



50TH

Annual AOA Research Conference— Abstracts, 2006

This issue of JAOA—The Journal of the American Osteopathic Association (August 2006) contains abstracts for poster presentations that will be given at the 50th Annual American Osteopathic Association (AOA) Research Conference. Poster presentations will be provided by AOA research fellows (abstract series F), as well as osteopathic physicians, medical students, educators, clinicians, and researchers on the following general topics: osteopathic manipulative medicine and osteopathic principles and practice (series P), clinical studies (series C), basic sciences (series B), and medical education (series M).

The 2006 AOA Research Conference will take place from Monday, October 16, to Wednesday, October 18, during the AOA's 111th Annual Convention and Scientific Seminar in Las Vegas, Nev. The theme of this year's conference is "Osteopathic Medicine in the American Health Care System." For more information on the Annual Convention and Scientific Seminar, please see the DO-Online Web site (<http://www.DO-online.org>).

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AOA Research Fellowships F01

Determining the Intra-observer Reliability of the Evaluation of Cranial Strain Patterns

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Purpose: To perform a prospective, randomized, single blinded, observational study to determine the intra-observer reliability of common palpatory tests used to diagnose strain patterns of the cranium.

Methods: Two board certified Neuromusculoskeletal Medicine/Osteopathic Manipulative Medicine (NMM/OMM) specialists examined 24 subjects each subdivided into three diagnostic groups of eight subjects: those with a history of headaches, with asthma, or with neither (healthy control group). Examiners diagnosed each subject's cranial rhythmic impulse (CRI) rate, cranial strain pattern (CSP), and quadrants of restriction (QOR). To blind the examiners, the subjects were divided into subgroups based on hair length and style. Examiners evaluated four subjects at a time with each subject being evaluated three times per session in a random order. Kappa coefficients (κ) were used to measure intra-observer reliability.

Results: Overall, the cranial strain pattern diagnosis showed the highest reliability ($\kappa=0.67$). Within individual diagnostic groups, the cranial strain pattern diagnosis showed the highest reliability for the healthy group ($\kappa=0.82$), followed by headache

($\kappa=0.67$) and asthma ($\kappa=0.52$) groups. CRI rate showed only fair reliability ($\kappa=0.23$). CRI rate by diagnostic groups, asthma had the lowest reliability ($\kappa=0.10$), while headache and healthy groups had fair reliability ($\kappa=0.23$ and 0.29 respectively). The three of the four quadrants of restriction (QOR) showed moderate overall reliability ($\kappa=0.44-0.52$), while the left posterior quadrant had only fair reliability ($\kappa=0.33$). In both the headache and healthy groups, the left anterior quadrant showed substantial reliability ($\kappa=0.60$ and 0.61 respectively) while the left posterior quadrants showed fair reliability in all three groups.

Comment: There are no previous studies on the intra-observer reliability of CSP. These results indicate good intra-observer reliability can be obtained when evaluating cranial strain patterns. Yet the intra-observer reliability for CRI and QOR were found to be only fair. This finding for intra-observer reliability for CRI was consistent with previous studies. Now that intra-observer reliability has been documented, studies need to be done to establish the inter-observer reliability for QOR, CRI and CSP.

Acknowledgment: Supported through a grant from the AOA (#F03-08).

F02

Intraocular Pressure Correlated With Osteopathic Manipulative Treatment

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◆ Indicates posters entered in the Student Prize Competition, a judged event that takes place during the Poster Session at the Research Conference.

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Purpose: To determine if a relationship exists between intraocular pressure (IOP) and osteopathic manipulative treatment (OMT) in patients with and without glaucoma.

Materials and Methods: Patients with primary open angle glaucoma (POAG) (n=76 eyes) and patients without POAG (n=58 eyes) were randomized into a treatment group, a sham-treatment group, and a control group. The treatment and sham-treatment groups had IOP measurements immediately prior to and after OMT, and again one week later. The control group had IOP measurements once, and again one week later. Each pressure measurement for a given patient was performed by the same person who was blinded to the group the patient was assigned. All pressures were measured using a Goldman tonometer.

Results: Using the independent-samples t-test, patients with POAG had a significant difference between treatment and sham groups (treatment (mmHg), 18.30 ± 4.78 ; sham, 16.34 ± 3.90 ; $P < .013$). There was no significant difference between treatment and control groups (treatment, 18.30 ± 4.78 ; control, 17.20 ± 3.22 ; $P < .193$) and sham and control groups (sham, 16.34 ± 3.90 ; control, 17.20 ± 3.22 ; $P < .255$). For patients without POAG, there was a significant difference between treatment and sham groups (treatment, 13.85 ± 2.32 ; sham, 15.52 ± 1.86 ; $P < .000$) and treatment and control groups (treatment, 13.85 ± 2.32 ; control, 16.25 ± 1.88 ; $P < .000$). There was no significant difference between sham and control groups (sham, 15.52 ± 1.86 ; control, 16.25 ± 1.88 ; $P < .104$).

Conclusions: There was a significant difference between treatment and sham-treatment groups for both patients with and without POAG; however, there was no significant difference between treatment and control groups and sham-treatment and control groups for patients with POAG. There was a significant difference between treatment and control groups and sham-treatment and control groups in patients without POAG.

F03

The Role of Osteopathic Manipulative Treatment in the Treatment of Fibromyalgia Syndrome

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Hypotheses: Adding Osteopathic Manipulative Treatment (OMT) to the treatment of Fibromyalgia Syndrome (FMS) would: decrease the effect FMS had on the patient's activity shown by a decreasing Fibromyalgia Impact Questionnaire (FIQ) score; decrease the amount of symptoms and syndromes the patient experienced shown by a decreasing Symptoms and Associated Syndromes Questionnaire (SASQ) score; decrease the amount of time it would take the patient to walk

75 feet; and decrease the amount of pain medication the patient required.

Materials and Methods: Nine possible subjects were recruited. Of these nine, five were enrolled. Of the five enrolled subjects, four completed the protocol. One subject dropped out for personal reasons. Each subject was evaluated and treated for 8 weeks. Each week, the patient filled out the FIQ and the SASQ and recorded their medication use in a diary. On weeks one and eight, the amount of time it took the subject to walk 75 feet was recorded. Osteopathy in the Cranial Field (OCF) treatment was given weekly. The OMT protocol was designed to address somatic dysfunction in the sphenobasilar synchondrosis, occipital-atlantal and atlantal-axial joints, 2nd cervical vertebrae and sacrum. The clinical endpoint of the model was improved symmetry, amplitude, vitality, and rate of the Cranial Rhythmic Impulse. All findings were recorded.

Results: Two of the four subjects had decreases in their FIQ scores from 28 to 14 and from 80 to 23. All four subjects had decreases in their SASQ scores from 29 to 25, from 31 to 29, from 37 to 30, and from 42 to 30. All four subjects also decreased the amount of time it took them to walk 75 feet with the decrease ranging from 1.5s to 10.1s. One subject showed a dramatic decrease in pain medication use going from using 2 medications to not using any medication. The remaining subjects varied in medication use.

Conclusion: This study supported the hypotheses, by showing a reduction in all proposed categories, but due to the small number of subjects, statistical significance cannot be properly assessed. The results suggest that OCF may be of benefit for patients with FMS. Expanding this study over a longer period of time, with a larger subject pool will provide definitive answers as to the benefit of OMT in FMS.

F04

Immediate Effects of Osteopathic Manipulative Treatments on Immune Function in a Healthy Population: A Pilot Study

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Objectives: The purpose of this pilot study was to investigate the immediate effects of Osteopathic Manipulative Treatment (OMT) on immune function in a healthy population.

Methods: This was a prospective, randomized, blinded and controlled clinical trial. Fifty healthy individuals, ages 18 to 40, were recruited primarily from the UNTHSC campus. Exclusion criteria included any disease, treatment or habit that influences immune function and any condition for which OMT is contraindicated. Subjects were randomly assigned to one of two groups: OMT or Rest (control). Blood and saliva samples were collected pre- and post-intervention (thirty minutes of OMT or Rest). Samples were analyzed for a complete blood count (CBC) with a differential using a Coulter Counter, salivary IgA using ELISA, and B-lymphocyte percentages, and T-lymphocyte sub-population concentrations: CD4, CD8 and natural killer cells using flow cytometry.

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Results: Fifty subjects were recruited, 25 in each group. This study demonstrated the feasibility of this protocol. Additionally, strong relationships for future collaborations were developed. No statistically significant differences in outcome measures were identified between the two groups, nor were any apparent trends identified.

Conclusion: Although this study proved the feasibility of this protocol and established a solid framework for future research, further studies must be conducted. It is imperative that the efficacy of OMT in acute and chronic infection, chronic pain, and immunocompromised populations continued to be studied. Animal studies have proven to be insightful, and should continue to provide valuable information on the mechanism of action of OMT. In addition, the long term results of osteopathic manipulative treatments over a longer period of time should be examined. This pilot study offers valuable information that should direct future studies.

Acknowledgment: This study was funded by the Osteopathic Research Center and approved by the UNTHSC Institutional Board.

Osteopathic Manipulative Medicine/Osteopathic Principles and Practice P01

The Effect of CV-4 Upon Cutaneous Bloodflow Velocity

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The CV-4 procedure purports to affect the cranial rhythmic impulse, a phenomenon that has been shown to be concomitant with the low-frequency Traube-Hering (TH) oscillation in blood flow velocity. This study examines the affect of the CV-4 upon blood flow velocity. Fourier-transform spectra were extracted from laser-Doppler flowmetry of each of three component parts of the experimental CV-4 procedure (Control, Treatment, Response). The component spectra are compared statistically in each of three low-frequency and four high-frequency spectral parameters derived therefrom. Also included in the analysis are the mean frequency of the dominant flowmetry (TH) wave, and the mean duration of the CV-4 procedure. Human subjects (informed consent obtained) were paired with 28 individual physicians for the performance of the CV-4 procedure. Flowmetry records (Transonic® Laser-Doppler; BLF21, Ithaca, NY) were obtained tracking the course of the procedure, 20 of which were useable for inter-group comparisons. Fourier transform difference spectra were compared statistically using the One-Way ANOVA (SPSS Inc, Chicago, IL). The mean frequency of the TH wave-form visible in the blood flowmetry record was 7.10 ± 2.07 cpm. The mean CV-4 procedure length was 4.43 ± 2.22 minutes. The CV-4 procedure was shown specifically to affect the low-frequency oscillations in blood flow velocity. Following its application, the amplitude of the TH wave increased

[0.10 Hz frequency relative area units: control minus treatment (0.08010 units) compared with control minus response (-0.03358 units), $P=0.011$]. It is concluded that cranial manipulation affects the autonomic nervous system because the TH waves have been demonstrated to be mediated through autonomic activity (Akselrod et al, 1985). Since palpation alone does not greatly effect blood flow velocity oscillations, there is a quantifiable difference between palpation alone and cranial treatment utilizing the CV-4 procedure. It can be said that the practitioners of cranial manipulation who participated in this study affected their subjects in a quantifiable manner with the CV-4 procedure.

[Akselrod S, Gordon D, Madwed JB, Snidman NC, Shannon DC, Cohen RJ. Hemodynamic regulation: investigation by spectral analysis. *Am J Physiol.* 1985;249(4 pt 2):H867-H875.]

P02

Osteopathic Manipulative Medicine for Carpal Tunnel Syndrome: Changes in Nerve Conduction

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Purpose: The goal of this clinical trial was to assess for physiologic and anatomic changes in CTS in response to OMT.

Methods: This prospective, randomized, controlled, clinical trial (RCT) included two experimental groups, OMT and placebo sub-therapeutic ultrasound. Adults were enrolled who were between 21 and 70 with a clinical diagnosis of CTS and increased conduction latency (slowed conduction) of the median nerve. Outcome measures were changes in median motor and sensory distal latencies measured by Nerve Conduction Studies (NCS). Subjects receive six treatments. NCS were taken at entry to the study (baseline), midpoint, and endpoint.

Results: Thirty-seven of a planned 50 subjects were randomized to groups. Thirty-one subjects were included in the final data analysis. Preliminary analysis found no significant difference in NCS values over the three testing intervals. Analysis of 15 subjects with a single treatment provider found significant improvements in some NCS for the OMT group.

Conclusions: The results of this study indicate possibility for improvement of CTS with OMT, but no conclusive statements about the efficacy of OMT can be made. This preliminary study enabled us to identify multiple areas in the research design and methodology that could be improved, and provides the framework for future studies. This study contributed to achieving a 3-year NIH-NCCAM R21 to study OMT for CTS in a larger number of subjects.

◆ Indicates posters entered in the Student Prize Competition, a judged event that takes place during the Poster Session at the Research Conference.

P03

Operator Differences in the Use of Thoracic Lymphatic Pump Technique in Persons With COPD

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Hypothesis: There will be no significant difference between operators using a thoracic lymphatic pump technique in persons with COPD.

Methods: A standard description of the thoracic lymphatic pump technique was developed. Operators A and B reviewed and practiced the protocol several times together at the onset of a study testing the effects of manipulation techniques on pulmonary function parameters in persons with COPD. Pulmonary function parameters were measured at baseline and 30 minutes after a five-minute session in which this standardized thoracic lymphatic pump technique was applied. Subjects were randomly assigned.

Results: Each operator treated 12 subjects. The mean residual volume (RV) decreased by 243 ml ±281 (standard deviation) for operator A, but increased by 144 ml ±455 for operator B, *P*=0.0094. The mean total gas volume (TGV) decreased by 214 ml ±150 for operator A and increased by 88 ml ±391 for operator B, *P*=0.0193. The mean total lung capacity (TLC) decreased by 258 ml ±256 for operator A, and increased by 99 ml ±376 for operator B, *P*=0.0153. The mean forced expiratory volume in one second (FEV1) decreased by 85 ml ±0.11 for operator A and decreased by 20 ml ±97 for operator B, *P*=0.0458. Post study review found differences in how operators A and B interpreted and used the protocol technique during the study. Operator A used progressive compressive forces on exhalation, with slow release on inhalation, while operator B used steady pumping throughout, without compressive forces.

Conclusion: The “compressive” operator A version of the technique reduced lung volumes in persons with COPD, while version B did not. Differences in how techniques are applied can lead to significant differences in physiologic effects. This highlights the need for stricter protocol standardization in research, and the need to study different versions and applications of osteopathic techniques. Lines of research organized in this manner will begin to sort out how to perform OMT in order to maximize physiological effects.

Acknowledgment: Supported by an Osteopathic Heritage Foundation grant.

◆ P04

The Immediate Effects of the Splenic Pump Technique on Blood Cell Counts in Normal Adults

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Hypothesis: The splenic pump technique will alter blood cell counts in healthy adults at 30 and 60 minutes posttreatment relative to a sham treatment. The technique will increase leukocyte and reticulocyte counts, decrease hemoglobin, hematocrit, erythrocyte and platelet counts, and change differential cell counts, but not change blood cell indices.

Materials and Methods: This was an unblinded IRB-approved study in which normal adults (age 20–67, n=107) were randomized to receive a splenic pump (n=52) or sham treatment (n=55). The treatment group received 100 repetitions of the splenic pump over a five-minute period. The sham group received light touch for five minutes. Blood for CBC and reticulocyte counts was drawn before treatment and at 30 and 60 minutes posttreatment. Analysis consisted of non-parametric ANCOVA.

Results: Compared to the sham group, the treatment group demonstrated lower MCVs at 30 minutes posttreatment [treatment median (1st quartile, 3rd quartile) 88.1 fL (86.3, 91.7); sham 88.7 fL (86.4, 91.0); *P*=0.01] and higher neutrophil counts at 30 minutes posttreatment [treatment 3.7×10^3 cells/mm³ (2.9, 4.6); sham 3.2×10^3 cells/mm³ (2.4, 4.2); *P*=0.02]. At 60 minutes posttreatment, the treatment group had higher neutrophil percents [treatment 58.1% (53.8, 61.6); sham 54.1% (48.9, 60.2); *P*=0.02], lower lymphocyte percent [treatment 31.3% (28.1, 35.5); sham 34.3% (28.9, 40.4); *P*=0.01], and higher basophil counts (all medians and quartiles 0 cells/mm³; treatment mean ± standard deviation $0.02 \pm 0.04 \times 10^3$ cells/mm³; sham $0.01 \pm 0.02 \times 10^3$ cells/mm³; *P*=0.05). There were no other statistically significant differences between the groups.

Conclusions: In normal healthy adults, the splenic pump did not alter leukocyte, reticulocyte, erythrocyte or platelet counts, hemoglobin or hematocrit levels as hypothesized. There were statistically significant differences in MCV, neutrophil percents and counts, lymphocyte percents and the basophil counts, but these modest changes may not constitute clinically meaningful differences in a healthy population. Subsequent studies with the splenic pump in a non-healthy population would enhance our understanding of the clinical relevance of the technique.

Acknowledgment: Supported by the ATSU Strategic Research Initiative, A.T. Still Research Institute.

◆ P05

The Immediate Effects of the Splenic Pump on Serum Level of C-reactive Protein in Normal Adults

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◆ Indicates posters entered in the Student Prize Competition, a judged event that takes place during the Poster Session at the Research Conference.

Hypothesis: Splenic pump technique will cause increased serum C-reactive protein (CRP) levels in normal adults when compared to sham treatment.

Materials and Methods: In this IRB-approved unblinded study, normal adults (age 20–67, n=107) were randomized to receive the splenic pump technique (n=52) or light touch (n=55) for five minutes. The splenic pump consisted of 100 bimanual compressions and relaxations of tissues overlying the spleen. The serum level of CRP was measured at baseline, and at 30 and 60 minutes posttreatment. A non-parametric ANCOVA was used to test for statistical differences between the groups, and Friedman's tests were used for within-group comparisons.

Results: The median (first quartile, third quartile) CRP levels in the treatment group were 0.428 mg/L (0.192, 1.956) at baseline, 0.512 mg/L (0.180, 1.943) at 30 minutes post-treatment, and 0.460 mg/L (0.182, 1.766) at 60 minutes post-treatment. The corresponding medians in the sham group were 0.440 mg/L (0.150, 2.040), 0.480 mg/L (0.142, 2.025), and 0.446 mg/L (0.140, 2.195). The changes within the treatment group and the sham group were not significant ($P=0.30$ and $P=0.06$, respectively). There was no statistically significant difference in the median serum levels of CRP between the treatment group and the sham group ($P=0.46$ and $P=0.80$ at 30 minutes and 60 minutes, respectively).

Conclusions: The splenic pump technique does not appear to change the level of C-reactive protein at 30 or 60 minutes post-treatment compared to sham treatment.

Acknowledgment: Funded by ATSU Strategic Research Initiative, A.T. Still Research Institute.

◆ P06

CV-4 Induced Physiological Change as Measured by Transcutaneous Laser Doppler Flowmeter

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Background: Osteopathic manipulation techniques, such as Compression of the Fourth Ventricle (CV4), influence autonomic balance. Prior studies demonstrate that laser Doppler flowmeter (LDF) accurately measures autonomic balance by measuring the Traube-Hering (TH) wave (.08-.15 Hz) of peripheral blood flow velocity. Authors investigated the ability of CV4 technique to alter autonomic balance.

Objective: To evaluate whether the CV4 technique alters autonomic balance as measured by the LDF in comparison to rest and touch (sham) only.

Methods: 25 healthy subjects (convenience sample) gave informed consent to participate. LDF measured skin blood flow velocity before, during and after the CV4 technique. 18/25 subjects experienced both a sham and a CV4 technique, 7 experienced a CV4 only. Lying horizontal, a five min. baseline was recorded, followed by a five min. sham procedure, five min. resting interval, five min. CV4 technique and a final five

min. post technique resting period. Three minutes of data were selected from each time frame. A spectral analysis was performed using DFT for each time frame and compared to other time frames using a paired sample t-test.

Results: There is a significant difference in autonomic balance during the CV4 ($P<.000$). There is no significant difference between the baseline and post technique autonomic balance ($P<.645$), nor between baseline and the sham procedure ($P<.727$), or between the sham procedure and post technique ($P<.630$). The 08-.15 Hz component of blood flow velocity greatly decreased in amplitude in 24/25 subjects during a CV4 technique. The duration of altered autonomic balance during the CV4 was also longer than any change in autonomic balance measured at rest, during sham, or after the interventions ($P<.000$). Mean time frame of decreased amplitude of TH wave during CV4 is 64.6 seconds, compared to 11.68 seconds during rest and 14.92 seconds for sham.

Conclusion: Decreased autonomic balance and its duration during CV4 are measurable and distinctly related to the technique. The LDF can quantify the therapeutic intervention known as the CV4 technique and differentiate its characteristic physiologic effect from resting state and sham interventions.

◆ P07

Validation of Transcutaneous Laser Doppler Flowmeter in Measuring Autonomic Balance

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Background: Transcutaneous Laser Doppler Flowmeter (LDF) is a non-invasive device to measure the Traube-Hering (TH) wave and heart rate. TH component represents baroreceptor activity; changes in TH reflect changes in autonomic balance. Heart rate variability (HRV) measured by EKG is the "gold standard" for measuring autonomic balance. To evaluate the validity of LDF as a device for measuring autonomic balance, it needs to be compared to the gold standard.

Objective: Evaluate LDF's sensitivity and specificity in measuring autonomic balance.

Methods: Blood flow velocity obtained from LDF and RRI from EKG were recorded from 22 healthy individuals (convenience sample) before, during, after provocative procedures. Head-Up Tilt Test and Cold Pressor Test were employed as standard provocative autonomic procedures. While supine, a five min. baseline recording was taken prior to tilt test. Subjects were passively raised 80° for five min. recording, and passively returned to supine for five min. recording. Cold Pressor Test was performed: immersion of subject's hand in cold water (10°C) for 60 sec. A five min. post provocation recording fol-

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lowed. HRV was analyzed from RRI using Fourier Transform. The spectral analyses from EKG were compared to spectral analyses of autonomic (TH) component of LDF wave. Subjects signed IRB approved informed consent prior to participation.

Results: Calculation software packages were used to quantify data gathered from spectral analyses. Using a paired-samples T-test, HRV calculated from EKG and cardiac component of LDF wave demonstrated a correlation of .97 ($P<.00$); this reflects LDF's ability to detect RRI with accuracy. TH component of LDF wave (.08-.15Hz) when compared with low frequency component of EKG/HRV (.08-.15Hz) demonstrated a correlation of .712 ($P=.00$); this reflects simultaneous changes between TH component of LDF wave and HRV. LDF is 95.45% sensitive and 90.91% specific in detecting changes in autonomic balance.

Conclusion: LDF is sensitive and specific in detecting autonomic balance changes. Cardiac and autonomic (TH) components of LDF wave accurately assess and quantify changes in autonomic balance.

◆ P08

Inhibitory Rib-Raising With Direct Sympathetic Nervous System Measurement

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The systematic evaluation of osteopathic manipulative therapy (OMT) on sympathetic neural activity (SNA) is limited. A specific OMT technique (inhibitory sympathetic rib-raising) has been theorized to regulate autonomic imbalance by applying direct pressure to the head of the rib near the sympathetic ganglion. This technique has historically been described to initially produce sympathoexcitation followed by a sympatholysis, yet no work has been published documenting this effect. An initial study in our lab found no change in SNA in healthy individuals who had low basal SNA. We hypothesized that rib-raising applied while SNA is elevated will effectively modulate SNA.

Methods: We used a cold stimulus to produce a pain-mediated elevation of the SNA which allowed us to determine whether OMT can directly reduce a state of elevated SNA. Fifteen healthy subjects by history and physical examination who were naive to OMT techniques and proposed effects were recruited. IRB approval was obtained. SNA was assessed using standard microneurography. Data were recorded continuously on a computer analysis system. After instrumentation and an initial 20 min period of rest, subjects were exposed to a two-minute cold stimulus (CS) by submersion of the hand in water randomized at 2°C, 10°C and 18°C. During each stimulus sustained inhibitory rib-raising or sham OMT was applied. The sustained inhibitory rib-raising OMT was performed for

2 min in an attempt to affect the thoracic sympathetic chain ganglia. Sham OMT was performed by placing the hands in the same position without the concurrent anterolateral force. Data recording began two-minutes prior to the stimulus, during the stimulus and for a four-minute recovery period. All data were reduced post hoc via a customized digital data acquisition system.

Results and Conclusions: There was no significant difference between the treatment groups ($P>0.05$) suggesting that the OMT did not directly affect sympathetic nervous system activity. However, there was a trend towards an initial sympathoexcitation ($P<0.11$) during the 10° cold-pressor stimulus. Additional subject recruitment to complete the study is currently ongoing and will achieve appropriate statistical power.

P09

Examiners Prioritize Displacement Over Pressure and Force Measurements When Diagnosing Rotational Thoracic Vertebral Motion

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Objectives: To quantify rotational vertebral motion testing and to determine how these measurements correlate with examiners' diagnoses.

Methods: Twenty examiners evaluated multiple thoracic vertebrae for rotational motion preference on one of seventeen subjects. Motion testing occurred through a pressure sensing pad used to obtain maximum pressure (kg/cm²) and force (Newtons) generated. Simultaneously from the head of the examination table, digital video imaging was collected so that reference points on the palpating thumbs could be seen throughout the motion testing process and used to determine the displacement (mm) of the thumbs relative to baseline. Examiners' thumbs identified the transverse processes of randomly chosen thoracic vertebrae. Anterior pressure with the right and then left thumbs were applied in an alternating fashion for two trials on each transverse process. Baseline and peak pressures and forces generated by each thumb were determined, synchronized with displacement measurements, and compared to examiners' observations.

Results: Maximum pressure, force, and displacement were determined for both thumbs individually and for the first and second trials per segment. The overall mean maximum pressure and force were 0.52 kg/cm² (95% CI: 0.37-0.66) and 24.6 N (95% CI: 16.7-32.6). There was no statistically significant difference between the thumbs on the pressure or force applied ($P=0.53$ and $P=0.15$, respectively) or when comparing first and second trials ($P=0.28$ and $P=0.43$, respectively). The overall mean for displacement was 8.3 mm (95% CI: 6.8-9.8). There were statistically significant differences in displacement between the two thumbs (1.2 mm; $P<0.001$) with the side of least displacement corresponding to the clinical diagnosis. There also was a statistically significant increase in displacement

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on both sides between trial 1 and 2 (0.9 mm; $P < 0.001$).

Conclusions: The results indicate that when osteopathic clinicians are evaluating segmental vertebral rotational motion, they tend to maintain similar thumb force and pressure during testing and focus on the secondary degree of displacement to determine diagnosis.

◆ P10

The Effects of OMT on Median Nerve Size and Function in Patients with Carpal Tunnel Syndrome

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Carpal Tunnel Syndrome (CTS) is the most common entrapment neuropathy. Conservative treatment includes NSAIDs, wrist splints, and exercises. Diagnosis can be made by using nerve conduction studies (NCS), or by showing median nerve swelling with ultrasound (US). In numerous studies OMT improved pain and Phalen tests, and was linked in pilot projects to trends in NCS improvement and decreased median nerve swelling.

Hypothesis: The addition of OMT to standard medical therapy for CTS will: decrease median nerve cross-sectional area (CSA), decrease Symptom Severity Scale (SSS) scores, and decrease Functional Status Scale (FSS) scores.

Methods: 23 patients with mild-to-moderate CTS diagnosed by NCS in 39 wrists were randomized into OMT ($n=21$) or sham laser ($n=18$) groups. US measurements of all median nerves were obtained, and all patients completed SSS and FSS prior to treatment. These subjects were provided eight weekly sessions of OMT (MFR/BLT) or sham laser. All patients were provided wrist splints, exercises, and ibuprofen if not contraindicated. Survey data on pain level, medication use, splint wearing, and exercises were recorded weekly. NCS, US, SSS, and FSS data were collected after the last treatment.

Results: There was a positive correlation between US measurements of the median nerve CSA and the severity of CTS as diagnosed by NCS ($P=0.012$). Comparison of pre-, post-, and post- minus pre-treatment groups revealed no significant difference in median nerve CSA. Regression analysis of the US measurements and SSS and FSS scores showed an increase in median nerve CSA was related to an increase in SSS in the laser post-treatment group ($P=0.007$). There were no significant changes in the OMT group. Head to head analysis of the post-treatment groups showed significantly higher SSS scores ($P=0.013$) and FSS scores ($P=0.005$) in the sham laser group.

Conclusions: While US was shown to correlate with the diagnosis of CTS, it did not detect any significant change in median nerve CSA in patients receiving OMT or sham laser after eight treatments. Weekly OMT was shown to slow, if not halt, the progression of symptoms and the decline in function in patients with CTS, while those receiving sham laser treatments had more symptoms and worse function.

P11

Effect of OMT on Myofascial Somatic Dysfunction and Objective Parameters of Severity in Patients with Carpal Tunnel Syndrome

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Background: Carpal Tunnel Syndrome (CTS) is a common entrapment neuropathy. Current conservative treatments are NSAIDs, wrist splints, and exercises. Myofascial trigger points (MTrPs), painful hyperirritable areas in skeletal muscles that can refer pain, decrease range of motion, and cause altered neural-vascular-lymphatic function, have been associated with CTS. In numerous studies, osteopathic manipulative treatment (OMT) has improved pain and Phalen's test measures. OMT has also been linked to decreased median nerve swelling and to trends in nerve conduction study (NCS) improvement.

Methods: Patients with mild-to-moderate CTS diagnosed by NCS were randomized into OMT ($n=14$ wrists) or sham laser ($n=14$ wrists) groups. Ultrasound measurements of the median nerve were obtained from all subjects. The subjects were provided 8 weekly treatments of pan-corporeal OMT (myofascial release/balanced ligamentous tension) or forearm sham laser. All patients were provided wrist splints, hand exercises, and ibuprofen if not contraindicated. At the time of the NCS, MTrPs common in CTS were recorded and scored (0=none; 1=inactive; 2=latent MTrP; 3=active MTrP) at baseline and 1 week after final treatment. Survey data on pain level, NSAIDs and splint use and exercises were recorded weekly throughout.

Results: There were 29 hands with a positive NCS (14 in OMT group, 14 in sham group, 1 excluded for severe CTS rating). There were also 12 symptomatic hands with negative NCS. There was no difference in total MTrP load in subjects with ($n=29$) or without ($n=12$) a positive NCS ($P=0.23$). A statistically significant reduction in total MTrP load was achieved in the OMT group ($P < 0.01$). Conversely, conservative+sham care resulted in more myofascial dysfunction with "no myofascial activity" shifting towards more inactive ($P=0.01$) and latent ($P=0.001$) MTrPs. The adductor pollicis is the most abundant MTrP ($P < 0.01$). Individual patient analysis was highly suggestive ($P=0.06$) of a trend towards MTrP reduction in those treated with OMT compared to the individual treated with sham laser.

Conclusion: OMT is successful in reducing MTrP load while those receiving standard care alone got significantly worse. This study suggests addition of OMT would benefit standard conservative care for mild-to-moderate CTS.

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◆ P12

Effectiveness of Osteopathic Manipulative Treatment for Parkinson Disease

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Hypotheses: We predict a significant difference in Unified Parkinson Disease Rating Scale (UPDRS) and Quality of Life Inventory (QLI) scores in favor of Parkinson Disease patients who receive OMT.

Methods: We used a randomized, single blind, IRB approved, placebo-controlled parallel-group design. Twenty-seven PD patients were randomized with 14 placed in the treatment and 13 in the placebo (sham) group. All participants were given the same pretest measures of symptoms (UPDRS) and quality of life (QLI). Experimental participants received standardized OMT that included soft-tissue articular and muscle energy techniques. Sessions were 30 minutes 1 day per week for 6 weeks. The control group received a sham treatment consisting of standard OMT diagnostic techniques alone. All participants were re-administered the same pretest measures to assess effectiveness of OMT at the end of the study.

Results: Mean pretest UPDRS scores were 27 (n=14, SD=9) for the treatment group and 36 (n=11, SD=17.8) for the control group. Mean posttest UPDRS scores for the treatment group were 24 (n=14, SD=8.6) and 32 (n=11, SD=18.4) for the control group. Pre-post UPDRS scores were not statistically significant by ANCOVA, $F(2,24)=0.183$, $P=.834$. Pre-post estimated marginal means for the UPDRS treatment group were 3 (n=14) and 4 (n=11) for the control group. Mean pretest QLI scores were 65 (n=14, SD=18) for the treatment group and 78 (n=11, SD=28) for the control group. Mean QLI posttest scores for the treatment group were 61 (n=14, SD=17) and 72 (n=11, SD=26) for the control group. QLI pre-post scores were not statistically significant by ANCOVA, $F(2,24)=0.702$, $P=.506$. Pre-post estimated marginal means (EMMs) for the QLI treatment group were 2 (n=14) and 7 (n=11) for the control group. Mean likert scores on the outgoing patient survey for the control group was 25 and 34 for the treatment group.

Conclusions: EMMs reveal a trend towards improvement that is clinically significant and will inform treatment. Comments often revealed that participants found OMT most useful for increased mobility. This medical benefit derived from the OMT appeared to be retained and transferred to other ADLs. Future studies will examine this in greater detail using a larger sample size and greater demographic heterogeneity.

**Clinical Studies
C01****Tai Chi and Low Impact Exercise Reduce Functional Limitations in the Elderly**

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Introduction: The Nagi Model of Disablement suggests that active pathology leads to physiological impairment, then to functional limitations and finally to disability. However, recent data suggest that physical inactivity is on par with chronic disease as a cause of disability, and that increased physical activity is associated with higher levels of functional mobility. Studies show that increased physical activity, even when begun late in life, results in improved physical fitness and functional ability.

Methods: Baseline assessments on 84 community-dwelling individuals over the age of 50 were conducted. The physical assessment consisted of the following tests: grip dynamometer (upper body strength), chair stand (lower body strength), sit-and-reach (flexibility), up-and-go (dynamic balance and agility) and the two minute step (endurance). Participants also completed a self report questionnaire on functional limitations. After the assessment, participants were randomized into one of three groups: tai chi (31), low impact (30), or control (23). Participants assigned to exercise groups were expected to attend three one-hour classes per week over the 12 week intervention. 12 people withdrew (14.3%), most for medical reasons unrelated to the study. After week 12, all physical measures were repeated on the 72 remaining participants.

Result: Better grip strength, balance, lower body strength, endurance, and less difficulty carrying heavy items were evident in the low-impact group compared to the Tai Chi group. Participants in the Tai Chi group reported higher subjective health and more energy. Both exercise groups performed better than the controls on most measures of functional limitations.

Conclusion: Compared with the control group, both low-impact exercise and Tai Chi are safe and cost-effective ways to reduce both self-reported and performance-based measures of functional limitations.

C02**Diastolic Dysfunction: Congestive Renal Failure?**

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Background: The majority of cases of volume overload arise from heart failure (HF), which represents one of the leading causes of hospitalizations among the elderly in the United States. When patients have normal ejection fraction and evidence of left ventricular hypertrophy (LVH) on echocardi-

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gram, they are commonly labeled as having diastolic dysfunction. We evaluated the prevalence of chronic kidney disease (CKD) among patients with presumed diastolic dysfunction. It was our hypothesis that patients with diastolic dysfunction had CKD and dysregulation of the renin-angiotensin-aldosterone system (RAAS) as the cause of their fluid overload and LVH.

Methods: The trial included 116 subjects at a community-based hospital with presumed diastolic dysfunction on echocardiogram in the absence of cardiac arrhythmias or significant valvular disease. The Modification of Diet in Renal Disease (MDRD) equation was used to estimate the glomerular filtration rate (GFR) among eligible subjects.

Results: The mean estimated GFR among patients with diastolic dysfunction was 49.16 ml per minute per 1.73 m². Despite the average serum creatinine level of 1.43 mg/dl, 91 (78.4%) patients met the estimated GFR criteria for CKD with an estimated GFR of <60 ml per minute per 1.73 m². The average GFR among non-diabetic patients was 53.7 ml per minute per 1.73 m², while the average GFR among diabetics was 45.7 ml per minute per 1.73 m².

Conclusions: A disproportionately high number of patients with diastolic dysfunction met the criteria for CKD. Cardiovascular disease is more prevalent with declining renal function. The pathophysiology of volume overload may be more than simple restrictive filling of the heart. A breakdown in the RAAS causes increased salt and water reabsorption which is directly linked to volume overload seen in patients with characteristics of diastolic dysfunction on echocardiogram. Also, erythropoietin deficiency and increased levels of angiotensin II seen in CKD leads to LVH and is the primary reason for non-compliance of the left ventricle and the resultant symptoms of diastolic HF. The symptoms of volume overload seen in diastolic dysfunction may, in fact, be primarily related to CKD.

C03

Duloxetine and the Control of Pain

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Background/Aims: Serotonin (5-HT) and norepinephrine (NE) have been implicated in the etiology and treatment of depression. However, these two monoamines are present in descending pathways in the spinal cord that are involved in the perception of pain, particularly chronic pain, that may be the result of central sensitization. Duloxetine, a potent and selective dual 5-HT and NE reuptake inhibitor (SNRI), has been approved for treatment of major depressive disorder (MDD) and management of diabetic peripheral neuropathic pain (DPNP). This presentation reviews the data from all randomized placebo-controlled clinical studies of duloxetine in MDD, with a focus on pain symptoms, and in DPNP and fibromyalgia

syndrome (FMS), to elucidate duloxetine's therapeutic potential in reducing pain.

Methods: Review of data from 11 clinical trials in patients with MDD, 3 trials in DPNP, and 2 trials in FMS. The primary outcome measure was HAM-D17 total score for 10/11 MDD studies. In the additional MDD study, involving elderly patients, the primary outcome measure was a prespecified composite cognitive score based on 5 cognitive tests that measured verbal learning and memory, selective attention, and executive functioning. Primary pain measures were used for DPNP and FMS studies.

Results: Duloxetine was superior to placebo on the a priori primary outcome measure(s) in 7/11 MDD studies, 3/3 DPNP studies, and 1/2 FMS studies. Outcomes on multiple secondary measures were positive in 3/4 remaining MDD studies and the other FMS study.

Conclusions: Duloxetine is effective for treatment of MDD, including painful physical symptoms, for management of DPNP, and treatment of FMS. This is consistent with its mechanism of action as an SNRI, presumably working on emotional symptoms within forebrain regions and pain symptoms within the spinal cord.

◆ C04

Prevalence of Abnormal Findings on Knee MRI in Asymptomatic NBA Basketball Players

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Variations in the prevalence of knee abnormalities in asymptomatic elite basketball players exist. The purpose of this study was to evaluate the knees of asymptomatic National Basketball Association (NBA) players via magnetic resonance imaging (MRI) and confirm or dispute findings reported in previous literature. It is believed that a variety of significant abnormalities affecting the knee exist despite an asymptomatic patient and these findings can be accurately identified on MRI. Two months prior to the 2005 season, bilateral knee MR imaging examinations of fourteen asymptomatic NBA players (28 knees) were evaluated for abnormalities of the articular cartilage, menisci, patellar and quadriceps tendons. The presence of joint effusion, subchondral edema, cystic lesions and the integrity of the collateral and cruciate ligaments were also assessed. Twenty-five knees had evidence of one or more abnormality (89%). Fourteen knees demonstrated chondromalacia (50%) and corresponding subchondral edema in 7 knees (25%). One knee had evidence of a meniscal tear (3.6%). Tendonosis of either the patellar or quadriceps tendon was evident for 13 knees (46%). The results support a high incidence of articular cartilage abnormalities primarily involving the patellofemoral joint of

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professional basketball players with a low incidence of meniscal tears. This emphasizes the potential hazards involving the knee joint in professional basketball players and the importance of using clinical correlation when assessing the knee of an injured athlete.

C05

A Fixed Dose Study of the Efficacy and Safety of Duloxetine for the Treatment of Generalized Anxiety Disorder

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Purpose: We examined the efficacy and safety of duloxetine, a reuptake-inhibitor of serotonin and norepinephrine, for treatment of generalized anxiety disorder (GAD).

Methods: In a 9-week, double-blind study, patients with DSM-IV defined GAD were randomized to receive duloxetine 60mg/day (DLX-60mg, N=168), duloxetine 120mg/day (DLX-120mg, N=170), or placebo (PBO, N=175). Primary efficacy outcome the Hamilton Anxiety Scale (HAMA) total score. Secondary measures included response rate ($\geq 50\%$ HAMA reduction), Sheehan Disability Scale Global Functional Impairment score (SDS), and HAMA Psychic and Somatic Subscales.

Results: Compared with PBO, both DLX groups demonstrated significantly greater reduction in HAMA scores (Mean decrease DLX-60mg=12.8, DLX-120mg=12.5, PBO=8.4, $P < .001$), greater response rates (DLX-60mg=58%, DLX-120mg=56%, PBO=31%, $P < .001$), greater improvements in SDS global scores (Mean decrease DLX-60mg=7.8, DLX-120mg=7.0, PBO=3.8, $P < .001$), and greater reductions in HAMA Psychic (Mean decrease DLX-60mg=7.6, DLX-120mg=7.1, PBO=4.5, $P < .001$) and HAMA Somatic subscales (Mean decrease DLX-60mg=5.2, DLX-120mg=5.3, PBO=3.8, $P < .001$). Discontinuation rates due to adverse events were 11.3% for DLX-60mg, 15.3% for DLX-120mg, vs. 2.3% for PBO ($P < .001$). The three most frequent adverse events associated with duloxetine were nausea, dizziness, and dry mouth.

Conclusion: Duloxetine 60mg and 120mg once daily was a safe, effective treatment resulting in clinically significant improvements in symptom severity and disability associated with GAD.

C06

Time Needed for Diabetes Self Care

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Background: Diabetes mellitus has reached epidemic proportions in the US. Nearly 7% of the US population has diabetes. Diabetes educators serve in the trenches to help people

learn the skills and habits necessary to successfully manage their diabetes. Self-management is central to controlling this disease. A previous study by Russell et al showed that in focus groups with DM educators that time demands for diabetes self-management were substantial if patients followed educators' recommendations. In this study, the authors attempt to quantify the necessary time needed to complete all of the tasks for self-management by enrolling a larger sample of diabetes educators.

Methods: The modal patient for this study was an adult with type 2 diabetes on oral medications alone. This study was completed using a questionnaire administered to diabetes educators and dietitians to estimate time demands for self-care as directed by the ADA and the CDE.

Results: At the time of submission 42 questionnaires have been completed. The time needed for the required elements as directed by the ADA was 190 minutes (3 hours and 10 minutes). Ninety-two minutes were for home glucose monitoring, record keeping, medication management, oral and foot care, problem solving and exercise. An additional 98 minutes/day were needed for meal planning, shopping and preparation. There was substantial variation in the estimates of each component of self-care.

Conclusions: The recommendations for DM self-care are likely too time intensive for most people to successfully complete. To make the demands more realistic we will need to find ways to combine tasks, or, alternatively, develop a cultural change so that all adults can build self care into their daily lives.

[Russell LB, Suh DC, Safford MA. Time requirements for diabetes self-management: too much for many? *J Fam Pract.* 2005 Jan;54(1):52-56.]

C07

Diabetes Management in Multiple Extended Care Facilities in the Midwest

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Diabetes is a chronic disease of epidemic proportions. The elderly in the US are experiencing the greatest increase in diabetes incidence. Guidelines have been published for adults and children with diabetes, but there has been a lack of studies evaluating diabetes control and treatment in extended care facilities. The ADA treatment guidelines have not addressed different treatment goals in these facilities. Experience demonstrates that providing intensive control for these patients can be challenging and even dangerous at times. In this study, the investigators used a retrospective chart review to identify areas of strength and weakness of current diabetes treatment in multiple extended care facilities in the Midwest. Adults who have lived at the facility for at least 3 of the previous 12 months and were admitted for long term care met the inclusion criteria. At the time of submission 44 charts had been

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reviewed. Seventy percent of the people were women, 95% Caucasian and 77% had type 2 DM. Preliminary results show that 100% had some glucose monitoring, but schedules for monitoring were very erratic. The mean HgA1c was 6.9%, yet only 21% were meeting ADA finger stick glucose goals. Thirty-two percent had a blood pressure of <130/90, and only 14% were at a LDL goal of 100 mg/dl. Forty-three percent were taking aspirin, and only 5% were getting screened for nephropathy with a microalbuminuria evaluation. Podiatry evaluations and influenza vaccination were almost universal. Many of the ADA recommendations were not utilized in this population. It appears that greater intensity of care was focused on glucose control as compared to blood pressure and lipid control. However, in large clinical trials there is greater mortality benefit with blood pressure and lipid control in the elderly. Glucose monitoring was sporadic and not uniformly performed. Glucose goals were not being met despite excellent HgA1c levels. This raises concern for unidentified hypoglycemia. Further studies are needed to evaluate current levels of care. Evidenced-based guidelines should be developed to guide treatment for this population.

C08

Assessment of Community Knowledge of HIV/AIDS in a Rural Village of Tanzania

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Objective: To conduct a survey in a rural village of Tanzania in order to determine ways to prevent the spread of HIV/AIDS and increase testing and knowledge about the disease.

Methods: From the Shirati Village of Eastern Tanzania, 141 people were surveyed. The survey was written by two medical students, in conjunction with a local HIV doctor, and translated into Swahili. Surveys were handed out to people attending church and in the center of town. After surveys were collected, an educational pamphlet was given out with the correct answers.

Results: The average age of individuals surveyed was 25. Approximately 60% of people surveyed were single and 78% were men. The average number of sexual partners was 11. In terms of knowledge, 88% of individuals surveyed believe that a virus causes AIDS and it can't be cured; 91% believe you can contract HIV thru contact with blood and the birth process. Only 26% believe that you can tell if someone is HIV+ just by looking at them or contract HIV by holding hands, hugging, or sitting on a toilet. In terms of prevention, 2/3 of people believe that using a new condom properly every time can prevent HIV and 78% said they would use a condom if they were readily available. In terms of testing, 85% said they would be more likely to get tested if it were free and they would change their sexual behavior if they found out they were

HIV+. In terms of social stigma, nearly half said their relationships and/or opinions of others would change if they found out their friends and/or family members were HIV+.

Conclusions: Overall, individuals' knowledge of transmission, methods of prevention and testing were high; however, the stigma associated with the disease also remains high. In addition, making condoms and testing free and separate from the church would increase the likelihood that people protect themselves.

Future directions: Of all new infections in Tanzania, 60% are among 15–24 year old females. Focus groups may help to assess this group's knowledge and gain input into designing effective prevention programs. Community support groups designed to reduce the stigma associated with HIV/AIDS is also paramount. Lastly, creating a welcoming place to distribute free condoms, in addition to holding educational workshops, will be critical to preventing the spread of disease.

C09

First-Dose Success of Vardenafil 10 Mg in Men with Erectile Dysfunction and Comorbidities in the RELY-I Trial

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Hypothesis: First-dose success is an important consideration when treating men with erectile dysfunction (ED), which often presents with comorbidities that may mitigate the effectiveness of phosphodiesterase-5 (PDE5) inhibitor therapies. The objective of the Reliability – Vardenafil for Erectile Dysfunction-I (RELY-I) study was to assess first-dose success and reliability of vardenafil (Var) (10 mg) in men with ED and its comorbidities.

Materials and Methods: The trial consisted of: 1) a 4-week treatment-free run-in period during which ≥ 4 attempts at intercourse were made on 4 separate days with $\geq 50\%$ unsuccessful attempts; 2) an open label, 1-week, single-dose, challenge period; and 3) a 12-week, double-blind, randomized phase during which responders were given Var 10 mg or placebo (PL). First-dose success rates for penetration and maintenance of erection to completion of intercourse were assessed using the Sexual Encounter Profile (SEP) 2 and SEP3, respectively, for ≤ 6 hours postdose during the challenge period. Through weeks 0–12, SEP2 and SEP3 success rates were assessed. Safety was assessed using adverse events (AEs).

Results: A total of 600 men with ED >6 months received a single 10 mg dose of Var during the 1-week challenge period. Of these patients, 32% had hypertension (HTN), 19% had dyslipidemia, and 16% had diabetes mellitus (DM). Least squares mean first-dose SEP2 and SEP3 success rates in the challenge phase were 87% and 74%, respectively. First-dose results by specific comorbidities in the challenge phase are provided in the table. For reliability of insertion (SEP2) over weeks 0–12, Var

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was statistically ($P<0.001$) and clinically superior compared with PL (83% vs 56%); for reliability of maintenance of erection to completion of intercourse (SEP3), Var also showed superiority (77% vs 42%, $P<0.001$). The most frequently reported AEs for Var were flushing (3.5%) during the challenge phase, and flushing (5.4%) and headache (5.0%) in the randomized phase.

Table
Challenge Phase: First-Dose Success Rates
of Vardenafil, 10 mg (N=600)

	No.	SEP2, %	SEP3, %
■ Overall	600	87	74
■ Comorbidities			
□ Hypertension	191	84	66
□ Dyslipidemia	116	84	72
□ Diabetes mellitus	95	75	58

Conclusions: Var 10 mg is efficacious, safe, and well tolerated, providing first-dose success and reliability in men with ED and frequently associated comorbidities.

◆ C10

Efficiency of Low Molecular Weight AGE Clearance During Hemodialysis Predicts Increases in Paraoxonase Activity in Chronic Renal Failure Patients

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Background: Patients with chronic renal failure show the highest increases in circulating AGEs due to a clearance defect. They also show low levels of paraoxonase activity.

Hypothesis: We hypothesized that paraoxonase activity is inhibited in the uremic milieu and that contributes to the lower activity seen in ESRD patients. We reasoned that PON-1 activity should rise after a successful hemodialysis intervention, irrespective of changes in HDL concentration or subclass distribution. Our second hypothesis was that low molecular weight substances in ESRD patients' serum but not in control subjects serum inhibit PON-1 activity.

Methods: We conducted an intervention study with 22 ESRD patients undergoing hemodialysis in whom paired pre- and postdialysis samples were studied along with 30 age-matched control subjects. We measured PON-1 activities, as well as lipid peroxidation, apoA, lipid profiles, HDL-subclasses and AGE products (both >10 kDa and <10 kDa) in each of the

patients and evaluated the correlations of uremia-associated substances (urea, creatinine) with paraoxonase activity. Ultrafiltrates (cut-off 10 kDa) of both pooled control serum and ESRD serum were incubated with PON-1.

Results: Dialysis produces a 5–40% increment in PON-1 activity (average $20\pm 8\%$) while no significant changes in either apoA-I or HDL subclasses distribution was observed. This change correlates ($r=0.86$, $P<0.001$) with the efficiency of dialysis as determined by either creatinine or urea rate of change. It also correlates ($r=0.87$, $P<0.001$) with changes in 350/440 fluorescence and pentosidine fluorescence in the <10 kDa serum fraction. These fractions produce a time and dose dependent inhibition of PON-1 activity ($25\pm 3\%$ vs $10\pm 2\%$ for control, $P<0.005$).

Conclusions: Our data give both in vitro and in vivo support to an inhibition of PON-1 by uremic toxins that are partially removed by dialysis and suggest that, at least in part, these toxins are low molecular weight AGE adducts. This research lends support to another mechanism for AGE-related pathogenicity, that would act synergistically with the prevalent HDL, LDL and hemodynamics changes in this disease.

Acknowledgment: Supported by Showa and Touro Universities.

◆ C11

Ischemia-Modified Albumin and Advanced Oxidation Protein Products Levels in Type 2 Diabetic Patients: Complementary Biomarkers for Metabolic Control?

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Background: Ischemia Modified Albumin (IMA) is a new biochemical marker of myocardial ischemia, but there is concern that IMA concentrations may be affected by ischemia occurring in tissues other than the myocardium. Advanced oxidation protein products (AOPP) are new markers of oxidative stress discovered in plasma of dialysis patients. They arise from the reaction between chlorinated oxidants and plasma proteins.

Hypothesis and aims: We had previously shown that IMA reference ranges in diabetic patients differ than those for non diabetics and that IMA may be affected by short term changes in glycemic control (Diabetes 2005, 54 A 537). Since diabetic patients have increased inflammation in parallel with oxidative and carbonyl stress, we hypothesized that AOPP levels should also be higher than in control patients. We correlated AOPP with IMA, AGEs and glycemic control.

Methods: We performed a case-control study with 41 diabetic patients (19 male and 22 female, 58 ± 9 years of age) and their age-matched controls. Basic metabolic panels, lipid panels and HbA1c were measured using standard methods. Deter-

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mination of AOPP is based on spectrophotometric detection. IMA was measured by a test based on an ischemia-induced decrease in cobalt 2+ binding to an N-terminal octapeptide in the albumin molecule, using our modification (Gugliucci et al. *Clin Chim Acta*. 2005;362:155–160). Determination of AGEs is based on the spectrofluorimetric detection.

Results: AOPP were 35.9 ± 17 for control subjects vs 52 ± 29 $\mu\text{mol/l}$ for diabetic patients, which represents a 44% increase ($P < 0.01$). IMA in diabetic patients was 0.56 ± 0.1 AU vs 0.45 ± 0.12 , a 24% increase, $P < 0.0001$. A significant correlation, $r = 0.36$ was found between IMA and glycemia in diabetic patients. No correlation was found between IMA and AOPP, HbA1c, or circulating AGEs.

Conclusions: We confirm our previous data showing higher IMA levels in type 2 diabetic patients in a larger series. Our data suggest that AOPP and IMA changes in diabetic patients reflect two different altered pathways and could be complementary biomarkers of oxidative and hypoxic stress in these patients.

Acknowledgment: Sponsored by Touro and Showa Universities.

◆ C12

Factors Affecting Maternal Choices in Home or Hospital Delivery in the Tarime District, Tanzania

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Background: The aim of this study was to investigate the factors influencing women's choice in delivering their children at home versus in the hospital in Shirati, Tanzania.

Methods: During a period of one month, 52 randomly selected mothers were surveyed. Sites included Shirati Hospital maternity ward, antenatal clinic, as well as mobile clinics in neighboring villages. Outcomes measured include maternal age, parity, education level, poverty indicators, cultural beliefs, distance from their home to the hospital, location of all deliveries and reason why. The place of delivery for each woman's pregnancy was determined.

Results: Although many women reportedly preferred to deliver in the hospital, the most prevalent factor determining their home delivery was lack of transportation to the hospital when in labor. It was found that 24 out of the 34 women who preferred to deliver in the hospital were prevented from doing so by transportation circumstances. Education level, poverty, and cultural beliefs had little influence on women's decision to deliver at home or in the hospital.

Conclusions: Based on the data analyzed, the main factor preventing women from having their children in the hospital is lack of transportation rather than education level, poverty, or cultural beliefs. With multiple women's suggestions in mind, improvements in transportation such as providing bicycles modified to carry pregnant women from each remote village to the hospital could improve delivery outcomes.

◆ C13

HIV/AIDS in Haiti: A Clinical Investigation

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Background: Heterosexual transmission of HIV in Haiti is the most common, followed by vertical transmission. It is estimated that every year there are about 13,000 pregnant women who are HIV-positive. The purpose of this study was to investigate on demographics of HIV and HIV/TB co-infections, and to assess current treatment modalities in Haiti.

Materials and methods: This study was conducted at the Hospital Albert Schweitzer (HAS) in Dechapelles, Haiti. Clinical data were collected from each patient visit as well as individual patients' files, which were made available from HAS administration. HAS is a major source of healthcare delivery in Haiti.

Results: On a daily average, 20 adult and 8 pediatric HIV-positive patients were presented at HAS. The average adult and pediatric CD4 counts were 128 and 188 cells/mm³ respectively. Analysis of data collection revealed 70% of HIV-positive patients also were TB-positive. Further, of the pool of patients we examined, 3.8% were HBV-positive; 1% were HCV-positive, and 1% were positive for syphilis. Patients who were admitted to the hospital commonly presented with wasting syndrome, generalized malaise, and pulmonary symptoms due to HIV/TB co-infection. The analysis of patients' data further revealed Haiti has the highest adult and pediatric HIV prevalence rate in the Caribbean region at about 5%, which is observed in Port-au-Prince and other areas of the country. Finally, our data shows malnutrition is a likely contributing factor in HIV/AIDS mortality, especially among children. Current therapy aimed at HIV-positive patient is combivir, a standard therapeutic regimen in Haiti. Since most patients in Haiti present with TB, the current guideline is to treat the TB, and then proceed to the management of HIV infection.

Conclusion: HIV/AIDS is highly prevalent in Haitian adults and pediatric populations. Majority of adult HIV-positive patients also present with TB co-infections. While combivir is currently the drug of choice, there is not any major clinical center to assess the drug's efficacy or evaluate the pattern of drug resistance. Combination of AIDS and NAIDS (nutritionally-acquired immunodeficiency syndrome) may contribute to a higher mortality rate in Haiti. Such patients would greatly benefit from osteopathic care.

C14

Controlled-release Hydrocodone and Acetaminophen Tablets Provide Significant Relief of Moderate to Severe Osteoarthritis Pain With BID Dosing

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◆ Indicates posters entered in the Student Prize Competition, a judged event that takes place during the Poster Session at the Research Conference.

Research, Inc, Pembroke Pines, FL; ²University Clinical Research, Inc, Deland, FL

Introduction: Numerous single-agent opioids are currently available in controlled-release formulations to manage chronic pain states. Despite their extensive use in pain management, combination opioid/non-opioid products are not yet available in controlled-release (CR) formulations. The objective of this Phase 2, randomized, multicenter, double-blind, placebo-controlled study was to determine the analgesic efficacy and safety of hydrocodone bitartrate 15 mg/acetaminophen 500 mg CR (HC/APAP CR) tablets compared with placebo in the management of moderate to severe osteoarthritis (OA) pain. **Hypothesis:** HC/APAP CR tablets administered twice daily will significantly decrease OA pain compared with placebo. **Materials:** HC 15 mg/APAP 500 mg OROS osmotic technology tablets.

Methods: The study consisted of 5 periods: 3-week screening/washout, 1-week titration (1 tablet BID), 3-week maintenance (2 tablets BID), 1-week study drug taper (1 tablet BID), 1-week Follow-Up. The primary efficacy variable was change from baseline to final evaluation (Day 29) in patient assessment of OA pain by visual analog scale (VAS) (0–100 mm).

Results: 120 subjects received at least 1 dose of study drug and comprised the ITT dataset: 58 in the HC/APAP CR group and 62 in the placebo group. Most subjects were white (79%) and female (72%), and the mean age was 57 years; no statistically significant differences were found between the treatment groups with regard to any demographic variable. Mean reduction in pain scores from baseline to final evaluation was greater for the HC/APAP CR group versus the placebo group; additionally, the differences between treatment groups were statistically significant at all scheduled evaluations, based on 1-sided tests. For the primary efficacy variable, the mean change from baseline to final visit in VAS score was –38.5 mm in the HC/APAP CR group compared with –26.7 mm in the placebo group ($P=0.033$). The mean percent change in VAS scores from baseline to final visit was –52.5% in the HC/APAP CR group compared with –36.0% in the placebo group ($P=0.027$). Overall incidence of adverse events, as well as the events of nausea, somnolence, pruritus, and lethargy were experienced by a greater proportion of subjects receiving HC/APAP compared with placebo. Discontinuations, at least in part due to adverse events, occurred in 31% of subjects ($n=18$) in the HC/APAP CR group and 10% of subjects ($n=6$) in the placebo group.

Conclusions: The HC/APAP CR group showed clinically and statistically significant reductions in VAS pain scores compared with placebo. The adverse event profile was characteristic of a mu-agonist opioid. BID dosing of HC/APAP CR tablets may provide an important and more convenient treatment alternative for subjects with moderate to severe chronic OA pain.

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Acknowledgment: This study was funded by Abbott Laboratories, Inc, Abbott Park, IL.

C15

Simultaneous Measurement of Glycated LDL and Glycated HDL Subclasses: a New Method

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Background: Protein modification by the Maillard reaction is involved in several disease processes, including diabetes and Alzheimer's disease, as well as in aging. The first glucose metabolite, glucose-6-phosphate (G-6-P), has been known for many years to be much more aggressive than glucose itself in studies performed in vitro. However, no studies had addressed the role of G-6-P in vivo.

Hypothesis: We hypothesized that G-6-P glycation is detectable in serum and occurs mainly intracellularly prior to secretion. Serum proteins are largely a secretion product of the hepatocyte and thus provide an accessible model for the proof of principle we aimed at in this preliminary study. Serum proteins can, moreover, be modified in the circulation throughout their life span.

Methods: We detected G-6-P modified proteins in serum from control subjects ($n=10$) and from type 2 diabetic patients ($n=10$). We employed 10% SDS-PAGE and Western blot using a specific antibody we produced and characterized previously (Nguyen et al. *Biochim Biophys Acta*. 2005;1762(1):94–102). This antibody does not cross-react with glucitolysine adducts in the dilutions employed here. In separate experiments we incubated serum with G-6-P to confirm the specificity in our model. **Results:** Serum incubated with G-6-P for 48 h showed the adducts while none was found in control serum incubated without G-6-P or with glucose. Multiple G-6-P modified proteins are detected in both control subjects and diabetic patients. No significant differences were detected between the 2 populations.

Conclusions: Our data give both in vitro and in vivo support to glycation of serum proteins by G-6-P. It opens several questions: Is G-6-P glycation an intracellular, an extracellular process or both? The low concentrations of G-6-P in serum would indicate that the intracellular process should predominate. The ER and Golgi are rich in G-6-P which participates as donor in several glycosylation reactions. The similar pattern shown in control and diabetic subjects further supports this idea. Alternatively, G-6-P glycation would occur in the circulation as a consequence of G-6-P leaking as a byproduct of cell turnover.

◆ C16

A Cross-Sectional Study of Somatic Dysfunction and Chronic Conditions in Older Adults

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Editor's message: In the original print publication, the incorrect text was published for clinical studies abstract C18, "Addition of laminin and fibronectin to SIS used as conduits in peripheral nerve repair," by Jacqueline C. Oxenberg, MS III, and colleagues. The error has been corrected here.

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Purpose: The purpose of this pilot study was to investigate the relationship between somatic dysfunction and chronic conditions in adults aged 65 and older who received Osteopathic Manipulative Treatment (OMT).

Methods: This was a cross-sectional study with information collected from medical records of patients seen for OMT in a Geriatric clinic at the University of North Texas Health Science Center between January 1, 2000–May 1, 2005. Data elements included demographics, Instrumental Activities of Daily Living (IADLs), Geriatric Depression Scale-Short Form (GDS-SF) scores, trauma history, chronic conditions, and somatic dysfunction.

Results: The sample included 139 adults, 83% of whom were females. In the sample, 118 (85%) subjects were white, 61 (44%) were married, and 112 (81%) were living at home. The average age was 77.4 years (SD=8.44). The mean number of chronic conditions per subject was 6.49 (SD=2.64). The most common areas of somatic dysfunction were the pelvis (84%), the sacrum (61%), and the ribs (60%). There were significantly more subjects with sacral somatic dysfunction that had GDS-SF cutoff scores above 5. IADLs were also significantly related to somatic dysfunction. Significantly more subjects that needed moderate assistance with meal preparation had somatic dysfunction in the upper thoracic (T1–T4) region ($P=.039$). Subjects with a history of motor vehicle accident (MVA) had significantly less lumbar somatic dysfunction ($P=.031$), but significantly more upper thoracic (T1–T4) somatic dysfunction ($P=.012$). Subjects with pulmonary/respiratory conditions had significantly more cranial somatic dysfunction ($P=.016$). Significantly more subjects with neurological/psychiatric conditions had somatic dysfunction of the diaphragm ($P=.043$).

Conclusions: The results of this study suggest several significant relationships between somatic dysfunction and chronic conditions (pulmonary/respiratory and neurological/psychiatric conditions) as well as trauma (MVA). Future research should be directed toward the prospective investigation of the onset of traumatic events and subsequent somatic dysfunction, depression, and associated medical conditions.

◆ C17

Chronic Obstructive Pulmonary Disease (COPD): Immediate Effects of Osteopathic Manipulative Treatment on Exercise Tolerance and Dyspnea

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Purpose: Contribute to our knowledge of the effects of OMT on breathing and exercise tolerance in patients with COPD. Hypothesis 1: One OMT intervention will have an immediate effect of improving dyspnea in a stable COPD subject, as mea-

sured by response to the Borg scale. Hypothesis 2: One OMT intervention will improve exercise tolerance in a stable COPD subject, as measured by distance in the six-minute walk test.

Methods: After obtaining informed consent, initial plethysmography and spirometry test were performed by a respiratory technician to verify that subjects met the pulmonary function test inclusion criteria. After acceptance into the study protocol, subjects were randomly assigned to either the Osteopathic Manipulative Treatment group or the no treatment (ie, rest) group. Demographics were then recorded and questionnaires were completed. Subjects filled out the St. George Respiratory Questionnaire, The American Thoracic Society Dyspnea Index and Borg Scale. The entire study took place in the Internal Medicine Department at UNTHSC-TCOM.

Results: Exploratory data analyses revealed equal variances in all descriptive measures. Between group differences were not statistically significant for any demographics or for the Quality of Life measures. No immediate effects of OMT were statistically significant following exercise for any of the outcomes of interest. Examination of individual cases within the sample provides clinically important information to contribute to our knowledge of how OMT versus rest may affect the six-minute walk and the Borg Scale for COPD patients.

Conclusions: Current OMT literature does not provide standards for dosage and frequency; therefore, this pilot study took the first step of looking at immediate effects. It is not possible to conclude appropriate dosage and frequency based on the findings of this study. Future studies with larger number of subjects and varying dosage and frequency will aid in determining these factors. Clinically important differences in the experimental groups provides information needed to design a larger study with improvements in design.

◆ C18

Addition of Laminin and Fibronectin to SIS Used as Conduits in Peripheral Nerve Repair

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This is a pilot study using laminin and fibronectin as an additive to small intestine submucosa (SIS) grafts used to repair transected sciatic nerve in a rat model. Previous studies in our lab have shown sciatic nerve regeneration to be successful using SIS grafts without growth factors. SIS is a biological material that may be used to correct problems of larger peripheral nerve gap injuries and improve regrowth by acting as a natural guide between the proximal and distal segments of the nerves, providing the proper growth environment with minimal antigenicity, thus decreasing chances of rejection. Other studies have shown the addition of fibronectin to various

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types of grafts further enhanced nerve regeneration by promoting Schwann cell growth. The goal of this study is to determine whether adding laminin and/or fibronectin to SIS grafts improves sciatic nerve regeneration. The experimental group had a 10 mm segment of the right sciatic nerve resected followed by placement and suturing of and SIS graft plus laminin and/or fibronectin laced in the gap as a conduit for nerve regeneration. The control group had the right sciatic nerve resected and reattached directly. Schwann cell growth and nerve regeneration were assessed using anti-s100 antibody, and fast cresyl violet stain to assess Schwann cell migration and neuron regeneration respectively. Preliminary results indicated that:

1. Schwann cell migration and accompanying neuron infiltration occurred up to approximately 2.5 cm over the 6-month healing time in experimental animals.
2. Healing in control animals was observed to be inhibited by the formation of collagen scar tissue.
3. Gait analyses show increased sciatic function in experimental groups of laminin and/or fibronectin compared to control groups.

These results exceed previously reported rates of peripheral nerve regeneration using non-SIS graft materials and show addition of laminin and/or fibronectin improve both sciatic nerve regeneration and sciatic function in gait analyses.

C19

Improving Symptoms, Pain, Functioning, and Strength for Persons with Carpal Tunnel Syndrome

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Purpose: The purpose of this study was to examine whether OMT could reduce symptoms, improve functioning, decrease pain, and improve strength in persons with CTS. Hypothesis 1: OMT will decrease symptom severity and pain. Hypothesis 2: OMT will improve daily functioning and strength.

Methods: Subjects (N=32) between 21 and 70 with confirmed diagnosis of CTS based on Nerve Conduction Studies (NCS), who had none of the medical exclusions consented and were randomized to two groups (OMT and Control). OMT group received one treatment, Control group received one sub-therapeutic ultrasound treatment. Power analysis called for 19 subjects per group. Symptom severity and functional status were self-reported using the Levine scales for CTS. Pain was measured with the visual analog scale. Grip strength was tested using a Jamar Dynamometer. Based on the literature, the average of three scores taken at each visit for grip strength, key

pinch strength, tripod pinch strength, and tip pinch strength was used for the analysis. Outcome measures were taken at entry to the study, mid point, and after the last treatment session.

Results: The OMT treatment group had complete data for 14 subjects, and the control group 18. The groups were similar in composition on gender, age, and body mass index (BMI). BMI correlated with symptom severity scores ($r = 0.392, P = .035$) and functional status scores ($r = 0.379, P = .043$). Baseline nerve conduction studies for median motor latencies (MML) differed significantly between groups ($P = .047$), and MML was related to symptom ($r = 0.437, P = .014$) and function scores ($r = 0.418, P = .019$). The OMT group reported significantly improved functioning at the end of the trial ($P = 0.019$) but between group changes were not significant. For the OMT group, grip strength changed significantly ($P = .013$). The control group made significant improvement in pinch and tripod pinch strength ($P = .034$).

Conclusions: This RCT was exploratory and preliminary; limited by a small sample size. This study, however, provided statistical support for trends in the efficacy of OMT for CTS and supported a successful R21 proposal to the NIH-NCCAM for a three year RCT for OMT in CTS patients.

◆ **C20**

Withdrawn at the Author's Request

◆ **C21**

Gender Differences in Onset of Diabetic Complications

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Introduction: Diabetes Mellitus Type II is one of the most common metabolic diseases in the US. Diabetic complications (nephropathy, neuropathy, and retinopathy) are influenced by duration of hyperglycemia and exacerbate morbidity and mortality in diabetics. Few studies have evaluated gender differences in the age of onset of hyperglycemia or diabetic complications. The hypothesis states that men with Type II diabetes have earlier onset hyperglycemia and accelerated nephropathic, neuropathic, and retinopathic complications.

Methods: This IRB-approved analysis of more than 375 medical records, conducted at South Pointe Hospital, used ICD-9 codes for Pre-diabetes and Type II diabetes with renal, neurological, and ophthalmic manifestations. Charts from January 2000 to January 2006 were reviewed for the onset of hyperglycemia and diabetic complications, determined by the initial date the ICD-9 code appeared in the medical record. Data was analyzed via 2-tailed t-tests using SPSS program.

Results: Pre-diabetes: 200 charts (78 males – 39%; 122 females – 61%) showed males developed hyperglycemia at age 60, 3 years earlier than females. Renal manifestations: 324 charts (154 males – 47.5%; 170 females – 52.5%) showed males developed diabetic nephropathy at age 66, 4 years before females.

◆ Indicates posters entered in the Student Prize Competition, a judged event that takes place during the Poster Session at the Research Conference.

Neurologic manifestations: 376 charts (156 males – 41%; 220 females – 59%) showed males developed neuropathic complications at age 63, 4 years earlier than females. Ophthalmic manifestations: 298 charts (121 males – 40.6%; 177 females – 59.4%) showed males developed diabetic retinopathy at age 63, 4 years before females. The 2-tailed t-tests revealed statistically significant differences in age of onset for diabetic nephropathy ($P=.001$), neuropathy ($P=.006$), and retinopathy ($P=.019$) but not for hyperglycemia ($P=.167$) between genders. **Conclusion:** Males develop diabetic neuropathic, nephropathic, and retinopathic complications earlier than females. Earlier interventions in diabetic men may improve disease outcomes. Males developed hyperglycemia earlier than females; these results were statistically insignificant. Further research will help determine if gender differences in hyperglycemia exist.

C22

Patient Expectations Between OMT, Subtherapeutic Ultrasound and Light Touch Placebo Controls

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Purpose: The purpose of this study was to determine attitudes towards 3 treatments (HVLA, placebo light touch, placebo subtherapeutic ultrasound) commonly used in OMT clinical research trials.

Methods: A randomized, cross-over design was utilized. Subjects were recruited from the Family Medicine Clinic, TCOM. Participants watched a video of 2 minute demonstrations of a High Velocity Low Amplitude (HVLA), placebo light touch (LT), and placebo subtherapeutic ultrasound (ULTRA) treatment for low back pain. Randomization of treatments controlled for order effect bias. Subjects indicated if they Strongly Agree, Agree, Disagree, or Strongly Disagree with 4 statements after each treatment: 1) I believe this treatment would allow me to get better quicker; 2) I believe this treatment would decrease my low back pain; 3) I believe this treatment would make me more able to do the things I want to do; 4) This seems like a logical way to treat low back pain. Repeated measures analysis of variance was performed, and a Partial Eta squared is presented for each question. Cohen's d was calculated for 2 groups at a time. The UNTHSC IRB approved this study.

Results: Thirty of 40 eligible subjects participated. Twenty-two (73.3%) were female, 16 (53.3%) were Caucasian, and 11 (36.6%) had completed college. The mean age was 43 (SD=15.11). Repeated measures ANOVA revealed no significant differences for statements 2 and 4. For statements 1 ($P=.025$) and 3 ($P=.039$), post hoc analysis revealed a difference between HVLA and LT. Partial Eta squared was 0.105, 0.072, 0.107, and 0.024 for each statement, respectively. For

statement 1, Cohen's $d=-0.29$ for HVLA and ULTRA, -0.49 for HVLA and LT, and -0.21 for ULTRA and LT. For statement 2, Cohen's $d=-0.11$ for HVLA and ULTRA, -0.38 for HVLA and LT, and -0.31 for ULTRA and LT. For statement 3, Cohen's $d=-0.41$ for HVLA and ULTRA, -0.47 for HVLA and LT, and -0.09 for ULTRA and LT. For statement 4, Cohen's $d=-0.21$ for HVLA and ULTRA, -0.19 for HVLA and LT, and 0 for ULTRA and LT.

Conclusions: There is a difference in treatment expectation between HVLA and LT for statements 1 and 3. Participants responded more positively after viewing the HVLA treatment than LT treatment suggesting subtherapeutic ultrasound is the better placebo when examining treatment expectation.

C23

Assignment of Copyright Permissions Pending

C24

Utilizing the AOA Clinical Assessment Program (CAP) to Examine the Delivery Effectiveness of Diabetic Care in Suburban and Urban Clinical Practices: A Pilot Study

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Background: Despite recent advances in diabetic care, there still exists a "quality chasm" in translating knowledge into practice. With the continual increases in the prevalence of type 2 diabetes, it is important that health care professionals effectively manage the care of diabetic patients utilizing evidence-based guidelines such as those provided by the AOA CAP.

Objective: In this pilot study, we have employed the AOA CAP to examine the delivery effectiveness of diabetic care in suburban and urban clinical practices.

Methods: In this random retrospective chart review, the AOA CAP parameters (www.do-online.org) were abstracted from 75 patient charts from both suburban (S) and urban (U) practices. Data was analyzed using chi-square tests for categorical variables and paired t-tests for continuous variables.

Results: HCC-S performed the same or better than the national standard in controlling HgbA1c, foot exam frequency (FEF), microalbuminuria screening (mAlbS), assessing/controlling hyperlipidemia (Hlp) and hypertension (HTN), and influenza vaccinations (InV). They did not meet the national standards in appropriate use of ACE-I. HCC-U performed the same or better than the national standards in FEF and HTN. They did not meet the national standards in HgbA1c, mAlbS, Hlp, ACE-I, or InV. HCC-S did significantly better than HCC-U in assessing HgbA1c ($P=0.007$), controlling HgbA1c ($P=0.001$), FEF ($P<0.001$), mAlbS ($P<0.001$), Hlp ($P=0.009$), controlling LDL <100 ($P=0.002$), and InV ($P<0.001$). HCC-U performed better than HCC-S only in controlling LDL <130 ($P=0.002$). There was

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no significant difference in HTN ($P>0.07$) or appropriate use of ACE-I ($P=0.519$).

Conclusions: HCC-S performed the same or better than the national standard in all but one parameter (ACE-I), whereas HCC-U performed at a lower level than the national standards in 9 of 13 parameters. However, when HCC-U is compared to a similar practice, it performed better in all parameters. This is consistent with a number of studies which highlight disparities in health care delivery.

C25

Efficacy of Rizatriptan for ICHD-II Menstrual Migraine

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Background: It is commonly believed that migraine attacks occurring during the perimenstrual period are more refractory to treatment. In retrospective analyses, the efficacy of rizatriptan for coexisting migraine and menses was similar to that for nonmenstrual migraine.

Hypothesis: In patients with menstrual migraine, as defined by the 2004 revision of the International Classification of Headache Disorders (ICHD-II), rizatriptan, 10 mg will be superior to placebo, as measured by the percentage of patients with 2-hour pain relief (primary) and 24-hour sustained pain relief (secondary).

Methods: The two protocols (MM1 and MM2) were randomized, parallel, placebo-controlled, double-blind studies approved by a central Institutional Review Board. Adult patients with menstrual migraine (both menstrual-related and pure subtypes) as defined by ICHD-II criteria were assigned to either rizatriptan 10-mg tablet or placebo in a 2:1 ratio. Patients were to treat a single menstrual migraine attack of moderate or severe pain intensity.

Results: A total of 707 patients (MM1: 357, MM2: 350) met study criteria for menstrual migraine and treated a menstrual migraine attack. The percentage of patients reporting 2-hour pain relief was significantly greater for rizatriptan than for placebo (MM1: 70% vs. 53%, MM2: 72% vs. 49%, $P<0.001$ for both studies). The percentage of patients reporting 24-hour sustained pain relief was also significantly greater for rizatriptan than for placebo (MM1: 46% vs. 32%, $P=0.016$; MM2: 46% vs. 33%, $P=0.024$).

Conclusion: Rizatriptan 10 mg was effective for the treatment of menstrual migraine, as measured by 2-hour pain relief and 24-hour sustained pain relief. To our knowledge, these were the first prospective studies to utilize the ICHD-II menstrual migraine criteria in clinical research.

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C26

Oxymorphone Extended Release Improves Pain Outcomes in Chronic, Moderate to Severe Low Back Pain Patients Regardless of Age, Gender, or Previous Opioid Use

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Hypothesis: Oxymorphone extended release (ER) is efficacious for treating moderate to severe low back pain (LBP) irrespective of patient age, gender, or previous opioid use.

Methods: Data from 2 large, placebo-controlled trials of oxymorphone ER were combined for analysis. One trial included only opioid-naïve patients, and the other only opioid-experienced patients. In each trial, adults were treating moderate to severe chronic LBP with a stabilized dose of oxymorphone ER q12h. Stabilized patients were then randomized to continue their oxymorphone ER dose or receive placebo q12h in a double-blind fashion for 12 weeks. Opioid rescue medication was allowed p.r.n. during the first 4 days and b.i.d. thereafter. Pain intensity (0–100-mm Visual Analog Scale [VAS]), patient assessments of study medication, and adverse events (AEs) were recorded.

Results: Demographic characteristics in the oxymorphone ER and placebo populations were not significantly different except for age (50 y vs 47.2 y, respectively, $P=0.0394$) and gender (men 43% vs 57%, respectively, $P=0.0116$). Following randomization, 69% (120/174) of oxymorphone ER and 39% (65/169) of placebo patients completed 12 weeks of double-blind treatment. Lack of efficacy caused 12% of oxymorphone ER patients and 43% of placebo patients to discontinue ($P<0.0001$). During double-blind treatment, the oxymorphone ER group had significantly lower mean VAS scores than the placebo group ($P<0.0001$; mixed effects regression model of VAS with treatment, visit and their interaction as factors). There were no significant effects of age, gender, and previous opioid use on mean VAS scores. After randomization, a significantly higher percentage of oxymorphone ER patients rated their study medication good to excellent (92% vs 68% with placebo, $P=0.0289$). More oxymorphone ER patients experienced moderate opioid AEs (13.7% vs 4.7% with placebo; $P=0.012$).

Conclusions: This combined analysis of opioid-experienced and -naïve patients more closely mimics a real world setting and further supports the efficacy of oxymorphone ER for moderate to severe LBP. Oxymorphone ER was generally well tolerated and significantly improved pain outcomes regardless of age, gender, or previous opioid use.

C27

A Randomized, Double-blind, Placebo-controlled Trial Evaluating the Efficacy and Safety of Oxymorphone Extended Release in an Opioid-naïve Chronic Low Back Pain Patient Population

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Hypothesis: The clinical benefits of opioid analgesics can be compromised by poor tolerability in opioid-naïve patients. In this efficacy study of oxymorphone extended release (ER), we evaluated whether gradual dose titration improved tolerability and minimized dropouts during treatment initiation of opioid-naïve patients with moderate to severe chronic low back pain (CLBP).

Materials and Methods: Patients =18 years of age with CLBP and a screening pain intensity of =50 on a 0- to 100-mm Visual Analog Scale received oxymorphone ER 5 mg q12h PO for 2 days. The dose was then titrated upward to a stabilized dose that reduced average pain to ≤40 mm for 3 of 5 consecutive days and was well tolerated. Stabilized patients were randomized to 12 weeks of double-blind treatment with either their stabilized dose of oxymorphone ER or placebo q12h. Oxymorphone immediate release q4–6h was provided PRN for the first 4 days of the double-blind treatment period and twice daily thereafter.

Results: 205/325 (63.1%) treated patients completed titration. The mean age was 50.1 years, 88.9% were white, and 49.2% were men. The mean ± standard deviation stabilized dose of oxymorphone ER for patients completing the titration phase was 40.0 ± 25.8 mg/d. 18.2% of patients discontinued because of adverse events (AEs). The average pain intensity of stabilized patients decreased from 69.4 mm at screening to 22.7 mm at stabilization ($P < 0.0001$). The 12-week double-blind period was completed by 71/105 (67.6%) oxymorphone ER and 47/100 (47.0%) placebo patients. The placebo group experienced a significantly higher increase in pain intensity than the oxymorphone ER group (least squares mean change of 26.9 ± 2.4 mm vs 10.0 ± 2.4 mm, respectively; $P < 0.0001$). Discontinuation rates due to AEs were 8.6% and 8.0% in oxymorphone ER and placebo groups, respectively. Nausea (11.4%) and vomiting (7.6%) were the most frequent AEs during double-blind treatment with oxymorphone ER. Most AEs were rated mild or moderate in intensity.

Conclusions: Oxymorphone ER q12h provided effective pain relief in opioid-naïve patients with moderate to severe CLBP. By following the clinical practice of “start low and titrate slowly,” opioid-related AEs were minimized.

C28

Efficacy and Sustained Tolerability of Oxymorphone Extended Release in Opioid-Experienced Patients With Chronic Low Back Pain

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Hypothesis: Up to 30% of patients using current opioid regimens experience inadequate pain relief (Cherny N et al. *J Clin Oncol.* 2001;19:2542–2554) necessitating new analgesic options. We hypothesize that a new opioid formulation, oxymorphone

extended release (ER), can be titrated to a stable, effective, and well-tolerated dose in opioid-experienced patients with chronic low back pain (CLBP).

Methods: Patients >18 y with moderate to severe CLBP received oxymorphone ER q12h at a daily dosage that approximated their prestudy opioid requirement. Patients were titrated to a stabilized dose that reduced pain to ≤40 mm on a 0- (no pain) to 100- (worst pain imaginable) mm Visual Analog Scale for 3 of 5 consecutive days. Stabilized patients were randomized to 12 weeks of double-blind treatment with either their stabilized dose of oxymorphone ER or placebo q12h. Oxymorphone immediate release (q4–6h) was available PRN for the first 4 days of the double-blind period and then limited to twice daily thereafter.

Results: During the titration period 57.0% (143/251) of patients were stabilized on oxymorphone ER, and 18.7% discontinued due to adverse events (AEs). Most (98%) stabilized patients were titrated in ≤1 month. After randomization, 70% of oxymorphone ER patients and 24.7% of placebo patients completed double-blind treatment. More patients discontinued due to a lack of efficacy in the placebo group than in the oxymorphone ER group (53.4% vs 11.4%, $P < 0.001$). Discontinuations from AEs were 10.0% and 11.1% for oxymorphone ER and placebo-treated patients, respectively. Pain intensity increased significantly more in the placebo group than in the oxymorphone ER group (least squares mean change from baseline [\pm standard error] to final visit was 31.6 [\pm 2.93] vs 8.7 [\pm 2.95], $P < 0.0001$). Ratings of study medication were more favorable for oxymorphone than placebo ($P < 0.0001$). The most frequently reported AEs were pain exacerbation (5.7%) and constipation (5.7%).

Conclusions: This 12-week study demonstrates that patients requiring around-the-clock opioid analgesics can be successfully switched to oxymorphone ER q12h and titrated to a stabilized dose that safely and effectively maintains adequate pain relief.

C29

Restless Legs Syndrome (RLS) Symptom Improvement With Ropinirole in RLS Patients With Disturbed Sleep

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Hypothesis: Restless Legs Syndrome (RLS) is characterized by an irresistible urge to move the legs that begins or worsens at night. Difficulty falling asleep and disturbed sleep are common. Patients may be prescribed insomnia medications that are unlikely to treat the underlying disorder. Ropinirole, a dopamine agonist, is FDA-approved for treatment of moderate-to-severe primary RLS. The effect of ropinirole treatment on RLS symptoms was evaluated in RLS patients with disturbed sleep.

◆ Indicates posters entered in the Student Prize Competition, a judged event that takes place during the Poster Session at the Research Conference.

Materials and Methods: Data were pooled from three 12-week, double-blind, randomized studies (TREAT RLS 1, 2 and US) in patients with moderate-to-severe primary RLS. Ropinirole, 0.25–4.0 mg/day, or placebo, titrated as needed and tolerated, was taken once daily 1–3 hours before bedtime. Efficacy was assessed in post-hoc analyses of patients who reported at least moderately disturbed sleep at baseline on Questions (Q) 3 (sleep not quiet/restless), 7 (trouble falling asleep) or 8 (awoken and trouble falling asleep again) of the Medical Outcomes Study (MOS) Sleep Scale.

Results: Of 930 patients (ITT population), at least moderately disturbed sleep was reported by 49% (Q3, n=453), 44% (Q7, n=411), and 39% (Q8, n=367) at baseline on the MOS Sleep Scale. A significant improvement in favor of ropinirole vs placebo was seen in change from baseline on the International Restless Legs Scale (IRLS) total score at Week 12 last observation carried forward (a priori primary endpoint) in patients with sleep disturbance (adjusted mean treatment difference [95%CI]: Q3 -2.9 [-4.6, -1.2], $P<0.001$; Q7 -3.4 [-5.2, -1.6], $P<0.001$; Q8 -4.6 [-6.5, -2.7], $P<0.001$). Ropinirole treatment in patients with a disturbed sleep response on the MOS Sleep Scale was also associated with a significantly greater proportion of patients rated as much (2) or very much (1) improved on the Clinical Global Impression–Improvement scale (responders) compared with placebo (adjusted odds ratio [95%CI]: Q3 1.8 [1.2, 2.6], $P=0.003$; Q7 2.2 [1.4, 3.2], $P<0.001$; Q8 2.2 [1.4, 3.4], $P<0.001$). Mean (SD) ropinirole dose at last visit was similar between groups reporting disturbed sleep (Q3: 2.0 [1.1] mg/day, Q7 and Q8: 2.0 [1.2] mg/day).

Conclusions: Ropinirole improves RLS symptoms and the overall disease condition in patients with sleep disturbance (identified at baseline).

C30

Response Rates of the Highly Cardioselective β 1-adrenergic Receptor Blocker Nebivolol in Mild-to-Moderate Hypertension

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Background: Nebivolol is a novel, long-acting, highly cardioselective β 1-adrenoreceptor blocker that induces an endothelium-dependent vasodilation. Nebivolol is an antihypertensive currently available in Europe and under review in the US. This study evaluated the response rates of nebivolol at doses up to 20 mg QD in patients with mild-to-moderate hypertension, defined as sitting diastolic blood pressure (SiDBP)=95 and <109 mm Hg.

Methods: The study (CSR NEB-302) was a randomized, double-blind, placebo-controlled, multicenter, parallel-group, dose-ranging study in patients with mild-to-moderate hypertension. The intent-to-treat (ITT) population included 909

patients, who were stratified across treatment arms by the following baseline characteristics: nebivolol metabolism (poor vs extensive metabolizers), diabetes status, race, age (<65 years vs >65 years), and gender. Following a 28- to 42- day, single-blind placebo run-in, patients were randomized to treatment with placebo or nebivolol (1.25, 2.5, 5, 10, 20 mg) once daily for 12 weeks. A group treated with 30/40 mg QD was included in secondary efficacy and safety analyses. The primary endpoint was the change in average trough SiDBP from baseline to end of double-blind treatment. A responder was defined as a patient whose average SiDBP taken at trough drug plasma level was <90 mm Hg at study end or had decreased by =10 mm Hg from baseline. Response rates were analyzed using logistic regression.

Results: Response rates follow (ITT population, last observation carried forward). Baseline mean SiDBPs (mm Hg) were: placebo (n=81) 100.3; nebivolol 1.25 mg (n=83), 98.9; 2.5 mg (n=82), 99.8; 5 mg (n=165), 99.6; 10 mg (n=166), 99.5; 20 mg (n=166), 99.4; 30/40 mg (n=166), 99.3. Response rates (n) were: placebo, 20 (24.7%); nebivolol 1.25 mg, 38 (45.8%, $P=0.008$); 2.5 mg, 41 (50%, $P=0.001$); 5 mg, 83 (50.3%, $P<0.001$); 10 mg, 89 (53.6%, $P<0.001$); 20 mg, 99 (59.6%, $P<0.001$); 30/40 mg, 107 (64.5%, P N/A). Nebivolol was well tolerated: 82.7% of patients in the placebo group and 85.7% of patients in the nebivolol groups completed the study.

Conclusions: Compared with placebo, response rates for nebivolol at trough plasma levels in patients with mild-to-moderate hypertension were significantly greater at all doses tested in a dose-dependent fashion.

◆ C31

Glycated LDL and HDL Subfractions in Type 2 Diabetic Patients: a Pilot Study Using a Novel Nitroblue Tetrazolium Method

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Background: Hyperglycemia increases the formation of oxidized LDL and glycated LDL, which are important modulators of atherosclerosis and cardiovascular disease. From the existing methods for glycated Lp measurement none explores the actual lipoprotein particles nor are they able to distinguish between HDL subclasses. HDL2 and HDL3 play different physiological roles and have been shown to be associated with different phases of reverse cholesterol transport.

Hypothesis and aims: We hypothesized that our recently developed method allows for the simultaneous measurement of glycated lipoproteins and for their monitoring in diabetic patients.

Methods: We performed a case-control study with 20 diabetic patients (10 male and 10 female, 58 ± 9 years of age) and their age-matched controls. Basic metabolic panels, lipid panels and HbA1c were measured using standard methods. Determination of glycated Lp is performed on a 4 step slab dis-

◆ Indicates posters entered in the Student Prize Competition, a judged event that takes place during the Poster Session at the Research Conference.

continuous gel (3%, 6%, 13% and 17.5% acrylamide). Serum is run without denaturation for 16 hours. The gel (after equilibrating in carbonate buffer) is incubated with the NBT reagent for 2 h. Gels are washed and densitometric analyses are performed after scanning and processing the gels using ImageJ NIH software (MD, USA).

Results: Our results demonstrate that this new method allows detection, in the same gel, of glycated LDL and glycated HDL2 and HDL3, without denaturation of the particles. In this series type 2 diabetic patients displayed increased lipoprotein glycation achieving statistical significance for HDL 2 and LDL ($P < 0.05$).

Conclusions: Our data confirm the usefulness of our method in a small pilot clinical study. The method we propose has the main novelty of being the first to measure glycation of HDL subclasses. Our study should pave the way for further work on larger series of type 2 and 1 diabetic patients, where correlation with markers of metabolic control and complications are warranted.

Acknowledgment: Supported by Touro and Showa Universities.

◆ C32

AIDS Orphans: Assessment of Families in Crisis in Rural Tanzania

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Introduction: Currently, there are 14 million children who have lost 1 or both parents to AIDS and 80% are living in sub-Saharan Africa. In 2015, it is estimated that rates will rise to 25 million AIDS orphans. In Tanzania alone, there were nearly 1 million orphans in 2003. Typically these children are absorbed into extended families, which are already overwhelmed by poverty and therefore unable to provide adequate health care and education.

Objectives: To conduct a needs assessment in a rural Tanzanian village and determine the factors that put orphans and their caregivers at risk for poor health and life outcomes.

Methods: Forty-four head of households and forty-nine orphans, living in the Nyamagongo village of Eastern Tanzania, were interviewed for this study. Two investigators and a translator interviewed villagers with surveys translated into Swahili.

Results: 43% of the orphans interviewed had lost both their parents to HIV/AIDS. The average age of a child when their parent(s) died was 6. Females comprised over 80% of orphan head of households. 98% of households did not receive any financial assistance from the government or outside organizations. 86% head of household care providers ranked education assistance as the highest unmet need.

Conclusions: The HIV/AIDS epidemic is wiping out generations of people and placing enormous socioeconomic burdens on families that assume the responsibilities of caring for these

orphans. This is especially evident for female head of households and those living in rural villages. The greatest unmet need identified through this study was the lack of educational assistance. Many children orphaned become caregivers and are made to work instead of attend school, thereby perpetuating the cycle of poverty and poor health outcomes.

Future directions: We envision that future medical students returning to the region will help develop and implement programs, in conjunction with community leaders, based on our preliminary findings. The development of micro-grants for women and/or educational vouchers may help families become more self-sufficient and allow children to stay in school. In addition, comprehensive HIV/AIDS-related prevention and educational programs may help decrease the rates of AIDS orphans.

◆ C33

The Use of Diagnostic Ultrasound for Diagnosis of Carpal Tunnel as Compared to Nerve Conduction Studies

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Background: Carpal Tunnel Syndrome (CTS) is a common neuropathy in the general population. Methods for diagnosis include clinical examination and nerve conduction analysis. The combination of results from these methods confirms a diagnosis of CTS. In previous studies ultrasound findings correlated with detecting CTS based on the cross-sectional area of the median nerve.

Hypothesis: Diagnostic ultrasound correlates with nerve conduction studies for diagnosing CTS and could therefore be an alternate technique.

Methods: Each subject interested in the study underwent bilateral nerve conduction analysis of the upper extremities during the first visit. The subjects were also screened by ultrasound at the level of the volar crease (externally) corresponding to the hook of the hamate internally, 1cm distal and 1cm proximal to the hook of the hamate. The subjects were then randomized into treatment groups and underwent either Osteopathic Manipulative Treatment (OMT) or sham laser treatment for 8 weeks. In addition to treatment, all subjects were provided wrist splints, exercises and ibuprofen. One week after the completion of treatment, the subjects had another nerve conduction analysis of both upper extremities, as well as a second ultrasound testing.

Results: Following analysis the null hypothesis was rejected indicating there is a positive correlation between the Ultrasound measurement of median nerve cross sectional area and the Nerve Conduction Study ($P = 0.025$) grading using stan-

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standard numerical parameters for diagnosis of CTS. There was also a positive correlation between median nerve cross sectional area measured by Ultrasound and Nerve Conduction Study ($P=0.012$) scoring based on clinically significant parameters to diagnose CTS. There was no statistical difference in median nerve measurement by Ultrasound between the laser and OMT groups before or after treatment.

Conclusions: The results of this study suggest that Ultrasonography is as reliable as Nerve Conduction Studies for the diagnosis of CTS. Ultrasound is painless and can be conducted in a physician's office. This may be a technique that is more cost effective for the patient since they will not have to travel and submit insurance claims to another office for testing.

◆ C34

The Use of Musculoskeletal Ultrasound in Carpal Tunnel Syndrome to Detect Response to Conservative Treatment

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Background: Carpal tunnel syndrome (CTS) is the most common entrapment neuropathy. Current conservative treatments include NSAIDs, wrist splints, stretching, exercise, and OMT. While diagnosis is commonly clinical, the gold standard is electromyography (EMG) and nerve conduction studies (NCS). However, these procedures are painful, time-consuming, and require a specialized physician. Research suggests that musculoskeletal ultrasound (US) may be useful in detecting CTS by measuring swelling of the median nerve. US is quick and painless, and can be performed by primary care providers.

Hypothesis: In patients responding to conservative treatment for CTS, US will be able to detect a decrease in swelling of the median nerve by showing a decrease in cross-sectional area (CSA).

Methods: 23 patients with mild-to-moderate CTS diagnosed by NCS in 39 wrists were randomized into OMT ($n=21$) or sham laser ($n=18$) groups. US measurements of the median nerve were obtained from all subjects, and they completed CTS Symptom Severity Scales (SSS) and Functional Status Scales (FSS) prior to treatment. These subjects were provided eight weekly sessions of pan-corporeal OMT (myofascial release/balanced ligamentous tension) or forearm sham laser. All patients were provided wrist splints, hand exercises, and ibuprofen if not contraindicated. Survey data on pain level, medication use, splint wearing and exercises were recorded weekly throughout. NCS, US, SSS, and FSS data were collected one week after the last treatment.

Results: There was a positive correlation between US measurements of the median nerve CSA and the severity of CTS

as diagnosed by NCS ($P=0.012$). Comparison of pre-treatment, post-treatment, and post- minus pre-treatment groups revealed no significant difference in median nerve CSA. There were no significant changes in NCS.

Conclusion: While US measurement of median nerve CSA did correlate with the severity of CTS as diagnosed by NCS, it was not sensitive enough to detect any change in the size of the median nerves in patients who responded positively to conservative treatment in this study.

C35

DVT Standardized Prevention Works to Save Lives

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Introduction: Thromboembolic events (TE), including deep venous thrombosis (DVT) and pulmonary embolism (PE), remain a potentially lethal complication in the care of the trauma patient. We have previously shown a decrease in TE rates for traumatically injured surgical ICU patients after introducing a standardized approach to DVT prophylaxis on the trauma service. We hypothesized that this approach would produce a sustainable decrease in TE, even after changing to a hospital wide prophylaxis form.

Methods: We previously collected prospective data on all trauma patients after implementation of a DVT prophylaxis guideline. We continued data collection for an additional 18 months (1/04–6/05) after the original study. In July 2005, the hospital implemented a mandatory DVT prophylaxis guideline for all patients, which replaced our service specific protocol. We continued data collection after that change, which included DVT and PE rates as well as prophylaxis type and timing.

Results: Most TE occurred in patients receiving appropriate prophylaxis or at risk for anticoagulation. The mandatory hospital wide guideline improved assessment to 99% of patients and decreased TE with inappropriate prophylaxis from 21% to 0%. TE rates initially improved but have not changed significantly over time. However, this may reflect more aggressive surveillance and detection in later time periods. Surgical ICU patients still have the highest risks of developing TE.

Conclusion: TE prophylaxis guidelines, when used effectively, can minimize TE. Given the risks in trauma patients, TE rates will likely never be zero, but prophylaxis errors can be eliminated.

C36

Beneficial and Detrimental Effects of Hemodialysis: a Study on Oxidative Stress and Fibrinolysis Biomarkers

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Background: In spite of technical advances in dialysis treatment modalities and better patient care management including correction of anemia, suppression of secondary hyperparathy-

◆ Indicates posters entered in the Student Prize Competition, a judged event that takes place during the Poster Session at the Research Conference.

roidism, lipid and oxidative stress profiles improvement, the morbidity and the mortality of dialysis patients still remain elevated. Many studies have addressed the issue of dialysis as an inducer of oxidative stress.

Aim: To shed some more light on this issue, in this work we measured changes produced by dialysis in several oxidative stress markers and in plasmin activity.

Methods: We conducted an intervention study with 22 ESRD patients undergoing hemodialysis in whom paired pre- and postdialysis samples were studied along with 30 age-matched control subjects. We measured AOPP, lipid hydroperoxides, total antioxidant capacity of plasma, HDL subclasses and ischemia-modified albumin (IMA). We also measured streptokinase-induced plasmin activity and evaluated the correlations of uremia-associated substances (urea, creatinine).

Results: AOPP and lipoperoxides (Lox) were significantly higher (average 2- to 3-fold for AOPP, 30% for Lox) in renal patients than in controls, and decreased significantly after dialysis. The antioxidant capacity of plasma was decreased after dialysis. We detected no significant dialysis-induced change in IMA or HDL subclasses. Plasmin activity increased from 146.4 ± 30.1 to 160.1 ± 30.1 mU/l ($P < 0.001$). This change correlates ($r = 0.38$, $P < 0.02$) with the efficiency of dialysis. Low molecular weight fractions of renal failure patients serum produce an immediate in vitro inhibition of plasmin activity ($30\% \pm 5\%$ vs $10\% \pm 4\%$ for control, $P < 0.05$).

Conclusions: Our data show that dialysis has beneficial effects on some markers, no effect in others and deleterious in some. IMA, a marker of ischemia is not sensitive to oxidative stress in this patient population. Dialysis increases plasmin activity and this may be, in part, due to removal of a low molecular weight inhibitor. Fibrinolysis defects may contribute to an early and frequent development of atherosclerosis in patients with chronic renal failure, to chronic dysfunction of the renal transplant, or to peritoneal fibrosis in patients on peritoneal dialysis.

◆ C37

The Five Coordinate Construct: A New Approach to Hairline Reconstruction in Hair Restoration Surgery

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Background: Achieving natural looking results is an important aspect of hair restoration surgery. Many methods of neo-hairline positioning have been described in the literature. Currently hair restoration surgeons use visual approximations and other interesting methods to determine their patients' neo-hairlines. Although these approaches are continually used in clinical practice, they do not necessarily produce results that are consistent with the natural hairlines seen in the public arena.

Objectives: In this study we describe a new method for building a five-coordinate construct that is consistent with the average Type I hairline dimensions (minimal to no recession

according to the Norwood-Hamilton Scale) seen among men between the ages of 25–45 regardless of race. This construct can be used as a guide to creating a neo-hairline on patients seeking hair restoration.

Methods: Our method of establishing the average five-coordinate construct involves measuring distances between specific cephalic landmarks and different points along the hairlines of 40 male subjects with Type I hair recession. Using the collected data, we establish specific dimensions of the average male Type I hairline and demonstrate an approach to creating a stencil that can be used for hairline coordinate positioning on patients with moderate hairline recession.

Results: Our results indicate that there is significant consistency among hairline dimensions of 40 male subjects with Type I recession, relative to the size of their cranial vaults when using the landmarks mentioned above. These data can be used as the basis for calculating the dimensions of a five-coordinate construct which is proportional to the head size of a random patient.

Conclusion: The Type I five-coordinate construct may be used by hair restoration surgeons to better aid in the natural positioning of the neo-hairline on men with mild to moderate hairline recession. Another advantage of this approach is that it can be applied to patients with varying cranial sizes and shapes. Further studies to establish a construct for different grades of recession may allow surgeons to set up various neo-hairlines to determine which one would suit the patient the best with the amount of donor hair that is available.

◆ C38

Diabetes Report Card for an Academic Medical Institution

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Objective: The goal of this study was to determine the current compliance of physicians at an academic medical center with the 2004 American Diabetes Association (ADA) guidelines for the treatment of type 2 diabetes mellitus and to identify which aspects of the current guidelines these providers are meeting and those which they are underutilizing.

Methods: A retrospective chart review was performed on all patients with type 2 diabetes (ICD 250.00-250.03) seen by physicians at an academic medical center between June 1, 2004 and May 31, 2005. Subjects were identified from billing information, and data was abstracted by manual chart review performed by medical students. Charts were reviewed to assess compliance with the 2004 ADA Standards of Care.

Results: A total of 642 charts were reviewed. Approximately 90% of the charts reviewed in this study had evidence of home glucose monitoring. In addition, 88% had a documented HgA1c level and 79% had lipids checked annually. Furthermore, 65%

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of patients received a foot exam, 26% received a dilated eye exam, 19% had an annual EKG, and 49% had an annual microalbumin check. Our results showed that 43% of patients were taking an aspirin every day, 13% had documented flu shots, and 23% had documented pneumococcal vaccinations. **Implications:** The physicians in this study demonstrated compliance with many of the ADA guidelines. However, there are many areas in which adherence to the guidelines should be improved.

C39

Retrospective Outcome Analysis of Osteopathic Manipulation in a Treatment Failure Setting

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This study was initiated to confirm a pattern of clinical improvement that was observed in a group of chronic pain patients when Osteopathic treatment was added to their treatment protocol.

Hypothesis: Osteopathic Manipulation in the Cranial Field integrated with physical therapy can positively influence functional and pain outcomes for patients with chronic neck and back pain with a history of treatment failure utilizing standard pain interventions and physical therapy alone.

Methods: The charts of 150 consecutive patients referred for osteopathic care in a major tertiary Pain Center were reviewed for inclusion in the outcome analysis. 40 patients met the inclusion criteria that included a history of cervical or lumbar pain of duration longer than 6 months with treatment failures in other Interventional Pain and Rehabilitation settings in the Boston metropolitan area. Exclusion criteria included use of high dose opioid medications, prior osteopathic manipulation, and outstanding litigation. Outcomes included numeric pain scores on a 10 point scale and assessment of functional capacity using a 5 point scale graded by both the patient and physical therapist. A score of 1 represents poor function, 2 represents fair function, 3 represents moderate function, 4 represents good function, and 5 represents excellent function. Assessments were done by the treating physical therapist as well by the patient's self-assessment before initiating treatment, at the end of treatment and at 6 month follow up by phone call. Standard pain management was provided by the attending Physiatrist. Additional treatment involved 3 to 10 sessions of osteopathic manipulation in the cranial field using balanced ligamentous tension by the primary author combined with a course of physical therapy working on progressive stretching and strengthening of postural stabilizing muscles.

Results: Retrospectively, there were significant improvements seen in pain scores and functional scores pre and post treatment. The mean pain score at the start of treatment was 5.76 and at the completion of treatment 2.05 (P 0.003). PT rated function was moderate or less at the start of treatment (mean 39.02). At the conclusion of treatment a function rating of good or excellent function in 85% of the participants (mean 74.39, P 0.006). At 6 months follow-up, only 30% of the subjects responded to a telephone assessment of pain and function. Of those that responded, 92% had maintained their post treatment function or had improved function. The average pain score remained low at 2.4.

Conclusion: This retrospective outcome assessment suggests that the inclusion of osteopathic manipulation in the cranial field integrated with physical therapy may have a significant effect on reversing treatment failures in patients with chronic neck and low back pain.

C40

Ropinirole Can Produce Rapid, Complete and Sustained Relief From the Symptoms of Restless Legs Syndrome (RLS)

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Hypothesis: RLS is a chronic neurological condition characterized by a compelling urge to move the legs often accompanied by uncomfortable sensations. Ropinirole, a dopamine agonist, is FDA-approved for the treatment of moderate-to-severe primary RLS.

Materials and Methods: Data were pooled from three similarly designed, double-blind, 12-week, placebo-controlled studies (TREAT RLS 1, 2 and US) of ropinirole in adults with primary RLS. Ropinirole (0.25–4.0 mg/day) or placebo was titrated as needed and tolerated and taken once daily 1–3 hours before bedtime. Efficacy was assessed using the International Restless Legs Scale (IRLS) total score and the Clinical Global Impression–Improvement (CGI-I) scale.

Results: At Day 2 (TREAT RLS 1&2)/Day 3 (TREAT RLS US), Weeks 1 and 12 mean (SD) doses were: ropinirole 0.25 (0.01), 0.45 (0.1) and 2.0 (1.2) mg/day, respectively; placebo 0.25 (0.03), 0.47 (0.1) and 2.9 (1.3) mg/day, respectively. There were statistically significant treatment differences in favor of ropinirole after 2 nights of treatment, in mean change in IRLS score from baseline (adjusted mean treatment difference [AMTD] -2.4 ; 95% CI: $-3.6, -1.2$; $P < 0.001$) and proportion of responders (rated as 1, very much, or 2, much improved) on the CGI-I scale (adjusted odds ratio [AOR] 3.0; 95% CI: 1.8, 5.0; $P < 0.001$). At Week 1, significantly more patients in the ropinirole than placebo group (8% vs 1%; AOR 6.3; $P < 0.001$) had an IRLS score of 0 (no symptoms). At Week 12, AMTD for change from baseline in IRLS score (primary endpoint) was -3.2 (95% CI: $-4.3, -2.1$; $P < 0.001$); AOR for responders on the CGI-I scale was 2.0 (95% CI: 1.5, 2.6; $P < 0.001$).

♦ Indicates posters entered in the Student Prize Competition, a judged event that takes place during the Poster Session at the Research Conference.

Conclusions: Ropinirole treatment provides statistically significant rapid and sustained relief of RLS symptoms, compared with placebo, with improvements seen after 2 nights through 12 weeks, and more patients reporting complete relief of RLS symptoms as early as Week 1.

Acknowledgment: Supported by GlaxoSmithKline R&D.

C41

Ropinirole Improves the Symptoms of Restless Legs Syndrome (RLS) and Is Associated With Improved Quality of Life and Daytime Functioning

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Hypothesis: Restless Legs Syndrome (RLS) is a chronic condition characterized by an irresistible urge to move the legs and can be associated with a negative impact on quality of life (QoL), as well as impaired daytime functioning. Ropinirole is the only FDA-approved treatment for primary RLS. The effect of ropinirole treatment on QoL and daytime functioning of patients with primary RLS was assessed in these analyses.

Materials and Methods: In three 12-week pivotal studies (TREAT RLS 1, 2, and US), patients with moderate-to-severe primary RLS were randomized to once-daily ropinirole (n=464), 0.25–4.0 mg/day, or placebo (n=466), titrated as needed and tolerated, and given 1–3 hours before bedtime. Data were pooled and analysis investigated treatment response using International Restless Legs Scale (IRLS) total score (primary endpoint). QoL was assessed using the Johns Hopkins RLSQoL questionnaire and daytime functioning was assessed by responses to IRLS Item 9 (severity impact of RLS symptoms on ability to carry out daily affairs; post hoc analysis).

Results: Mean (SD) ages were 54; approximately two-thirds of the population were women. Mean (SD) dose at last visit was 2.0 (1.2) mg/day in the ropinirole group and 2.9 (1.3) mg/day in the placebo group. At Week 12 last observation carried forward (LOCF), there was a significant treatment benefit in favor of ropinirole, compared with placebo, for change from baseline in IRLS total score (adjusted mean treatment difference [AMTD]: -3.2; 95% CI: -4.3, -2.1; $P < 0.001$) and in RLSQoL questionnaire overall impact score (AMTD: 4.6; 95% CI: 2.6, 6.7; $P < 0.001$). The most often reported adverse events in the ropinirole group were nausea, headache, and somnolence.

Conclusions: Ropinirole improves RLS symptoms and was accompanied by enhanced overall quality of life and better daytime functioning as compared with placebo.

Acknowledgment: Supported by GlaxoSmithKline R&D.

Basic Sciences

B01

A β Fibril-promoting Agents

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Alzheimer's disease (AD) is classified as a protein misfolding disorder where a progressive accumulation of aggregates of amyloid β (A β) peptides can be seen. A β is derived from the proteolytic processing of the neuronal transmembrane protein called amyloid precursor protein (APP). The elevation of A β in AD results in aggregation and deposition into plaques. Aggregates of A β are present as a fibril structure. While A β aggregation occurs spontaneously, we hypothesize that markers of AD tissue degeneration, such as acrolein and methylglyoxal, may act as fibril promoting agents. Oxidized lipids and carbohydrates generate acrolein and methylglyoxal, respectively. This hypothesis was tested using intrinsic fluorescence polarization as a unique method for tracking A β fibrillation. Since A β (1-40) and A β (1-42) do not contain the natural fluorophore tryptophan, we examined the fluorescence polarization of the single tyrosine residue (Tyr10). A β fibrillation was also tracked by traditional methods of analysis. Thioflavin T fluorescence, light scattering and size exclusion chromatography all correlated with tyrosine fluorescence polarization validating its effectiveness as a technique for monitoring A β aggregation. After a 30min incubation at room temperature, methylglyoxal (115mM) increased tyrosine fluorescence polarization of A β (1-42) by 2.5-fold. Similarly, there was a 1.9-fold increase in tyrosine fluorescence polarization of A β (1-42) by acrolein (100mM) after 7hr at room temperature. Additionally, acrolein (0.5mM) further increased tyrosine fluorescence polarization of preformed A β (1-40) fibrils. These observations suggest that methylglyoxal and acrolein promote fibril formation of the two major A β peptides. We conclude that byproducts of neurodegeneration may contribute to the pathogenesis of AD by accelerating the formation of A β fibrils.

◆ B02

Prehensile Tail Use in New World Monkeys: The Howler Monkey of the Coribicci in Western Costa Rica

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A basic understanding agreed upon by most physical anthropologists is that the beginnings of bipedalism arose from primitive primates whose upper limbs had become mobile by use in the regions in trees below the primary branch structures. Two excellent examples of animals which fit this category are the contemporary primates Ateles and Brachiateles the arboreal howler monkey provides an interesting example of prehensile tail use without the concurrent association of a freely mobile shoulder girdle. In this case, the howler provides a view of a transition form between old world monkeys and those new world species which not only have a prehensile tail, but also, a mobile shoulder girdle. Data has been collected for this study every summer since 2002 with the exception of

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AOA COMMUNICATION

2004. Approximately 9,000 observations have been made of these animals in their native habitat during these field sessions. These animals seldom come to the ground. Approximately 94% of all feeding employs tail use. Often, this is used in two modes: hanging by the tail with all four limbs free and supporting the body in long stretched reaches for fruits. During travel, the prehensile tail is used about 68% of the time to aid in support of the animal. During quadrupedal walking, young animals employ the prehensile tail for support 40% of the time. Juveniles who are more adapt in arboreal travel, employ tail use during quadrupedalism approximately 20% of the time, as do adults. Bridging is an interesting travel form particularly suited to tail use. During this locomotor modality, animals cross from one branch network in the canopy to that of an adjacent tree. In Infant II's (which are free from the mother) approximately 98% of all bridging employs tail use. In juveniles, 91% of bridging involves the use of the tail; in adults, 87% involve tail use.

Acknowledgment: Funding was provided for these research efforts by an EPSCoR Grant from the NSF and by funding from the Intramural Grants Program was WVSOM.

B03

Lymphatic Pump Treatment Increases Leukocyte Numbers in Thoracic Duct Lymph

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Our long range goal is to evaluate the effectiveness of osteopathic manipulative therapy (OMT) at enhancing the immune response against a variety of infectious and inflammatory diseases. In this study, the thoracic duct of five mongrel dogs was isolated so the immediate effects of lymphatic pump on leukocyte output could be measured. Lymph flow was measured by timed collection and lymph was collected over ice under 1) resting (control) conditions, 2) during application of the osteopathic abdominal lymphatic pump procedure. Baseline leukocyte numbers were $1.7 \times 10^6 \pm 0.57 \times 10^6$ cells/ml, while lymphatic pump manipulation significantly increased leukocyte numbers in the lymphatic fluid to $6.2 \times 10^6 \pm 1.6 \times 10^6$ cells/ml. Flow cytometry and differential cell staining revealed macrophages, neutrophils, lymphocytes, T cells and B cells were significantly enhanced ($P < 0.05$) during LPT. In addition, LPT enhanced lymphatic flow rate approximately five-fold (2.7 ml/min) compared to the baseline lymphatic flow rate (0.5 ml/min). By combining the total cells per ml with the lymphatic flow rate per min, LPT enhanced total cells from $0.75 \times 10^6 \pm 0.11 \times 10^6$ total cells per minute to

$17 \times 10^6 \pm 6.9 \times 10^6$ total cells per minute. Similar trends were observed in macrophages, neutrophils, lymphocytes, T cells and B cells during LPT. In conclusion, we have demonstrated that lymphatic pump manipulation significantly increases leukocyte numbers in lymphatic fluid during treatment, approximately 23-fold, suggesting that lymphatic pump increases leukocyte output via the lymphatic system, which then releases these cells into peripheral blood circulation. This significant release of immune cells into the lymphatic system during LPT may be one of the immune mechanisms responsible for the increased immune responses observed in patients given OMT. Ongoing and future studies will determine if lymphatic pump manipulation increases protective immune responses during immunization and infection challenge.

◆ B04

Plastic Fragments of A β -polyacrolein in the Alzheimer's Brain

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The accumulation of senile plaques is thought to contribute to the pathogenesis of Alzheimer's disease (AD). Plaques consist predominately of amyloid- β (A β) peptide. We propose a novel mechanism of plaque formation involving the formation of plastic composite material. Human serum albumin or A β peptide fragments (1-40 and 1-42) were incubated with acrolein (16-750 mM) under unstirred conditions in culture flasks and microplates at room temperature for various times. The samples were then examined under phase contrast light microscopy. The resulting structures were stained with Congo Red and the Bradford reagent to determine presence of protein, and a carbonyl reagent for the presence of acrolein. We observed thin plastic fragments that were extensively punctated. Planar aggregates stained for protein and for cross- β structures suggesting an A β -polyacrolein colloidal mixture. Depending on acrolein concentration and incubation time, we observed uniformly sized planar aggregates (approx. $10 \mu\text{m}^2$) or monolayers ($>100 \text{mm}^2$) of thin polyacrolein films embedded with A β oligomers. The plastic is derived from the polymerization of acrolein ($\text{H}_2\text{C}=\text{HC}-\text{CH}=\text{O}$), which is found in high concentrations in AD brain. Polyacrolein forms composite material by aggregating with amylospheroids of A β . Acrolein and A β colocalize in AD brain. The surfactant and redox properties of A β may act as a catalyst for the polymerization of bifunctional acrolein. A β may also lead to a localized increase in concentration of acrolein by its ability to peroxidize lipids. The ability of A β and acrolein to readily form plastic-like complexes suggests that A β -polyacrolein latexes may exist in neural tissue contributing to the pathogenesis of AD.

B05

Injurious Strain and Counterstrain in an In Vitro Human Fibroblast Model: Regulation of Interleukin Secretion

◆ Indicates posters entered in the Student Prize Competition, a judged event that takes place during the Poster Session at the Research Conference.

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The cellular basis for osteopathic manipulative therapies (OMTs) remains elusive. Previously, we described an in vitro cellular strain model useful in deciphering potential cellular mediators of OMT. In the current study, we further this work by investigating interleukin (IL) secretory profiles from human fibroblasts that were exposed to various strain regimens. Fibroblasts were exposed to 1) an eight-hour hammering strain, 2) a 60 second counterstrain, or 3) both profiles. We hypothesized that hammering would increase pro-inflammatory IL secretion and that counterstrain would reverse this effect and/or increase anti-inflammatory IL secretion. Conditioned media obtained immediately post-hammering (IPH), 24 hours post-hammering (24PH), 24 hours post-counterstrain (24CS) and 24 hours post-hammering plus counterstrain (24HCS) were analyzed by cytokine array. Hammering notably (ie, >2-fold change) increased secretion of pro-inflammatory IL-1 alpha, IL-1 beta and IL-16. While secretion of IL-16 was observed in the IPH group, secretion of IL-1 alpha and IL-1 beta were delayed (seen in 24PH). Hammering also induced anti-inflammatory IL-1 receptor antagonist secretion. Simulated OMT (24CS) induced secretion of two dual action cytokines possessing both anti- and pro-inflammatory properties (IL-13 and IL-16) and also stimulated secretion of five pro-inflammatory ILs (IL-1 alpha, IL-3, IL-6, IL-7, IL-15). The combination of hammering followed by counterstrain induced secretion of fewer pro-inflammatory ILs compared to counterstrain alone. The current study clearly shows that strain profiles designed to mimic injury and OMT elicit different types and quantities of interleukin secretion. The ultimate inflammatory outcome post-strain appears to result from the balance between pro- and anti-inflammatory cytokine actions. These data suggest that OMT may result in anti-inflammatory-based clinical outcome in the long run, but within 24 hours of counterstrain the data may also support a role for ILs in post-treatment flare by modulating local fibroblast-derived ILs.

Acknowledgment: This work was funded by National Institutes of Health Grant P-01 AT2023 (National Center for Complementary and Alternative Medicine) to PRS.

◆ B06

Heterobiaxial and Equiradial Strain Models of Osteopathic Manipulative Treatments (OMTs): Dichotomous Regulation of Human Fibroblast (HF) Cell Morphology and Cytokine Secretion

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Hypothesis: We hypothesize that equiradial strain induces equivalent changes in fibroblast morphology, proliferative capacity and cytokine secretions as compared to heterobiaxial strain.

Introduction: There is a paucity of data regarding cellular mechanisms explaining OMTs. Utilizing human fibroblasts

and an in vitro cellular strain apparatus, we reported that acyclic heterobiaxial strain for 48 hours at 110% of resting cell length induces morphological alterations and increases proliferation and IL-6 secretion. As various OMTs strain cells and tissues differentially, we sought to determine if acyclic equiradial strain induces equivalent alterations.

Materials and Methods: Cultured HF were equiradially strained acyclically for 48 hours at 110%. HF morphology and actin architecture were microscopically assessed, while proliferation was estimated using Owen's reagent and by quantifying the cellular protein to dsDNA ratio. Cytokine secretion was assessed from strained and control HF cultures using a cytokine antibody array.

Results: Equiradial strain resulted in no morphological alterations and no change in HF proliferation. However, there was $42 \pm 5\%$ decrease in IL-6 content in conditioned media of strained cells. Additional downregulated cytokines included FGF-6, IL-15, IFN- γ , PDGF-BB, RANTES, TNF- α , and TNF- β . Cytokines whose secretions were increased with strain include angiogenin, BMP-4, Eotaxin, and FGF-7.

Conclusions: When comparing heterobiaxial and equiradial strains of equal magnitude, frequency and duration, we observed divergent effects on HF morphology, proliferative response and cytokine secretion. Since various OMTs impart differential biophysical strains in vivo, we suggest that this translates to differential cellular responses with respect to fibrosis, cytokine secretion, etc. If true, such diversity in cellular responses may underlie the relative efficacies of each OMT in treating various somatic dysfunctions.

Acknowledgment: Funding for these studies was made possible by grants awarded to PRS from the NIH and the National American Heart Association.

◆ B07

Beta-Catenin Mediates Guidance and Branching Dynamics of Optic Axons

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N-cadherin is required for targeting and branching of developing optic axons within the tectal midbrain. However, the underlying molecular and cellular mechanisms of this process have not been elucidated. The N-terminal domain of beta-catenin contains the alpha-catenin binding site, which is required for coupling of Cadherin to the actin cytoskeleton. Our goal is to define the role of the N-terminal domain of beta-catenin in targeting and branching dynamics of optic axons in *Xenopus laevis* tadpoles. A deletion mutant of beta-catenin consisting of only the N-terminal domain (NTERM) (which binds to alpha-catenin but not to Cadherin) was constructed. A plasmid containing the mutant NTERM with a GFP tag was injected into eye buds of one-day-old embryos. This resulted

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in expression of NTERM in single optic axons in one week old tadpoles. Live images of optic axons in one-week-old tadpoles were taken with conventional and confocal microscopy. We find that a majority of NTERM optic axons (NOA) did not reach their specific targets within the dorsal tectum. Higher magnification imaging with a confocal microscope demonstrated these NOA that mistargeted in the tectum did not branch. The remaining NOAs targeted correctly in the tectum, and extended a reduced number of branches compared to controls. This data suggests that the Cadherin-beta-catenin pathway is requisite for branching involved in guiding ventral optic axons to topographic-specific synaptic regions. This pathway is also essential for the secondary branching that establishes synaptic connections of optic axons with tectal neurons.

◆ B08

The GSK-3beta Binding Site of Beta-Catenin is Essential for Mapping and Shaping Arbors of Ventral Optic Axons in the Dorsal Tectum

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Recent studies have shown that the Wnt signaling pathway is required for targeting of axons in the developing nervous system. In the Wnt beta-catenin pathway, Wnt signaling inhibits GSK-3beta phosphorylation of beta-catenin. This facilitates beta-catenin stabilization, translocation into the nucleus and induction of gene transcription. We hypothesize that GSK-3beta phosphorylation of beta-catenin is necessary for specific mapping of optic axons in the retino-tectal map. Conversely, disruption of this Wnt beta-catenin pathway should lead to abnormal axonal mapping in the tectum. Beta-cat 107 is a deletion mutant consisting of the N-terminal 107 amino acids of wild type beta-catenin that contain the GSK-3beta binding site. Beta-cat 107 was tagged with GFP and overexpressed in individual ventral optic axons of *Xenopus* tadpoles. The projection and arborization of the axons was observed in live tadpoles using conventional and confocal microscopy. Optic axons expressing beta-cat 107 follow the path of the wild-type axons into and through the dorsal tectum. However beta-cat 107 axons target farther and more medioposteriorly in the tectum. In addition, beta-cat 107 axons extend the same average number of branches as wild-type control axons, but these branches are directed more medially than in controls. Inhibition of the interaction between GSK-3beta and wild-type beta-catenin by overexpression of beta-cat 107 shifts mapping of ventral optic axons in the dorsal tectum and alters their arbor shapes. This suggests that normally, inhibition of GSK-3beta phosphorylation of beta-catenin induces mapping of optic axons to specific targets in the dorsal tectum by shaping axonal arbors. Thus, the Wnt signaling pathway acts as a topographic-specific cue in the retinotectal projection.

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◆ B09

Simultaneous Measurement of Glycated LDL and Glycated HDL Subclasses: a New Method

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Background: In diabetes patients, glycation of apolipoproteins correlates with other indices of recent glycemic control, including HbA1c. For several reasons, increased glycation of apolipoproteins may play a role in the accelerated development of atherosclerosis in these patients. Glycated LDL and HDL could prove very useful in measuring the effect of hyperglycemia on cardiovascular disease, its risk factors, and its complications. Comparing different glucose-lowering and lipid-lowering drugs in respect to their influence on glycated LDL could increase knowledge of the mechanism by which they alter cardiovascular risk.

Hypothesis: We propose an alternative method that allows for simultaneous estimation of early glycation of native LDL, HDL2 and HDL3 particles in the same patient sample. These data can be adjusted to apoA and apoB measured by conventional methods or to the cholesterol values.

Methods: On a 4 step slab discontinuous gel (3%, 6%, 13% and 17.5% acrylamide), serum is run without denaturation for 16 hours. The gel (after equilibrating in carbonate buffer) is incubated with the NBT reagent for 2 hours. Gels are washed and densitometric analyses are performed after scanning and processing the gels using ImageJ NIH software (MD, USA).

Results: When we compared serum run in our system and processed with lipid (Sudan Black), glycation (NBT) and protein staining (Coomassie), the glycation signal matches with protein and lipid (thus, lipoprotein). When pure HDL or LDL is run in parallel with the sera, each band matches when reacted with NBT, Sudan Black and Coomassie, certifying the identity of the bands. The CV for glycated LDL and HDL measurements are 5–10%.

Conclusions: None of the existing methods for glycated Lp measurement explores the actual lipoprotein particles nor are they able to distinguish between HDL subclasses. HDL2 and HDL3 play different physiological roles and have been shown to be associated with different phases of reverse cholesterol transport. Our method could be used in clinical research focusing of the atherogenic role of lipoprotein glycation, to correlate with other lipid parameters, HbA1c, fructosamine as well as clinical outcomes.

Acknowledgment: Supported by Touro University.

◆ B10

***Ilex Paraguariensis* “Mate” Drinking Increases Paraoxonase-1 Activity in Normal Subjects: a Pilot Study**

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Background: The antioxidant activity of high-density lipoprotein is largely due to its paraoxonase 1 (PON1) content. Exper-

iments 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 PON1 activity is an independent predictor of new CHD events. Studies from our laboratory, which have since been confirmed by others, have demonstrated the antioxidant properties of *Ilex paraguariensis* "mate" with regard to human LDL oxidation in vivo. This beverage has gained popularity in the United States in the last few years. It is used as a very popular folk beverage dating from pre-Columbian times, in large regions of South America.

Hypothesis: We hypothesized the high polyphenol beverage "mate" (*Ilex paraguariensis*) acutely increases serum PON1 activity in healthy subjects. We based our idea on the recent observation that dietary antioxidants can affect PON1 levels and activities by reducing oxidative stress (which inactivates PON1), and also directly by affecting paraoxonase gene expression.

Methods: In this pilot study, capillary blood was obtained from healthy human volunteers (n=6) when fasting and sampled again at 1 h after drinking 1/2 liter of mate or water or coffee and milk in a 60 min period. PON-1 activity was determined from the initial velocity of p-nitrophenol production at 37°C and recorded at 405 nm in a VERSAmax (Tunable) Microplate Reader.

Results: PON-1 activity increased significantly after mate drinking in all subjects, averaging a $10\% \pm 1\%$ vs $0 \pm 1\%$ for control breakfast or water ($P < 0.05$).

Conclusions: Our data give in vivo proof of principle for a positive effect of *Ilex paraguariensis* on PON-1 activity. PON1 activity could be a target for dietary modulation or pharmacological intervention. The magnitude of the effect, though modest, is similar to that found for several studies with statins. A larger study with kinetic data and evaluation of HDL composition and PON-1 mass is warranted. If proven in this ongoing study, our preliminary results would suggest another way to increase PON-1 activity and thus HDL protection of LDL oxidation.

Acknowledgment: Supported by Touro University.

◆ B11

Microbial Flora of Common Household Spiders and Their Potential for Spider Bite Wound Infections in Humans

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There is an increasing incidence in the number of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the United States. Skin and soft tissue infections caused by MRSA have often been described as being preceded by a spider bite. Several possibilities exist to explain this phenomenon. Among these are: 1) spiders introduce MRSA into the bite wound and thereby serve as a potential vehicle or vector for MRSA; 2) MRSA colonization is an event

secondary to the spider bite; and, 3) the spider bite is a misguided way for patients to explain the initial lesion of their skin or soft tissue infection. We hypothesized that if spiders were able to serve as vehicles or vectors for MRSA infections, they would themselves be colonized with this pathogen. To test this hypothesis, we captured common household spiders and determined the patterns of normal microbial flora isolated from them. Spiders were collected from several dwellings by dwelling occupants, photographed for speciation and cultured for external and internal microbial flora. Of over 100 spiders collected, none were found to carry *Staphylococcus aureus* or MRSA. Relatively low numbers of microbial flora were isolated and only a single isolate with pathogen potential in human skin and soft tissue infections (*Aeromonas* species) was isolated. Spiders are unlikely to be a source of MRSA. Colonization of human spider bite wound by MRSA is likely to be an event secondary to the spider bite. Alternatively, MRSA infections may masquerade as spider or other insect bites in their early stages.

◆ B12

Novel Methods for the In Situ Evaluation of Proximal Tubule Epithelial Cell Viability and Barrier Integrity

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Objective: As part of our ongoing research to identify the mechanism by which cadmium (Cd) produces its nephrotoxic effects, we developed novel, in situ assays for cell viability and epithelial barrier integrity that involve perfusing the intact kidney with the cell viability indicator, ethidium homodimer, and the cell-surface (glycocalyx) marker, ruthenium red.

Methods: Rats were treated with either saline vehicle alone or Cd (0.6 mg/kg, 5 days per week, s.c.) for 12 weeks. As a positive control for detecting cell death and disruption of epithelial barrier function, other rats received HgCl₂ (0.437, 0.875, 1.75, 3.5 mg/kg, i.p.), doses that have previously been shown to cause mild-severe necrosis of the proximal tubule within 24 h. At the end of the treatment protocols, animals were anesthetized and the left kidneys were perfused in situ with a physiologic saline solution containing either ethidium homodimer (5μM) or ruthenium red (12.7 mM).

Results: Acute nephrotoxic doses of HgCl₂ had biphasic, dose-dependent effects on urinary protein excretion and urine volume. Furthermore, there was a dose-dependent increase in the percentage of ethidium-labeled cells in the kidneys of the Hg-treated animals. By contrast, the kidneys from Cd-treated animals showed no increase in the number of ethidium-labeled cells compared to controls. Ruthenium red staining in control rats was confined to the apical surface of the proximal tubule, whereas the kidneys of the Cd-treated

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animals showed diffuse labeling at the basolateral surface.

Conclusion: These results indicate that ethidium homodimer and ruthenium red can be used to measure cell viability and barrier integrity in the intact kidney, respectively. Furthermore, these results indicate that sub-chronic Cd exposure causes a loss of epithelial barrier integrity by mechanism(s) that occur prior to, or independently of cell death.

Acknowledgment: Supported by NIH Grant R01 ES006478.

B13

Fractalkine Is a Novel Chemoattractant for Rheumatoid Arthritis Fibroblast-like Synoviocytes and Signals Through MAP Kinases and Akt

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Purpose: Fibroblast-like synoviocytes (FLS) from rheumatoid arthritis (RA) patients are a major constituent of the hyperplastic synovial pannus that aggressively invades cartilage and bone during RA. Fractalkine (Fkn/CX3CL1) is expressed in RA synovial fluids (SFs) at 3- to 4-fold higher levels than those detected in SFs from patients with osteoarthritis or other rheumatic diseases, respectively. Interestingly, RA FLS express CX3CR1, the receptor for Fkn, yet no functions have been characterized for Fkn acting on RA FLS.

Methods: RA FLS were employed in chemotaxis assays, Western blotting, ELISAs, and Alexa Fluor 488 Phalloidin staining of F-actin to characterize the relationship between Fkn and RA FLS.

Results: Stimulation of RA FLS with 1 or 10 nM Fkn shows significant cytoskeletal rearrangement after 2 hrs, consistent with the slow migration associated with fibroblasts. Chemotaxis assays reveal that Fkn is a chemoattractant for RA FLS, effectively inducing significant migration of all 6 RA patients that we tested ($P < 0.05$). To elucidate a potential signaling mechanism, we stimulated RA FLS with 10 nM Fkn, and show by Western blot the activation of several specific mitogen-activated protein (MAP) kinases. We demonstrate that Fkn stimulates RA FLS phosphorylation of Jun-N-terminal kinase (JNK; $n=3$), extracellular signal-regulated kinase (Erk) 1/2 ($n=3$), and Akt ($n=3$ at both Ser 473 and Thr 308) in a time dependent manner in as little as 5 minutes with a maximal response typically around 15–30 minutes. Since we have shown previously that RA FLS can also produce Fkn, and since TNF- α , IFN- γ , and IL-4 have recently been shown to regulate nasal fibroblast secretion of Fkn, we assessed whether these cytokines alter Fkn production in RA FLS. Our results suggest that TNF- α , but not IFN- γ , can upregulate RA FLS secretion of Fkn in a time dependent manner ($n=3$; $P < 0.05$), and that IL-4 alone or in combination has no significant effect on Fkn secretion.

Conclusions: We have identified a novel role for Fkn in regulating RA FLS cytoskeletal structure and migration. Fkn specifically induces RA FLS phosphorylation of the MAP

kinases JNK and Erk 1/2, as well as full activation of Akt, a potent survival signal.

B14

Fractalkine Functions as a Chemoattractant for Osteoarthritis Synovial Fibroblasts and Stimulates Phosphorylation of MAP Kinase

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Purpose: Synovial fluid from osteoarthritis (OA) patients contains the CX3C chemokine, fractalkine (Fkn/CX3CL1). Expression of the Fkn receptor, CX3CR1, has also been demonstrated in OA synovial lining, albeit in low amounts. This study aimed to determine a biological function for this ligand/receptor pair in OA and to assess a potential signaling mechanism for Fkn in a predominant synovial cell type, OA fibroblasts.

Methods: OA synovial fibroblasts were studied using chemotaxis assays, Western blotting, and staining of F-actin to characterize a novel function for Fkn acting on OA fibroblasts.

Results: Chemotaxis assays demonstrate that the chemokine domain of Fkn, an established potent chemoattractant for monocytes, also effectively recruits OA synovial fibroblasts. Fkn induced significant migration of fibroblasts in all 10 of the OA patients that we tested ($P < 0.05$). Consistent with this finding, visualization of F-actin demonstrates that 1 or 10 nM Fkn induces noticeable reorganization of cytoskeletal structure in OA fibroblasts in a time-dependent manner. We see dramatic enhancement of F-actin staining within 30 min stimulation and we note maximal enhancement at around 2 hrs. These time points are consistent with the slower migratory rates associated with fibroblasts. To determine whether Fkn activates signaling mechanisms often associated with migration, we stimulated OA fibroblasts with 10 nM Fkn and generated cell lysates to analyze by Western blotting. We demonstrate that Fkn stimulates phosphorylation of the MAP kinases p38 ($n=3$), JNK ($n=3$), and Erk 1/2 ($n=3$). Examination of survival signals generated by the PI3 kinase-Akt pathway demonstrate that Akt is phosphorylated at both Ser 473 and Thr 308, suggesting full activation of Akt ($n=2$). Further, our data suggest that all of these phosphorylation events occur in a time dependent manner, with little or no activation within 1 min, and maximal activation typically between 5–30 min.

Conclusions: Fkn is a novel chemoattractant for OA fibroblasts, consistent with Fkn-induced alterations in cytoskeletal structure. Fkn induces phosphorylation of the MAP kinases p38, JNK, and Erk 1/2, as well as full activation of Akt, a potent survival signal.

◆ B15

Fractalkine Induces Cytoskeletal Rearrangements and MAP Kinase Signaling in Microvascular and Macrovascular Endothelial Cells

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Purpose: Neovascularization is a well established characteristic in rheumatoid arthritis (RA) synovial pannus. We have previously demonstrated that fractalkine (Fkn/CX3CL1) expression is significantly increased in the RA joint, and that fractalkine induces angiogenesis in vitro as well as in vivo. Moreover, endogenous Fkn present in RA synovial fluids contributes a significant portion of angiogenesis induced in vivo by these fluids. We established that Fkn induces migration and tube formation of human microvascular endothelial cells (HMVECs), and that these cells express CX3CR1, the Fkn receptor. Potential mechanisms through which Fkn achieves these angiogenic effects remain undefined.

Methods: HMVECs and human umbilical vein endothelial cells (HUVECs) were stimulated with Fkn and either analyzed by Western blotting or fixed at various time points for staining of F-actin.

Results: F-actin staining reveals that significant cytoskeletal rearrangements occur in HUVECs and HMVECs starting as early as 1 hour after stimulation with 1 nM or 10 nM Fkn. Maximal alterations in the cytoskeleton can be seen 2 hours after stimulation with Fkn. Stimulation of HUVECs or HMVECs with 10 nM Fkn demonstrates no significant alterations in phosphorylation of Akt at Ser 473 (n=3) or Thr 308 (n=3). In contrast, Western blotting demonstrates significant phosphorylation of specific MAP kinases. HUVEC stimulation with Fkn results in phosphorylation of JNK ranging from 1–30 min (n=4). In line with this finding, HMVEC stimulation with Fkn reveals phosphorylation of JNK (n=2) over a similar time course. Fkn also induces significant phosphorylation of Erk 1/2 in HUVECs (n=4) over a time course ranging from 1–15 min. A somewhat similar time course (5–15 min) was detected for Erk 1/2 phosphorylation in HMVECs (n=3).

Conclusions: Fkn induces significant alterations in both microvascular and macrovascular cytoskeletal structure. While not effecting Akt, Fkn specifically activates the MAP kinases JNK and Erk 1/2 in both macrovascular cell types. Our results suggest that the endogenous Fkn present in the RA joint may induce angiogenesis through activation of the JNK and Erk 1/2 pathways.

◆ B16

Resistance of p21 Null Mice to Arthritis May Be Associated With an Anti-angiogenic Microenvironment

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Purpose: Mice deficient in p21 (p21^{-/-}) are resistant to serum

transfer-induced arthritis (K/BxN) in comparison with control mice (WT). While p21^{-/-} mice have higher levels of chemokines in the joint than WT mice, the joint has fewer synovial macrophages. This is likely related to a demonstrable lack in circulating inflammatory monocytes in p21^{-/-} mice, and could also be related to defects in angiogenesis. A role for p21 in regulating angiogenesis has not been clearly determined.

Methods: To determine the role of p21 in regulating angiogenesis, we examined p21^{-/-} and WT fibroblast RNA extracted from quadruplicate cultures at passage 4 by microarray analysis using Affimetrix Gene Chips. Since synovial fibroblasts can produce the matrix that supports blood vessel development, we allowed p21^{-/-} or WT fibroblasts to produce matrix, and then co-cultured them with human microvascular endothelial cells (HMVECs) to assess influence on tube formation. For comparison, we co-cultured HMVECs with either synovial fibroblasts from humans with rheumatoid arthritis (RA) or those from osteoarthritis (OA) patients.

Results: Microarray analysis using RNA isolated from p21^{-/-} and WT fibroblasts revealed a marked increase in anti-angiogenic chemokines. Namely, we identified a 5.9-fold increase in CXCL9 (monokine induced by interferon (IFN)-gamma; MIG) and a 9.6-fold increase in CXCL10 (IFN-gamma-inducible protein; IP-10), two potent anti-angiogenic chemokines. We also show a 33.8-fold increase in CXCL2 (MIP-2 beta/KC), a chemokine previously identified as possessing either pro- or anti-angiogenic characteristics. Co-culture of HMVECs with p21^{-/-} fibroblasts induced significantly less tube formation than those cultured with WT fibroblasts ($P < 0.0001$). Interestingly, fibroblasts from RA and OA patients did not induce differences in tube formation when co-cultured with HMVECs.

Conclusions: Our results suggest that fibroblasts lacking p21 support significantly less tube formation when compared with WT fibroblasts, potentially through upregulation of anti-angiogenic chemokines. This implies that inhibiting angiogenesis, in addition to the previously noted defects in macrophages, is a potential mechanism through which p21^{-/-} mice resist arthritis.

B17

Cadmium Functions in an Angiostatic Manner on Human Umbilical Vein Endothelial Cells (HUVECs) Through Decreasing VE-cadherin Expression

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Purpose: The average US citizen ingests 10–30 ug of cadmium (Cd) per day, and substantially higher levels if they use tobacco products. The biological half-life of Cd is very long, estimated at 15–30 years, suggesting that the total body burden of Cd increases over time. The vascular endothelium appears to be

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AOA COMMUNICATION

a primary target for Cd, yet very little is known about the effects of Cd on angiogenesis, and even less is understood in regards to a potential mechanism. We hypothesized that Cd disrupts angiogenesis through decreasing expression of VE-cadherin.

Methods: HUVECs were introduced to Cd at various doses and tested in viability assays, chemotaxis assays, and tube formation assays in addition to identifying VE-cadherin expression via immunofluorescence.

Results: Toxicity studies on HUVECs in the presence of 0.1% serum suggest that a dose of 100 μ M Cd has no deleterious consequences after a 6 hr exposure, whereas a dose of 1 mM Cd appears to kill approximately 50% of HUVECs. Despite the absence of a toxic effect, 50–500 μ M Cd significantly decreases migration of HUVECs to a powerful chemoattractant. We previously demonstrated that inclusion of Cd during the tube formation process significantly disrupts endothelial cell tube formation. We now further these findings by demonstrating that pre-incubating HUVECs with Cd for 48 hours, and then performing the assay in the absence of Cd, significantly disrupt PMA-induced tube formation even when doses as low as 100 nM Cd are used. Immunolocalization studies reveal consistent VE-cadherin expression around the perimeter of confluent HUVECs that are not treated with Cd, or those treated with 10 nM Cd. In contrast, VE-cadherin expression is significantly decreased following an overnight incubation with doses of Cd ranging from 100 nM – 100 μ M. In addition, noticeable gaps in HUVEC confluence can be identified when exposed to this range of Cd doses.

Conclusions: Cd acts in an angiostatic manner on HUVECs, having low toxicity, yet significantly disrupting HUVEC migration and tube formation. Moreover, Cd disrupts VE-cadherin expression on the cell surface of HUVECs, an adhesion molecule that is critical for angiogenesis.

◆ B18

Reduction in HERG Current by Extracellular Potassium and Extracellular pH

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The human ether-a-go-go-related gene (HERG) encodes a voltage gated potassium channel that is found in the human myocardium and is involved in the repolarization of cardiac myocytes during an action potential. Dysfunctioning HERG channels have been implicated in Long QT Syndrome, which is a syndrome characterized by a lengthening of the time between the Q and T waves on an EKG and can lead to the lethal arrhythmia Torsades de Pointes. Changes in extracellular electrolytes and extracellular pH have been shown to alter HERG channel function. However, the mechanisms by which these electrolytes alter HERG channel function are not well

understood. Current experiments focus on elucidating the interactions between extracellular potassium and extracellular pH.

Hypothesis: Both potassium and hydrogen interact with the extracellular pore of HERG channels. Thus a reduction in the extracellular concentration of the permeant ion (ie, K⁺) will result in greater current reduction with increased extracellular hydrogen.

Materials: Experiments were performed using two electrode-voltage-clamping of *Xenopus Laevis* oocytes expressing HERG channels.

Methods: Changes in current through HERG channels were measured after oocytes were bathed in solutions of either pH 6.0 or pH 7.4 with a potassium concentration of either 0mM, 2mM or 20mM (6 unique solutions).

Results: These experiments demonstrated that the greatest reduction in current through HERG channels after changing the extracellular solution from pH 7.4 to pH 6.0 occurred in 0mM K⁺. In addition there was minimal change in current reduction due to increased extracellular hydrogen between 2mM K⁺ and 20mM K⁺.

Conclusions: These experiments suggest that hydrogen ions are capable of occluding the permeation pathway, most likely by binding to an outer pore site. One possibility is that both hydrogen and potassium bind to the same site near the channel's outer pore and although potassium goes through the channel (conducting current), hydrogen remains at the outer pore and blocks the channel. These results suggest that patients who are hypokalemic and acidotic might be at increased risk of developing Long QT Syndrome and in turn Torsades de Pointes.

◆ B19

Evaluation of One-Year Survival Rates of Children With Burkitt's Lymphoma in Tanzania After Cyclophosphamide Monotherapy

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Objective: To assess one-year survival rates in Tanzanian children with Burkitt's Lymphoma (BL) receiving cyclophosphamide (CPM) monotherapy treatment. In addition, to assess the pattern of presentation of Burkitt's Lymphoma in Tanzanian children - mandibular versus abdominal involvement.

Design: A retrospective study of 47 children with BL, confirmed by fine needle aspirates (FNA), was performed. Treatment for children was carried out using CPM monotherapy (IV CPM at 40 mg/kg/dose at 14-day intervals for 4 weeks). Monotherapy (vs multiple treatment therapy) was used due to limited funding at Shirati Hospital, Tanzania. All 47 cases studied had information regarding the type of BL (mandibular vs. abdominal); however, information on survival was only obtainable in 28 cases.

Results: Out of 47 patients, abdominal BL was the most frequent site of involvement (76%), with 36 cases total (8 of which also concurrently displayed mandibular involvement). The

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M:F ratio was 3:2 and the median age of diagnosis was <7 y/o. Among the 28 cases with survival data, the overall survival rate was approximately 30%, with at least 50% of these children dying within the 1st year after treatment. Only 2 of the 8 cases with mandibular lymphoma survived (25%) and 6 of 21 abdominal lymphoma cases survived.

Conclusion: Through this study we were able to determine that monotherapy with CPM is not adequate to cure children with mandibular and/or abdominal BL in Tanzania. Other studies using multi-therapies have shown survival rates between 81%–90%. Unfortunately, poor record-keeping to U.S. standards precluded us from receiving comprehensive clinical data and accurate survival estimates for all children. A prospective study with proper staging, assessment of FNA, marrow and cerebrospinal fluid collection using modern techniques, plus adequate follow-up would help us better evaluate the current therapeutic value of CPM monotherapy versus short, intensive polychemotherapy (shown effective in other clinical studies). Further investigation is needed to evaluate the most effective treatment for Tanzanian children suffering from BL so as to minimize their morbidity and ensure their survival.

B20

Inhibition of Human Renal Cancer Cell Invasion by Sulfonamides

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Renal cell carcinomas are characterized both by a tendency to metastasize before giving rise to local signs or symptoms, and by their resistance to chemotherapy and radiotherapy. The zinc enzyme carbonic anhydrase (CA) catalyses the reversible hydration of carbon dioxide to bicarbonate and a proton. Both cytosolic and cell-surface CA isozymes may function, in association with bicarbonate and proton membrane transporters, to facilitate proton extrusion from cancer cells. This would both promote growth of the cancer cells, and acidify the extracellular milieu to promote cancer cell invasion and metastasis. We investigated the potential of highly specific, sulfonamide inhibitors of carbonic anhydrase as inhibitors of the invasion of human renal cancer cells, with a view to their employment as new chemotherapeutic agents. Seemingly ubiquitous in nature, CA is expressed in several different isoforms. In the normal human kidney, CA II is expressed in the cytoplasm, whilst CA IV and XII are expressed on the extracellular surface. The CA IX isozyme appears to be specific to cancer cells and is strongly expressed on the cell surface of most renal cell carcinomas, where its presence is considered to be diagnostic. We employed two renal cancer cell lines: 786 O cells which strongly express both the extracellular CA IX and XII isozymes, and Caki 1 cells which express neither. The effect of sulfonamide inhibitors on the invasive characteristics of both cell lines was examined *in vitro*, using Bio-

coat Matrigel invasion chambers. Acetazolamide (DIAMOX), at 10 micromolar concentration, inhibited the invasion of Caki 1 cells by up to 60%, but no significant effect on the invasion of 786 O cells was observed. Acetazolamide also inhibited the growth of these cells in culture, but only at much higher concentration. These data suggest that acetazolamide inhibits the invasion of renal cancer cells by inhibition of the cytosolic CA II isozyme. Thus, the development of specific sulfonamide inhibitors, targeted at this isozyme, may be of value in cancer therapy.

Acknowledgment: Support of the Hess Roth Kaminsky Foundation is gratefully acknowledged.

B21

Assessment of the Contribution of Lactate to Fatty Acid Synthesis by Mass Isotopomer Distribution Analysis (MIDA)

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Recent studies have revealed a variety of important roles of lactate in carbohydrate and fat metabolism. One intriguing problem is the relative contribution of glucose and lactate to fatty acid synthesis. We hypothesize that, at physiological concentrations, extracellular lactate competes with glucose as a source of acetyl-CoA, hence as a carbon source for the synthesis of fatty acids. In the present study adipocytes were isolated from the epididymal fat pad of rats, and incubated for 2 h with 5 mM U-¹³C-glucose in the absence and presence of 0.5 mM unlabeled lactate. Thereafter, lipids were extracted, palmitate and stearate were trans-esterified, and analyzed for the incorporation of ¹³C in their carbon chains by gas chromatography/mass spectrometry. The data were evaluated by an extended application of MIDA. Due to the high abundance of ¹³C in the glucose substrate, newly synthesized palmitate and stearate were highly labeled, containing 12–16 and 14–18 heavy carbon (¹³C) atoms in their skeletons, respectively. The labeling (¹³C-enrichment) of the precursor acetyl-CoA pool was derived by MIDA calculations from the enrichment pattern of these new species, without a need of correction for the natural abundances. In fact, it was found that both palmitate and stearate were synthesized from the same highly labeled acetyl-CoA precursor pool, the calculated enrichment of which was 90%. Addition of unlabeled lactate to the incubation decreased this enrichment of acetyl-CoA by 12–16%. Thus, in the presence of labeled glucose, the contribution of extracellular lactate (at low physiological concentration) to the synthesis of fatty acids was also 12–16% under these circumstances. In conclusion, the use of stable isotope-labeled glucose and lactate and the MIDA technique provide a new approach to assess the respective contributions of these substrates to fatty acid synthesis by adipocytes.

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◆ B22

Comparison of the Kinetic Properties of Fola and FolM, Two Dihydrofolate Reductase Isoenzymes in *Escherichia coli*

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Background: Folic acid is essential for cell life due to its role in production of DNA, RNA and other cellular components. Unlike humans, bacteria synthesize folic acid de novo. Trimethoprim and pyrimethamine inhibit dihydrofolate reductase (DHFR) and provide antibacterial effects. Bacteria acquire resistance to drugs through DHFR gene mutations and amplification. Recently, a second gene, folM, was found in *E coli* that encodes a new DHFR.

Hypothesis: Our hypothesis was that DHFR encoded by folM would have a different affinity for its folate substrates than DHFR encoded by folA. Our goal was to investigate and compare the kinetic properties of DHFR isoenzymes encoded by the folA and folM genes using varying levels of substrates: NADPH and dihydrofolate (H2Fol) with 1, 2 or 3 glutamates in the folylpolyglutamate "tail."

Methods: PCR was used to create histidine tagged plasmid-encoded versions of DHFR encoded by folA and folM, respectively. These were transformed into *E coli*, which was used to over-express the proteins. Cells were grown, disrupted, and each DHFR was purified to near-homogeneity in one step through utilization of a histidine-tag selective nickle column. Substrate H2Fol (glutamate chain length 1, 2 or 3) was chemically synthesized and purified. The NADPH dependent reaction was then performed varying either the H2Fol or NADPH concentrations. The change in absorbance at 340 nm was used to measure reaction progression. Results were analyzed using GraphPad Prism 4 to generate Km and Vmax for each enzyme with folate substrates of various chain lengths.

Results: FolM-encoded DHFR has different kinetic properties from folA-encoded DHFR; in general, Fola is a better enzyme catalytically than FolM.

Conclusion: The purpose of a second DHFR enzyme such as FolM in *E coli* is not fully understood. The existence of folM, however, provides a secondary target of amplification or mutagenesis enabling cells to develop resistance to drugs which target DHFR. A better understanding of folM and its role in metabolism of folic acid may lead to better pharmacologic defense against bacteria that have evaded traditional therapeutic means.

Acknowledgment: This research was supported by Midwestern University and by NIH R15 GM71009 to JMG.

◆ B23

Toll-like Receptors and Cytokine Production by Human Dermal Fibroblasts Stimulated With *Candida Albicans* Extract

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Candida albicans skin test antigen (CAST or Candin®) is an extract used for anergy panel skin testing. It has been shown to have efficacy when injected intralesionally for the treatment of recurrent or recalcitrant verrucae.

Hypothesis: CAST engages Toll-like receptors and subsequently stimulates inflammatory cytokine responses in human dermal fibroblasts.

Materials and Methods: Human dermal fibroblasts were acquired from Cambrex, Inc. (Boston, MA) and grown to 80% confluency in 25 cm² flasks. Cells were then treated with either CAST at a dilution 1:10 or 1:100 in cell culture medium; lipopolysaccharide (LPS, a positive control) at 100 ng/ml of cell culture medium; or with fresh cell culture medium only (negative control) at various time points following treatment. Cell culture supernatants were then harvested and subjected to inflammatory antibody capture microarray analysis (Ray-Biotech, Norcross, GA). At the same time, fibroblasts were treated with Trizol® Reagent (Invitrogen) and mRNA was extracted for rtPCR analysis of Toll-like receptor expression.

Results: At 24 hours post-treatment with CAST at a 1:10 dilution, numerous proinflammatory cytokines and chemokines were up-regulated when compared to controls. Of a total of 40 inflammatory cytokines and chemokines assessed, 38 were upregulated, 1 was decreased and 1 had no change detected. Messenger RNA for several Toll-like receptors was also enhanced.

Conclusions: CAST engages multiple Toll-like receptors which results in proinflammatory cytokine and chemokine production. Dissecting the exact Toll-like receptors engaged for particular cytokine and chemokine responses by various antigens found in CAST could lead to new immunotherapeutic and adjuvant applications.

◆ B24

Aortic Reactivity in an Animal Model of Preeclampsia: Modulation by Magnesium Sulfate

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Hypothesis: We hypothesized that MgSO₄ would improve endothelium-dependent relaxation in an animal model of preeclampsia.

Materials and Methods: On gestational day 17, Sprague Dawley rats were anesthetized and osmotic minipumps were implanted to continuously deliver saline, NG-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor (50 mg/kg), L-NAME + MgSO₄ (60 mg/kg) or MgSO₄ alone (4-5 rats per group). Cardiovascular variables (systolic, diastolic, mean and pulse pressures, and heart rate were recorded

on gestational days 17, 18, and 21. On gestational day 21, rats were euthanized and aortic rings (4 mm) were removed from the thoracic aorta and mounted in a Radnoti muscle chamber. Vascular reactivity was assessed in response to phenylephrine and acetylcholine. Aortas were sectioned and stained for histological analysis. Repeated-measures multivariate analysis of variance was used for statistical comparisons.

Results: The multivariate analysis for overall effects of all five cardiovascular variables was significant ($P=0.006$). A day by treatment interaction was found for systolic ($F=9.0$, $P=0.0001$), mean ($F=3.17$, $P=0.0006$), pulse ($F=2.28$, $P=0.037$) pressures and heart rate ($F=9.88$, $P=0.0001$). Concentration-dependent increases in aortic active tension were seen in all 4 groups. The contractile responses to phenylephrine were potentiated in the saline and MgSO₄-only groups compared to the L-NAME and L-NAME + MgSO₄ groups ($P<0.05$). A loss of acetylcholine-induced endothelium-dependent relaxation was seen in the L-NAME and L-NAME + MgSO₄ groups compared to the saline and MgSO₄-only groups ($P<0.05$). There were no differences in aortic lumen areas, diameters or wall thickness between the 4 groups.

Conclusion: L-NAME-treated pregnant rats have decreased vascular responsiveness, which does not appear to be related to vascular wall remodeling. While MgSO₄ reduced blood pressure in this animal model of preeclampsia, relaxation of rat aorta by MgSO₄ was reduced when nitric oxide was blocked. Thus, magnesium's ability to lower blood pressure may be through a mechanism other than induction of nitric oxide.

◆ B25

Cardiorespiratory Effects of Differential Electrical Stimulation of the Rat Anterior Ethmoidal Nerve

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Electrical stimulation of the anterior ethmoidal nerve (AEN) can produce a cardiorespiratory response similar to the diving reflex. Both responses result in an increase in peripheral vascular resistance, bradycardia, and apnea. However, mechanical stimulation of the nose does not always produce this response, but rather can produce a sneeze or other interruption of respiration.

Hypothesis: The current study sought to characterize the cardiorespiratory responses produced after variation of AEN electrical stimulation parameters.

Methods: Three male Sprague-Dawley rats (287–322g) were anesthetized with urethane and instrumented with venous and arterial catheters. The left AEN was surgically isolated, and a stimulating bipolar Pt/Ir hook electrode was secured on the nerve with dental impression material. Heart Rate (HR) was calculated from arterial pulse pressure intervals. A fixed current stimulator was used to electrically stimulate the AEN at 50Hz. Varying current and pulse duration produced different cardiorespiratory responses. Respiratory rate (RR), mean arterial pressure (MAP), and HR changes classified the responses

and five categories were established. Current and duration could be traded off reciprocally to establish a strength-duration curve. Stimulus effectiveness equaled the product of current and duration.

Results: For each animal the lowest current-duration product resulted in only a 15% decrease in RR. The second subsequent stimulation product produced a response that was characterized by a slight (7%) increase in MAP, no change in HR, and a 27% decrease in RR. The third category included a 14% increase in MAP, an 8% decrease in HR with one or two dropped heart beats, and a 72% decrease in RR. The fourth category included a 17% increase in MAP, a 14% decrease in HR with the lowest HR falling to 165 BPM, and sustained apnea. The fifth and highest current-duration product stimulus resulted in an 11% increase in MAP, a sustained 40% decrease in HR, and sustained apnea. All animals consistently showed all five categories that correlated with an increase in current-duration stimulation product.

Conclusions: The results show that differential electrical stimulation of the AEN can reliably produce different cardiorespiratory responses.

◆ B26

Chemokine, CXCL16, Expression in Rat Endotoxin-induced Uveitis

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Hypothesis: Acute anterior uveitis is characterized by invasion of leukocytes into the anterior chamber of the eye. The CXC chemokine, CXCL16, is involved in recruiting leukocytes to sites of inflammation. CXCL16 functions by binding a specific receptor, CXCR6, on the surface of leukocytes resulting in their chemotaxis to the site of inflammation. We hypothesize that CXCL16 expression is upregulated in endotoxin-induced uveitis.

Materials and Methods: Immunohistochemistry was performed to determine the expression pattern of CXCL16 and its receptor CXCR6 throughout the course of uveitis in a rat endotoxin-induced uveitis model. In this model, inflammation, as determined by slit lamp examination, peaks at 24 hours following injection of LPS and continues for several days. We examined 12–13 rats at the time of injection (0 hour), 6 hours, 18 hours, 24 hours, and 72 hours after injection. Immunostained rat eyes were microscopically analyzed and staining was quantified on a 10-point scale with 1 indicating no staining and 10 indicating all cells staining. Additionally, CXCL16

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levels in the peripheral blood of rats were quantified using a specific rat CXCL16 ELISA.

Results: CXCL16 was expressed by cells of the ciliary body and iris. In the ciliary body, CXCL16 expression significantly increased at 6 hours following LPS injection and stayed significantly elevated through the 18-hour time point ($n=12$, $P<0.05$). In the iris, the expression of CXCL16 paralleled its expression in the ciliary body but did not reach statistical significance. CXCR6 was also expressed on cells in the iris and ciliary body. In the ciliary body, CXCR6 showed a biphasic expression, first being significantly increased at 6 hours and 18 hours following LPS injection before returning to baseline at 24 hours and rising again 72 hours after LPS injection ($n=11$, $P<0.05$). In the iris, CXCR6 expression paralleled its expression in the ciliary body but only reached significance at the 72-hour time point ($n=8$, $P<0.05$). In the peripheral blood of rats with endotoxin-induced uveitis elevated levels of CXCL16 (947 ± 69 pg/ml) were detectable at the 72-hour time point ($n=11$, $P<0.05$).

Conclusion: This data suggests a role for CXCL16 and its receptor, CXCR6, in the pathogenesis of acute anterior uveitis.

◆ B27

The Effects of Chronic Versus Binge Ethanol Exposure on Murine Limb Development

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Background: Alcohol is a common human teratogen, but the underlying pathogenesis is still unclear. Maternal consumption of ethanol can cause a variety of birth defects including limb malformations. Acute ethanol exposure in mouse embryos has shown two different phenotypes in the apical ectodermal ridge (AER) of limb buds. First, embryos have shown mislocalization of the AER. Second, embryos show loss of postaxial and sometimes preaxial AER. The AER loss phenotype is the most common limb anomaly seen from acute ethanol injections. This study focuses on the effects of chronic ethanol exposure on the AER. Also examined are the combined effects of chronic ethanol diet with acute ethanol injections on limb development.

Methods: We used a transgenic line of mice that express β -galactosidase in the AER. Everclear® (190 proof) was added to a commercial liquid diet preparation (BioServ) to contain 0%, 2.67%, or 5.3% ethanol. All mice were fed 0% ethanol diet for 2 days before adding ethanol. After exposing female mice to ethanol for 3–7 days, they were mated, and subsequently, some females were also injected with 0.020 mL 25% ethanol/g body weight at 9 days 8 hours post-coital (PC) or 10 days 6 hours, and again 4 hours later. Ethanol-containing diets were continued throughout pregnancy. Embryos were collected at 11–12 days PC and stained with X-gal to visualize AER formation.

Results: Chronic ethanol exposure caused primarily AER mis-

localization and occasional postaxial AER deficiency. AER anomalies were more frequent in forelimbs than hind limbs, but did not favor right versus left limb. Malformation frequency was dose dependent. Combination of chronic 2.67% ethanol diet and acute ethanol injections manifested significant and apparent additive ethanol-induced AER anomalies. However, chronic 5.3% ethanol diet combined with acute ethanol injections did not increase the frequency of AER anomalies relative to chronic diet alone.

Conclusion: Chronic ethanol diet can cause both AER phenotypes seen in acute ethanol injections. However, the mechanism of AER disruption may be different from that of acute ethanol exposure because the predominant limb anomaly seen is AER mislocalization rather than AER loss.

◆ B28

Morphology and Distribution of Neurons Expressing Serotonin 5-HT_{1a} Receptors in Rat Hypothalamus

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Disturbances in serotonergic neurotransmission in the hypothalamus are involved in mood disorders, circadian rhythm, neuroendocrine functions and sleep. Several serotonin (5-hydroxytryptamine, 5-HT) receptors have been identified in the central nervous system using pharmacological and molecular cloning techniques. Among those receptor subtypes currently recognized, 5-HT_{1A} receptors have received considerable attention since their importance in the etiology of mood disorders was observed. While previous studies have shown the presence of 5-HT₁ receptors in several regions of the rat brain, there is no detailed map of the cellular distribution of 5-HT_{1A} receptors in the rat hypothalamus. The present study provides for the first time detailed immunocytochemical mapping of 5-HT_{1A} receptors in the rat hypothalamus. Furthermore, the morphology of the 5-HT_{1A}-immunoreactive (IR) neural elements was also analyzed. Multipolar and fusiform 5-HT_{1A}-IR neurons were widely distributed in the preoptic area, tuberal region and in the posterior hypothalamus. Numerous perikarya were detected in the dorsal zone of the medial and lateral preoptic area, whereas the ventral part contained a significantly smaller number of 5-HT_{1A}-IR cells. The nucleus of the diagonal band of Broca, magnocellular preoptic nucleus and the medial zone of substantia innominata had a large population of 5-HT_{1A}-IR neurons. 5-HT_{1A}-IR perikarya were also found in the bed nucleus of stria terminalis. Densely packed 5-HT_{1A}-IR neurons populated the entire supraoptic and paraventricular nuclei. The anterior hypothalamic nucleus, lateral hypothalamic area, dorsomedial nucleus also contained loosely arranged 5-HT_{1A}-IR perikarya. Only few cells were found in the ventromedial nucleus, posterior hypothalamic and premamillary nuclei. In the present study we provided detailed mapping of 5-HT_{1A}-IR neurons in the rat hypothalamus. Moreover, we also described the morphology of the 5-HT_{1A}-IR neural elements in rat. Since rat is widely used lab-

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oratory animal in pharmacological models of mood disorders, future studies revealing the peptidergic characterization of the cells containing 5-HT_{1A} may have a tremendous impact on clinical pharmacology.

◆ B29

Genistein on Ischemic Tolerance, Blood Pressure and Uterine Weight

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Background: Genistein, a naturally occurring isoflavonic phytoestrogen, has been associated with reduced incidence of cardiac disease and thus may be a possible alternative treatment for postmenopausal women with heart disease.

Hypothesis: Genistein will increase the rate and extent of cardiac recovery following no-flow ischemia, reduce blood pressure and increase uterine weight in treated animals.

Materials and Methods: The left ventricular working heart system was used to examine the effects of genistein on heart function in intact female (IF) (n=9) and ovariectomized (OVX) (n=11) Sprague-Dawley rats. The rats were treated either with genistein (0.25mg/kg) dissolved in DMSO or DMSO alone, daily for two days. Blood pressures were obtained using a tail cuff system both before initial treatment and then two days later prior to sacrifice. Following treatment, hearts were perfused under aerobic conditions then subjected to 25 min of global no-flow ischemia and 30 min of reperfusion. Hearts were perfused with Krebs Henseleit buffer containing 11 mM glucose, 1.2 mM palmitate and 3% albumin. Cardiac function was monitored every five minutes.

Results: There was no difference in recovery between IF given genistein or DMSO alone. However, there was a significant improvement in recovery of HR, dP/dt and CO, following ischemia in the OVX rats given genistein compared to OVX rats given DMSO alone. The following are percent recover data for HR, +dP/dt, and CO respectively:

- OVX + genistein: 79.8 ± 5.1%, 75.0 ± 5.5%, 48.8 ± 7.0%
- OVX + DMSO: 70.5 ± 2.2%, 34.3 ± 7.8%, 21.0 ± 4.1%

OVX rats treated with genistein had significantly increased uterine weights (0.26±0.02g) compared to untreated rats (0.19±0.02g). IF groups showed no changes in uterine weight. Both systolic and mean arterial blood pressures were significantly reduced in genistein-treated IF, but not in the genistein-treated OVX rats.

Conclusions: Our data shows that injections of genistein over two days, significantly increases uterine weight in OVX rats and better facilitates hearts to recover following no-flow ischemia (without changes in blood pressure), suggesting a potential therapeutic role in postmenopausal women.

◆ B30

Altered Fibrinolysis Induced by Macrophage-mediated Nitration of Plasminogen: Another Link Between Inflammation and Thrombosis?

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Background: Despite many years of study, clinical trials of new drugs to prevent thrombosis have often been disappointing. Part of the problem lies on our incomplete understanding of the regulation of fibrinolysis, including plasminogen activation and/or inhibition in vivo. We have previously shown that in vitro nitration of plasminogen in plasma by peroxynitrite resulted in decreased plasmin activity.

Hypothesis: We hypothesized that macrophages may be agents of plasminogen nitration and designed this study to prove this hypothesis.

Methods: We first better characterized our previous observations using purified plasminogen instead of whole plasma, studied the time and concentration dependency of these reactions, and co-incubated plasminogen with macrophages as well as non-inflammatory cells as controls, to assess nitration and impaired activity.

Results: When plasminogen (10 μmol/L) is incubated in the presence of SIN-1 (0.01-02 mmol/L), plasmin activity (generated by streptokinase) is reduced in a time- and concentration-dependent fashion. We performed experiments incubating human plasminogen in the presence of murine RAW264.7 macrophages, allowing for free diffusion of reactive oxygen species, while preventing the action of proteases. In this way we show that incubation of plasminogen with macrophages also decreases plasmin activity, while increasing nitration of the molecule, an effect that is already apparent after 2 h and reaches a plateau of 60% inhibition at 24 h incubation. This effect appears specific for macrophages since 31EG4 murine mammary cells employed in parallel and under the same conditions, failed to produce any deleterious changes in plasminogen.

Conclusions: Our data on quick functional inactivation of plasminogen by nitration, mediated by macrophages, adds a new pathophysiological dimension to our previous work showing plasminogen as a target for peroxynitrite damage. Nitrosative stress may be implicated in impaired fibrinolysis. New therapeutic approaches for nitrosative stress in atherosclerosis and diabetes should limit the formation of superoxides and peroxynitrite.

Acknowledgment: Supported by JDRF 5-2004-236.

Medical Education

M01

Teaching Osteopathic Manipulative Treatment to Allopathic Family Medicine Residents: The Role of the Community Preceptor

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◆ Indicates posters entered in the Student Prize Competition, a judged event that takes place during the Poster Session at the Research Conference.

Background: Military Medicine has a higher proportion of osteopathic physicians, including trainees, than found in civilian practice with up to one third of Family Medicine residents having graduated from osteopathic programs. Exposure to osteopathic residents frequently inspires interest in Osteopathic Manipulative Techniques (OMT) in their allopathic colleagues. Previous studies have demonstrated that allopathic residents can be trained in a defined set of osteopathic principles and skills. Based on both resident desire and demonstrated success at other facilities, the decision was made to establish an elective rotation in OMT. However, an insufficient number of osteopaths on faculty made successful implementation difficult.

Methods: A survey was developed to assess allopathic residents' interest in learning OMT principles and skills. At the completion of the survey, the sole osteopathic faculty member at the Naval Hospital Jacksonville Family Medicine Residency Program established a curriculum for a one month elective in OMT. The curriculum was composed of didactic and procedural instruction by both in-house resources and a proposed community-based osteopathic preceptor. A survey was developed to assess allopathic residents' knowledge and perceived skill in OMT both prior to the rotation and at its completion.

Results: Based on initial survey results, 91% of allopathic residents reported an interest in learning OMT principles and skills. One community-based osteopathic family physician was identified, who frequently uses OMT techniques, and a Memorandum of Understanding was established. Allopathic residents were offered the newly established OMT rotation. Eight of 11 allopathic residents have elected to participate in this rotation as part of their educational experience in Family Medicine.

Conclusion: Previous research has demonstrated that family medicine graduates desire more confidence in managing musculoskeletal issues. Exposure to osteopathic physicians prompts interest in allopathic physicians to learn OMT principles and skills. In an allopathic family medicine residency program, with limited osteopathic faculty support, community-based preceptors can be successfully incorporated into a curriculum for OMT.

M02

Correlation of Values with Specialty Choices of Medical Students

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Hypothesis: Changes in the health care environment have directed increasing attention to the recognized oversupply of

specialists and relative lack of primary care physicians. To promote efforts that will increase the production of primary care physicians, an approach addressing key factors in medical student specialty choice is needed. Utilizing a value rated questionnaire might identify those physician values that correlate to student preference of primary care.

Methods: For this long-term study the investigators used a 38-item validated physician value questionnaire (Hartung and Savickas) which identifies 6 value preferences: prestige, service, autonomy, lifestyle, management, and scholarly pursuit. The questionnaire will be administered over the next 4 years to all first-year medical students who are also asked to declare their specialty choice if known. A follow-up survey will be given to those same students after their fourth year match to analyze for differences in value responses as well as to actual matched residencies.

Summary Results: To date the questionnaire was completed by 69 first year students. Twenty-three (23) students declared a specialty choice on the survey with 46 students undecided. The results indicate first-year medical students who declared a primary care specialty choice possessed physician values highest in service and lowest in prestige and scholarly pursuit.

Conclusions: Results from a value questionnaire could facilitate early identification, either prior to or at matriculation, of students who might ultimately pursue primary care. This could promote development of mentorship and support programs and perhaps aid medical school admissions committees to ensure a higher workforce in primary care in the future.

M03

The Survey of Preferences in Religion and Instructional Teaching (SPIRIT) Study

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Hypothesis: The investigators hypothesize that students at Kansas City University of Medicine and Biosciences College of Osteopathic Medicine (KCUMB) will be in favor of including spiritual topics into their education. We also hypothesize that the majority of students will be affiliated with a major religion, and that their faith will affect medical decision making, thereby influencing patient care.

Materials and Methods: With IRB approval, each student in all 4 classes (approx. 950) was sent an email requesting participation via a link to a 17 item online survey.

Results: 370 students completed the survey, with the majority (85%) identifying with a particular religious faith. 90% stated they were of a Christian denomination, 2% (3) stated they were atheist, and 19% (26) agnostic. Overall, 96% of participating students consider themselves spiritual (defined as believing in any higher power), and 75% pray regularly. Well over half (60%) of participants stated they feel their religious beliefs will impact their medical decision making, and that having these beliefs confers an advantage on physicians treating

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patients of the same religion (72%). Slightly over half (54%) felt that religion/spirituality should play a role in medical policy making. Finally, 238 (64%) believed that medical school classes regarding spirituality should be required. The religion that participants were most interested in learning more about was Islam, followed by Buddhism and Judaism.

Conclusions: Many students perceive themselves as spiritual/religious, and believe their faith will affect their medical decision making. Selection bias may have affected the study, as students who are more spiritual may have been more likely to participate. Of those students who pray, most do so daily. A majority of the participants feel coursework regarding spirituality should be mandated as part of the medical school curriculum. It remains to be seen whether attitudes will change as students graduate. Osteopathic medical schools, based on our data, should consider offering coursework on the major religions the students reported they were most interested in learning about, and to which the vast majority of respondents do not belong.

M04

Survey to Establish the Utilization of Advanced Directives Among Family Practitioners

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Hypothesis: There has been a significant movement in recent literature aimed at molding physicians to engage their patients with end-of-life planning. Despite physician and patient education programs, only a minority of patients complete an advanced directive. It is known that patient-physician discussions about future medical interventions increase patients' satisfaction, yet the dialogue is lacking. Furthermore, physicians are often unaware of advanced directives when patients do execute them. A survey of physician responses will help identify this department's utilization of advanced directives as well as elucidate reasons for omitting these physician-initiated discussions.

Methods: The investigators developed a 13-item questionnaire and distributed it to the 170 departmental full-time, volunteer and resident physician faculty. The questionnaire focused on initiation, documentation, and criteria used for Advanced Directives conversations with patients. Respondents were asked to indicate who should have primary responsibility for initiating such a discussion as well as their own training and familiarity regarding Advanced Directives. The questionnaire also elicited information regarding barriers that might affect the respondent's decision to/or not to initiate such a discussion.

Summary Results: To date the questionnaire was completed by 60 physicians. 47% of respondents indicated that they rarely or never initiate AD conversations. 42% of physicians did not think time was a factor in their decision to initiate these conversations; where as, 65% felt patients were too healthy at the

time for this dialogue. A full 91% used the patient's age and current health status as a criterion for initiating AD conversation. 92% of respondents believed that the primary responsibility for initiating conversation was on the physician. 83% of the respondents who indicated they "often or always" initiated AD conversations, had completed an Advanced Directive themselves.

Conclusions: Results from our survey reveal that these advanced directive conversations are lacking among physicians who do believe it is their primary responsibility to discuss it.

◆ M05

Assessing the Effectiveness of OMT Provided by Undergraduate Teaching Fellows as Measured by a Visual Analog Pain Scale

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Little research has been done among the osteopathic profession to assess the effectiveness of OMT provided by osteopathic medical students. Our null hypothesis is that undergraduate teaching fellows (UTF) in the Osteopathic Manipulative Medicine Department are not able to effectively treat patients using OMT as measured by a visual analog pain scale. To research this hypothesis, we performed a retrospective survey review from patients seen at an on campus osteopathic free clinic, in which faculty, staff and students of Western University are offered three free OMM treatments per semester. During a one hour session a patient receives an abbreviated history and physical related to the chief complaint by a UTF. The case is then presented to an OMM faculty member and a management plan is decided. The UTF treats the patient using OMT. Three days following treatment the patient is emailed a survey to report their pretreatment, immediate post-treatment and 72 hour post-treatment pain level using a visual analog pain scale from 0–10. Data was gathered from the returned surveys from patients seen during the Fall Semester of 2005 and the Spring Semester of 2006. There were 356 patients seen in clinic and 61 (17%) returned anonymous surveys. Using a 2-tailed paired T-test in SPSS, there was a significant difference between pretreatment pain (mean 4.5) and immediate post-treatment pain (mean 2.2; $P=0.00$) and 72 hour post-treatment pain (mean 2.3; $P=0.00$). There was no significant statistical difference between the immediate post-treatment pain scale and the 72 hour post-treatment pain scale, $P=0.73$. From the results obtained, we were able to reject our null hypothesis. After treatment by a UTF, patients had an average reduction of pain of approximately 50% not only immediately following treatment, but also sustained reduction of pain at least 72 hours after treatment. Our data supports the alternative hypothesis that undergraduate teaching fellows are able to effectively treat patients using OMT as measured by a visual analog pain scale.

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◆ M06

Attitudes, Knowledge, and Use of Osteopathic Manipulative Treatment by Osteopathic Sports Medicine Physicians: Is OMT Being Used in the Sports Arena?

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Purpose: While all osteopathic physicians are trained in osteopathic manipulative treatment (OMT), some choose not to use it. Recent surveys have shown a decline in the use of OMT by osteopathic physicians. There is currently no literature assessing whether osteopathic sports medicine physicians (OSMP) use OMT on a regular basis. One might believe that all of these physicians would take advantage of the benefits OMT may have for their patients. If OMT is not being used by OSMPs, then their rationale for not employing such a treatment must be further explored. This project aims to assess the frequency of OSMPs' use of OMT and factors associated with its use.

Methods: This study is a cross-sectional survey. Surveys will be mailed to osteopathic sports medicine physicians using mailing lists provided by the American Osteopathic Association

and the American Osteopathic Academy of Sports Medicine. The survey will be a 33-item self-reported instrument designed to be completed in approximately five minutes. Twenty-three of the items are scored on a Likert scale, with response options including "strongly agree," "agree," "undecided," "disagree," and "strongly disagree." The ten remaining items request OSMP demographic and medical practice information. Survey data will be collected and analyzed using the SPSS statistical software package.

Results: The survey response rate will be computed as a percentage using the number of respondents in the numerator and the number of potential respondents in the denominator. The latter will include those on the mailing lists less the surveys returned as non-deliverable. Basic descriptive statistics will be computed for socio-demographic characteristics, training, practice characteristics, and responses to the attitudes, knowledge and use survey items. Multivariate analysis and factor analysis will also be used to further explore the relationships among survey variables. Statistical significance will be assessed at the .05 level.

Conclusions: The results of this study may potentially provide empirical support for policies that incorporate more OMT training in Continuing Medical Education (CME) or within post-doctoral Osteopathic Sports Medicine Fellowships.

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