehensive Osteopathic Medical Licensing Examination Level 2-Performance Evaluation (COMLEX Level 2-PE). Handedness, more specifically inconsistent handedness, has been shown to have complex relationships with verbal, non-verbal, mathematical, memory and reasoning ability (Corballis MC, Hattie J, Fletcher R. Handedness and intellectual achievement: an even-handed look. *Neuropsychologia*. 2008;46[1]:374-378). Handedness may

therefore have an effect on COMLEX Level 2-PE pass rates.

Hypothesis: Starting as an interesting observation noted by the examination proctors, we hypothesized that COMLEX Level 2-PE pass rates would be equal for left-handed and right-handed candidates.

Methods: For the 4698 candidates who took the examination during the 2007-2008 testing cycle, proctors identified and recorded which candidates wrote with their left hands while completing their postencounter SOAP Notes. Based on these candidate ID numbers recorded by the proctors, candidates were divided into two groups: left-handed and right-handed. An independent samples *t* test was then conducted to compare the mean pass rates of right-handed and left-handed candidates for each of the following: the overall clinical skills examination, the Humanistic Domain, and the Biomedical/Biomechanical Domain.

Results: Proctors identified 444 candidates who were left-handed (9.5% of all candidates). For overall pass rate, there was no significant difference between right-handed (94.2%) and left-handed (92.8%) candidates, $t(521.60)=1.076, P=.282$. For the Humanistic Domain pass rate, there was no significant difference between right-handed (96.3%) and left-handed (96.2%) candidates, $t(4696)=.147, P=.883$. For the Biomedical/Biomechanical Domain pass rate, there was no significant difference between right-handed (97.0%) and left-handed (95.9%) candidates, $t(514.62)=1.074, P=.283$.

Conclusion: We did not investigate inconsistent handedness (ambidexterity) or mixed-handedness (ie, throwing with right hand, but writing with the left). However, we observed that candidate handedness while writing SOAP Notes did not have a significant effect on the Humanistic Domain, Biomedical/Biomechanical Domain, or overall COMLEX Level 2-PE pass rates.

Acknowledgments: We wish to thank our proctors for identifying the research question and recording data throughout the testing cycle.

◆ ME4

Health Conditions Related to the Use of Unsanitary Water in Rural Mexico: A Study of Tamaula, Guanajuato, Mexico

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The purpose of the research project was to engage the population in the rural community of Tamaula, Guanajuato, Mexico in a short-term study of their health conditions related to the use of unsanitary water. Tamaula is a small, isolated rural ranching town located in the mountains near the city of Irapuato. The local Irapuato government does not provide Tamaula with many necessary public services, and the community has no public well or potable water and no sewage system or sanitary bathrooms. To better understand the impact

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of the use of unsanitary water in rural areas, each head of household in the rancho was interviewed individually or with family members to determine prevalent waterborne illnesses in the community and what age groups are affected. Data were collected from in-person household surveys and self-reported health problems to determine the number of people affected by health problems related to drinking the water and related to bathing in unclean water. Water was supplied to each household every 9 to 10 days from the city of Irapuato. This water was mainly used for drinking and cooking. Water from a water reserve to which was added powder lime stone was used for washing clothes and bathing; however, when the chlorinated water supplied by the city had been depleted, especially in large families, this water was used for cooking. From a total of 250 people, 30 individuals were reported to have a health condition related to the use of unsanitary water. A total of 20 people were affected with illnesses caused by drinking unclean water and 10 people were affected with skin irritations, reactions caused by bathing with unclean water. The age groups reported to be most affected by diseases were the youth (aged <10 years), individuals between ages 31 and 40 years, and elderly people (aged 51 years or older). The most common illnesses were gastritis, diarrhea, and skin irritations. Three people were reported to have hepatitis, and 1 person had Typhoid fever. It is clear that the lack of access to clean water and the use of unsanitary water lead to the contraction of waterborne diseases. The age groups affected by reoccurring illnesses were the children and the elderly. These individuals were part of large families who were obligated to use water from the water reserve. Although vaccines are administered to children by the government, waterborne disease are still common in rural Mexico indicating action need to be taken to better the public healthcare system.

◆ ME5

Exploring the Relationship Between Perceived Quality of Clinical Clerkship Hospital Site and NBOME Subject Examination Performance

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Background: Each year, New York College of Osteopathic Medicine of New York Institute of Technology (NYCOM) sends nearly 300 third-year medical students to more than 30 hospitals in and around New York. Upon completion of the clerkships, NYCOM obtains subjective feedback and evaluations of the clerkships from the students. Through evaluating yearly feedback data, it has been established that there is a disparity in perceived clerkship quality. Based on this feedback data, students often rank their choices of clerkship hospital sites. A computerized lottery system selects the final clerkship schedule for each student. Although not always given their

top choices, students must generally attend their assigned hospitals. Many students not rotating at their choice hospitals often feel disadvantaged regarding successful completion of the National Board of Osteopathic Medical Examiners (NBOME) subject examinations, as compared to their peers who are seemingly fortunate in rotating at their top choice hospitals.

Hypothesis: This study tests the hypothesis that the mean NBOME subject examination scores of students who completed their rotations at hospitals of perceived higher or lower quality will not be different than the class average.

Materials and Methods: The feedback data from the previous 3 years was gathered using a multi-modal clerkship assessment scale in which the hospitals were rated on 15 items using a 5-point Likert scale. The hospitals were then compared according to perceived clinical education quality. For each of the 6 core clerkships, the 3 highest-scoring and the 3 lowest-scoring hospital sites were used in the analysis. Mean NBOME subject examination scores at these hospital sites were calculated and compared against the mean NBOME subject examination scores of the entire class using regression analysis.

Results: The results indicate that there is no statistical significance between the mean NBOME subject examination scores of students who completed their rotations at hospitals of perceived higher or lower quality and the mean NBOME subject examination scores of the class as a whole.

Conclusion: The study lead to rejection of the null hypothesis. The results may be accepted as encouraging or even empowering to students who may be concerned about how the clinical education they receive may impact their examination scores. The findings suggest that the responsibility of earning good scores on the examinations lies with the student, regardless of the hospital site he or she attended.

◆ ME6

Awareness of Health Literacy–Related Issues Among Medical Students

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Background: Many patients have inadequate health literacy skills and are unable to understand basic health information. Lack of understanding results in increased hospitalizations, adverse health outcomes, medication errors, and higher medical expenses.

Hypothesis: Since poor health literacy is a significant and widespread problem affecting the health outcomes of patients,

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we expect that medical students have awareness about it.

Methods: An 11-item survey about health literacy was created and distributed to 600 first- and second-year medical students attending New York College of Osteopathic Medicine of New York Institute of Technology (NYCOM). The questions explored three areas: perceptions, factual knowledge, and intervention awareness. This project was approved by the NYCOM institutional review board. More than 100 surveys were returned and analyzed using SPSS.

Results: As a whole, students had accurate perceptions about health literacy 50% of the time. Around 60% of respondents answered factual knowledge questions correctly. The vast majority of students were not familiar with common interventions such as “Ask Me 3” and “Teach Back Method” that aim to overcome health literacy related barriers.

Conclusion/Discussion: The results of this study indicate that students are broadly aware of health literacy-related issues. However, despite resource intensive national campaigns, supposed solutions to health literacy-related problems have not made it to the medical education community. This study suggests that there is a need for including didactics in medical school curriculums that address strategies to use when interacting with patients who have poor health literacy skills.

◆ ME7

Ghanaian School Children’s Attitudes and Perceptions of Hygiene and Transmission of Infectious Disease

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Hypothesis: Worldwide, the leading infectious diseases in the pediatric population include lower respiratory infections, diarrheal diseases and malaria. It is accepted that early educational intervention can significantly improve children’s hygiene and health outcomes in these preventable diseases. Analysis of deaths for children under five demonstrates that the burden is heaviest in Africa, whose region accounts for 9 out of 10 worldwide child deaths due to malaria, 4 out of 10 child deaths due to diarrheal diseases, and 5 out of 10 child deaths due to lower respiratory diseases. Although attitudes, perceptions, and behaviors concerning infectious disease have been investigated in adult populations, the literature available on children’s understanding of infectious causes and transmission is minimal. This study will investigate the current beliefs, awareness and behavioral practices that Ghanaian school-aged children have regarding hygiene and infectious

diseases. The study also aims to evaluate the effectiveness of a health-education program, proposed by the Rohde Foundation (nongovernmental organization stationed in Oworobong, Ghana), targeting school-aged children. By improving children’s appreciation of the importance of proper hygiene in disease prevention and increasing their baseline knowledge regarding basic human anatomy, we hypothesize that the postprogram evaluation will show significant improvement in the children’s understanding of targeted infectious diseases and hygiene practices.

Materials and Methods: This is a prospective health and hygiene knowledge and behavior assessment which will be conducted before and after the educational program for school-aged children (5 to 17 years) during the course of the New York College of Osteopathic Medicine of New Jersey Institute of Technology research and medical mission in accordance with the Rohde Foundation. The proposed health program will focus on targeting school-aged children via “edutainment”—an entertaining educational program which will depict the importance of infectious disease and proper hygiene behavior.

Results: The survey data will be collected in June 2010 in Ohema, Awisasu, Afasu, and Oworobong I and II, the nearest surrounding villages of the Oworobong Health Clinic.

Conclusion: Given that needs assessment studies and early intervention are key to reducing the burden of disease, children’s health education has the potential to save millions of lives across the globe.

ME8

Edward Via College of Osteopathic Medicine Determinants of Health Survey and Outreach Strategies in El Salvador

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Hypothesis: The purpose of this research is to develop baseline data and related comparisons of healthcare needs in 5 selected rural underserved El Salvadorian villages in order to address prevention, treatment and continuous care. The research also develops trend analyses and model building leading to sustainable health.

Materials and Methods: A case study approach was used to collect data utilizing El Salvadorian Ministry of Health (MOH) promoters and VCOM doctors. The MOH survey instrument was used in Fall 2007 to collect information on health-related environmental factors; yet had the limitations of self-reporting by participants, lack of medical knowledge by promoters, and typically high school education or less. The MOH survey contained 5 sections including general, family demographics, health behaviors, family health and risk factors, and perceived health needs. Authenticated records from real-time patient care by VCOM doctors during one-

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week medical mission trips and continuous year-round care doctors provided in-depth and comparative data to consider, perceived health needs, and disease prevalence found by the MOH survey.

Results: Findings were derived from descriptive statistic and narrative analyses of the data. The findings provide meaningful data comparing perceived risk factors to disease incidence, core cluster health conditions across villages and targeted “hot spots” of prominent health issues. Mapping provided visual imagery for model interventions and appropriate level of care for sustainable health. Variables were analyzed to determine differences in gender, age, and other factors related to healthcare needs of segments of the populations.

Conclusion: Results benchmarked differences and common findings among the surveys in order to provide effective interventions such as treatment plans, medicines, education, research and outreach of various types. Outcomes include recommendations for improvement in utilization of Salvadorian VCOM faculty doctors in their regular village visits with the mobile clinic, and VCOM faculty and students during 1-week medical missions. Significance of the research is continuous improvement of health services and model approaches to health assessment and intervention for developing countries. Future research will continue to collect baseline data and analysis on model programs leading to continuous improvement of healthcare.

ME9

Edward Via College of Osteopathic Medicine Dominican Republic Emergency Medical System Survey and Training Workshop

Jeremy White, DO, PhD; James E. Powers, DO; Dean Sutphin, PhD; Dixie Tooke-Rawlins, DO

Edward Via College of Osteopathic Medicine, Blacksburg

Hypothesis: The Haiti Earthquake in January 2010 created an international response and highlighted the limited relief capabilities of both the nation of Haiti and the Dominican Republic, its neighbor on the island of Hispaniola. In response to this growing need for improved emergency medical services and disaster preparedness, the Edward Via College of Osteopathic Medicine (VCOM) has partnered with SESPAS, the Dominican Ministry of Health; and the PUNTACANA Foundation to conduct a survey of the Emergency Medical System in the Dominican Republic and provide Advanced Cardiac Life Support (ACLS) training to Dominican physicians. The purpose of this research was to assess the current state of Emergency Medical Services and the ACLS knowledge base of physicians working in the Dominican Republic and assess the impact of ACLS training on this knowledge base. Certified VCOM faculty members presented 3 days of ACLS training to 28 Dominican physicians employed at local hospitals. An Emergency Medical System survey was administered as a

pre- and post-test to all training workshop participants. The standardized survey instrument and researcher-developed component collected professional opinions from participating physicians regarding emergency medical response capabilities and tested physician knowledge base for core materials taught in ACLS.

Materials and Methods: The institutional review board approved the survey, and it was administered to participants before and after training, with identities coded to ensure confidentiality. Emergency Medical System Survey questions included general information such as degree, specialty area, years practicing medicine, medical school curriculum, and previous Emergency Medical Response training, as well as general information about the size, service, supplies, and facilities of each physician’s workplace. The survey requested physicians to report on their workplace disaster response plan and whether their workplace is capable of caring for patients in a selection of advanced cardiac conditions. The ACLS knowledge survey consisted of questions selected from the most recent American Heart Association ACLS precourse assessment. The posttest included a ACLS postsurvey and Likert-scale survey asking participants to rate the quality of the training and a narrative section soliciting response on barriers to implementation of knowledge gained as well as interest in future training opportunities.

Results and Conclusion: Survey results provide a benchmark of educational effectiveness of physician-led ACLS workshops in developing countries. This information will be beneficial to VCOM and SESPAS in designing future outreach or response efforts and to identify areas of need in the emergency medical response system. It provides a model for the next phase of training in the Dominican Republic.

Health Policy

◆ HP1

Knowledge and Opinions on End of Life Issues Among South Florida Residents

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Hypothesis: As a result of population aging and the increasing cost of healthcare, quality of life and end of life (EOL) care have become important medical and public health priorities. Planning for EOL can result in better patient outcomes and lowered healthcare costs. Despite this, many Americans do not create living wills, and hospice services are underutilized.

(continued)

◆ Indicates posters entered in the AOA Council on Research’s Student Poster Competition, a judged event that takes place during the poster session at the AOA Research Conference.

Little is known about what factors influence EOL care decisions. We hypothesized that selected demographics (ie, age, religious affiliation, and education) and previous knowledge and experiences with EOL care would influence patients' EOL planning (ie, EOL healthcare decisions and hospice utilization).

Materials and Methods: Using an observational, cross-sectional design, we recruited a community-based, ethnically diverse sample of 331 South Florida residents aged 18 to 84 years (mean [standard deviation], 44 [14.95] years) to complete an anonymous pen-and-paper questionnaire. Data were collected from community venues (eg, community centers, festivals, educational settings) from October 2009 to March 2010 to examine participants' knowledge and opinions on EOL issues, including planning for EOL, types of healthcare desired at EOL, knowledge and opinions on hospice services, and comfort discussing death and dying. Multiple regression was used to analyze the data.

Results: Regression models were constructed for the three outcome variables: (1) hospice care attitudes, (2) end of life care,

and (3) death and dying communication. Results showed that (1) older age, higher educational level, and previous knowledge of living wills and hospice services were associated with more favorable attitudes toward hospice care ($F=12.912$, $P<.001$); (2) older age, not having a religious affiliation, and prior knowledge of hospice care were associated with a preference for limited medical interventions at end of life ($F=8.855$, $P<.001$); and (3) higher educational level, having a living will, and prior knowledge of hospice care were associated with more comfort in communicating about death and dying ($F=18.812$, $P<.001$).

Conclusions: These findings show that having knowledge of EOL care issues (ie, knowing what hospice care is, hearing about or having a living will) may influence patients' planning for EOL care and hospice care utilization. Patient education on EOL care may increase utilization of hospice, enhance end of life planning, and improve outcomes for patients (eg, decreased pain, less dyspnea) and their families (eg, minimize conflict, decrease stress).

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