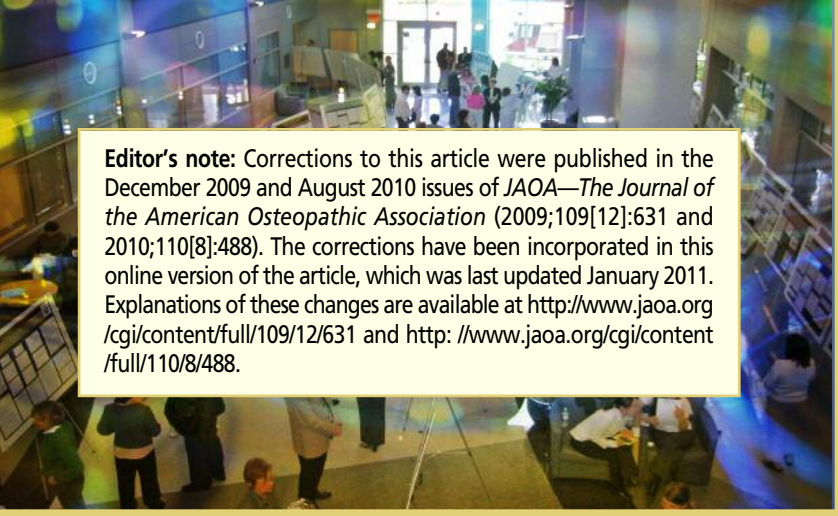

Editor's note: Corrections to this article were published in the December 2009 and August 2010 issues of *JAOA—The Journal of the American Osteopathic Association* (2009;109[12]:631 and 2010;110[8]:488). The corrections have been incorporated in this online version of the article, which was last updated January 2011. Explanations of these changes are available at http://www.jaoa.org/cgi/content/full/109/12/631 and http: //www.jaoa.org/cgi/content/full/110/8/488.

53rd Annual AOA Research Conference—Abstracts, 2009

This issue of JAOA—The Journal of the American Osteopathic Association *features abstracts from the posters that will be presented at the 53rd Annual AOA Research Conference. These posters represent the most recent work of numerous osteopathic medical clinicians, researchers, educators, and students.*

This year's abstracts are organized into five groups:

- ***series P****—osteopathic manipulative medicine and osteopathic principles and practice (see below)*
- ***series C****—clinical studies (see page 429)*
- ***series B****—basic sciences (see page 440)*
- ***series ME****—medical education (see page 458)*
- ***series HP****—health policy (see page 462)*

To enhance the readability of this special feature to the JAOA, *the abstracts have been edited for grammar and basic* JAOA *style. A key to the acronyms used for the colleges of osteopathic medicine appears on page 427. The content of these abstracts has not been modified; neither the AOA Council on Research nor* THE JOURNAL *assume responsibility for the abstracts' content.*

This year's AOA Research Conference, "The Translation of Genomic Science into Osteopathic Clinical Practice and Research," will take place in New Orleans, La, from Sunday, November 1, to Tuesday, November 3, during the AOA's 114th Annual Osteopathic Medical Conference and Exposition (OMED 2009), "The Road to Health Begins With Prevention."

For more information on the AOA Research Conference or other programs taking place during OMED 2009, access the conference's Web site at http://www.omedconference.org. The AOA Research Conference program can be accessed by selecting "Programs & Information" on the drop-down navigation bar and selecting "Non-specialty Affiliates." Information about the conference is also available through DO-Online (http://www.do-online.org).

Osteopathic Manipulative Medicine and Osteopathic Principles and Practice P1

Evaluation of Somatic Dysfunctions in Newborns: An Observational Study

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Hypothesis: Somatic dysfunctions represent important parameters for osteopathic practice. In the framework of a study on the effect of OMT in newborns, we designed an observational study of osteopathic dysfunctions in a subgroup of subjects.

Materials and Methods: After the application of eligibility criteria, 155 newborns were enrolled, including preterms admitted at the NICU after birth, in a period of 6 months. Osteopathic evaluation was performed once on each subject and all osteopathic dysfunctions in terms of grouping into cranial, trunk, column, pelvis, and upper and lower arms were recorded. Descriptive analysis and test of association based on chi-square test were performed.

Results: The highest percentage of single dysfunctions was observed on L5-S1 compression (61 events, 39.35%). After grouping into classes, the median dysfunctions in cranial field was two events (range 0-14) while the highest percentage was observed on SSB compression (36.77%); as far as the column was concerned, the median was one event (range 0-6) while the highest percentage was observed on medium dorsal segment (T4-T5-T6) (18.71%); in the pelvis, the median was one event (range 0-5) while the highest percentage was observed on intraosseum of the sacrum (36.77%); when considering the trunk, the median was zero events (range 0-2) while the highest percentage was observed on diaphragm (16.77%). No significant association between groups and dysfunctions, adjusting for gestational age ($\leq$37 vs $>$37) and weight at birth ($\leq$2500 vs $>$2500).

Conclusion: The present study was the first conducted looking at all the osteopathic dysfunctions affecting newborns. The results obtained reveal that no association was found between gestational age and somatic dysfunctions nor between weight at birth and somatic dysfunctions. This suggests that somatic dysfunctions may be due to extrinsic factor (eg, type and characteristics of labor, fetus position in uterus or mother's situation during pregnancy and labor) rather than weeks of birth or weight. The present study may represent a basis for further research, on a broader population, to build an osteo-

pathic dysfunctions database in newborns and to improve knowledge on the genesis of newborns' dysfunctions.

P2

MRI Assessment of Responses to Manipulative Treatment of Individuals With Low Back Pain

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Introduction: Despite the palpatory findings of altered tissue compliance of skeletal muscle in patients with low back pain (LBP) there is little empiric evidence describing whether asymmetries in paraspinal muscle activation levels exist (side-to-side differences in the degree of muscle activation), and whether osteopathic manipulative treatment (OMT) alters this paraspinal muscle asymmetry (MA).

Hypothesis: We hypothesized that subjects with acute LBP (<3-wk duration) would exhibit greater paraspinal MA compared to control subjects, and that OMT would attenuate this MA.

Methods: Muscle functional magnetic resonance imaging (MRI) was used to determine side-to-side differences in the transverse relaxation time (T2) of the psoas (Ps), quadratus lumborum (QL), erector spinae (ES), and multifidus (Mu) muscles in nine subjects with acute LBP and nine asymptomatic, age-matched controls. The subjects with LBP underwent a single OMT session and MRIs were repeated immediately following and 48 hours after the intervention to evaluate MA in T2. Pain (1-10 visual analog scale) was also compared before and after OMT.

Results: The LBP subjects exhibited greater MA for the QL than control subjects (P=.05; 29.1+4.3% vs 15.9+4.3% T2 MA). A mean difference in MA was also observed between LBP subjects and controls for the Ps muscle (22.7+6.9% vs 9.5+3.0% T2 MA); however, this difference failed to reach significance (P=.11). Mean MA differences observed for the ES and Mu muscles were small and also fell below significant levels (P=.24 and .60, respectively). OMT attenuated the MA in the Ps immediately and 48 hours following the intervention (P=.03; Pre: 22.7+6.9% T2 MA, Immediately Post: 6.0+4.9% T2 MA, 48-hours Post: 9.5+2.2% T2 MA). Small decreases in mean MA also occurred in the ES and QL, but fell short of statistical significance (P=.27). A slight increase was observed in Mu MA following OMT (P=.04, Pre: 1.8+0.4% T2 MA, Immediately Post: 3.0±0.6% T2 MA, 48-hours Post: 4.1+0.8% T2 MA). Pain was reduced immediately and 48 hours following OMT (P<.01; Pre: 3.0+0.9, Immediately Post: 1.5+0.5, 48-hours Post: 1.9+0.6).

Conclusions: T2-weighted MR images revealed significant MA in LBP subjects compared with controls. In LBP subjects, MAs fell in the Ps and possibly in the ES and QL following OMT and rose slightly in the Mu.

Acknowledgments: Ohio University IRB Approval #A 07F035 – Supported by the Osteopathic Heritage Foundation.

♦P3

Structural Asymmetry and Its Relation to Sports Injury

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Hypothesis: There is a statistical correlation between an adolescent athlete's preseason posture and subsequent occurrence of injury.

Methods/Materials: Adolescent athletes (N=256) were recruited; all signed an institutionally approved informed consent form (Midwestern University IRB). At their preseason physical examination, each participant was administered an osteopathic structural examination and an orthopedic evaluation of the knee. Asymmetry measures included levelness of mastoid processes (MP), acromion (ACR), inferior angle of scapula (IASCAP), iliac crest (IC), greater trochanter (TRO), medial malleolus (MM), arch of the foot (ARFT) including flat feet (individually or bilaterally), pelvic side shift (PSS), posterior/superior iliac spine (PSIS), standing flexion test (SFT), seated flexion test (SeatFT), lateral curves present (LC) and location of their convexity (LCCNVEX). Orthopedic tests included: Trendelenburg, Lachman's, posterior drawer, varus and valgus stress, McMurray's tests. Asymmetry was recorded as (higher on the) right, left, or equal; and positive, positive and equal, or negative. The incidence of all injuries incurred by athletes was then recorded.

Results: Examination revealed distinct left-positive findings for MP, ACR, TRO, ARFT, SFT, SeatFT; right-positive findings for IC and LC convexity. Measures IASCAP, MM, PSS, and PSIS were left-right equal. For the orthopedic tests, right-positive findings were 5% greater than left-positive for the Trendelenburg test; the other orthopedic tests were equal. Statistical significance was found for ACR (P<.034), MM (P<.022), and PSIS (P<.090). There were no correlations for the any of the orthopedic tests with injury.

Conclusions: Adolescent injuries occurring during the athletic season correlate with postural asymmetry present at the beginning of the athletic training season. If positive, these screening tests can identify high-risk subjects for sports injury, and allow appropriate measures to be taken before injury occurs. The incidence of injury as it correlates with clinically defined injury type will also continue to be followed, and is an ongoing part of this study.

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

Colleges of Osteopathic Medicine in the United States		
Abbreviation	**Full Name**	**Location**
■ **ATSU-SOMA***	A.T. Still University, School of Osteopathic Medicine	Mesa, Ariz
■ **DMU-COM**	Des Moines University—College of Osteopathic Medicine	Des Moines, Iowa
■ **KCOM**	A.T. Still University-Kirksville College of Osteopathic Medicine	Kirksville, Mo
■ **KCUMB-COM**	Kansas City University of Medicine and Biosciences College of Osteopathic Medicine	Kansas City, Mo
■ **LECOM** □ LECOM-Bradenton† □ LECOM-Seton Hill‡	Lake Erie College of Osteopathic Medicine Lake Erie College of Osteopathic Medicine-Bradenton Lake Erie College of Osteopathic Medicine-Seton Hill	Erie, Pa Bradenton, Fla Greensburg, Pa
■ **LMU-DCOM***	Lincoln Memorial University-DeBusk College of Osteopathic Medicine	Harrogate, Tenn
■ **MSUCOM** □ MSUCOM-DMC‡ □ MSUCOM-MUC‡	Michigan State University College of Osteopathic Medicine Michigan State University College of Osteopathic Medicine Detroit Medical Center Michigan State University College of Osteopathic Medicine Macomb University Center	East Lansing, Mich Detroit, Mich Clinton Township, Mich
■ **MWU/AZCOM**	Midwestern University/Arizona College of Osteopathic Medicine	Glendale, Ariz
■ **MWU/CCOM**	Midwestern University/Chicago College of Osteopathic Medicine	Downers Grove, Ill
■ **NSU-COM**	Nova Southeastern University College of Osteopathic Medicine	Fort Lauderdale, Fla
■ **NYCOM**	New York College of Osteopathic Medicine of New York Institute of Technology	Old Westbury, NY
■ **OSU-COM**	Oklahoma State University College of Osteopathic Medicine	Tulsa, Okla
■ **OU-COM**	Ohio University College of Osteopathic Medicine	Athens, Ohio
■ **PCOM** □ GA-PCOM†	Philadelphia College of Osteopathic Medicine Georgia Campus—Philadelphia College of Osteopathic Medicine	Pennsylvania, Pa Suwanee, Ga
■ **PNWU-COM§**	Pacific Northwest University of Health Sciences, College of Osteopathic Medicine	Yakima, Wash
■ **PCSOM**	Pikeville College School of Osteopathic Medicine	Pikeville, Ky
■ **RVUCOM§**	Rocky Vista University College of Osteopathic Medicine	Parker, Colo
■ **TOUROCOM//**	Touro College of Osteopathic Medicine	New York City, NY
■ **TUCOM-CA** □ TUNCOM†	Touro University College of Osteopathic Medicine—California Touro University Nevada College of Osteopathic Medicine	Vallejo, Calif Henderson, Nev
■ **UMDNJ-SOM**	University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine	Stratford, NJ
■ **UNECOM**	University of New England College of Osteopathic Medicine	Biddeford, Me
■ **UNTHSC/TCOM**	University of North Texas Health Science Center—Texas College of Osteopathic Medicine	Fort Worth, Tex
■ **VCOM**	Edward Via Virginia College of Osteopathic Medicine	Blacksburg, Va
■ **WesternU/COMP**	Western University of Health Sciences College of Osteopathic Medicine of the Pacific	Pomona, Calif
■ **WVSOM**	West Virginia School of Osteopathic Medicine	Lewisburg, WVa

* Provisional accreditation status granted September 2006.
† Branch campus. Parent institution is noted above.
‡ Additional location. Parent institution is noted above. Matriculating students in Fall 2009.
§ Provisional accreditation status granted December 2006.
// Provisional accreditation status granted August 2007.

(continued)

♦P4

Amelioration of Oxidative Stress and Motor Deficits With Osteopathic Manipulative Treatment in a Mouse Model for Human Parkinson Disease

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Hypothesis: Pitx-aphakia (*ak*) mice closely resemble the neuropathology, age-related progression, and behavioral consequences associated with Parkinson disease (PD) in humans. PD-related oxidative stress is linked to augmented urinary levels of the DNA-damage biomarker 8-hydroxydeoxyguanosine (8-OHdG) in humans. We hypothesize that *ak* mice mimic PD-related oxidative stress resulting in elevated urinary 8-OHdG. We further hypothesize that modified osteopathic manipulative treatment (OMT) of *ak* mice will significantly lower urinary 8-OHdG levels through improved fine motor coordination and rigidity.

Materials and Methods: To establish a urinary baseline of 8-OHdG in *ak* versus healthy control animals (strain C57BL/6J), 11am urine samples were analyzed for 8-OHdG and creatinine (glomerular filtration rate) using an enzyme-linked immunosorbent assay (ELISA). Subsequently, *ak* animals were randomly divided into an OMT treatment group receiving modified muscle energy using the active direct technique and a control group receiving a sham procedure to the corresponding body regions. To eliminate individual variability, all procedures were performed by the same, trained individual under professional supervision. Treatments were conducted as 1-minute sessions, each repeated three times. Urinary levels of 8-OHdG and creatinine were assessed before and after OMT/sham procedures. Lastly, all subjects underwent motor coordination and muscle rigidity "hourglass" testing. Urinary 8-OHdG/creatinine, motor skills competence, and rigidity were scored and statistically analyzed (*t* test, $P \leq .05$).

Results: Urinary 8-OHdG was significantly elevated in *ak* mice compared to healthy controls. There was however, no relevant difference in urinary 8-OHdG resulting from OMT treatment. OMT-treated *ak* mice performed significantly better in the rigidity testing than sham-treated controls, whereas no difference in motor coordination competence could be observed between the two groups.

Conclusion: We were able to establish 8-OHdG as a urinary biomarker for PD-related oxidative stress in *ak* mice. The antioxidant effect of treatment modalities and dietary supplements in respect to PD can now be conveniently tested in an animal model of the disease. OMT was effective in the improvement of muscle rigidity symptoms associated with PD. Studies that are more detailed may be necessary to link OMT to reduced systemic oxidative stress and improved motor coordination in PD.

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

P5

Creation of a Database Template for Performing a Retrospective Chart Review of an OMM Hospital Consultation Service

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Objective: Osteopathic manipulative treatment (OMT) has been utilized in clinic and hospital settings since the osteopathic profession was founded. However over the past few decades, the use of OMT in the hospital has declined. In order to understand this trend and to establish a baseline for future hospital-based studies, the current use of OMT in the hospital needs to be documented. The purpose of this project was to create a database collection tool to evaluate the details of a specialty-level osteopathic manipulative medicine (OMM) inpatient consultation service.

Materials and Methods: A database collection tool was created using the Microsoft Office Access database software. The major subject categories included demographic information, diagnoses and symptoms for which OMM consultations were obtained, patient comorbidities, illness severity, and OMT specifics.

Results: The database collection tool was created as one main form with five tabbed subsections. The main form contained patient demographic information and dates of service. The first subsection included admitting physician information, admission diagnoses, and patient illness severity information (eg, ICU status, use of mechanical ventilation).The second subsection included discharge information (eg, discharge diagnoses, outpatient follow-up).The third subsection included the specifics of the OMM consultation (eg, reason for consultation, final assessments, resident physician participation).The fourth subsection contained information regarding the OMT provided each day that the patient was seen by the OMM specialist, including areas of somatic dysfunction identified and osteopathic techniques used (eg, myofascial release, balanced ligamentous tension, high-velocity low-amplitude).The last subsection provided a place for notes regarding information that was not recorded elsewhere in the database. To date, 2195 OMM consultations have been partially recorded in the database and 595 chart reviews are complete.

Conclusion: A useful Access database collection tool was created. Charts reviewed reflect one decade of OMM hospital consultation service from 1998 through 2008.Ultimately the long-term goal of this project is to increase the use of OMT in the hospital setting by establishing the efficacy of OMT in this setting.

Acknowledgment: This research was supported by a grant from the ATSU Strategic Research Fund

P6

Retrospective Chart Review of the OMM Hospital Consultation Service at NRMC - Preliminary Results

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Objective: Originally all osteopathic physicians used osteopathic manipulative medicine (OMM) in the care of hospitalized patients. However over the past few decades, most OMM care has been provided by family practitioners and OMM specialists. The purpose of this study is to retrospectively evaluate the details of a specialty-level OMM inpatient consultation service.

Materials and Methods: 2195 inpatient OMM consultations that took place at Northeast Regional Medical Center (NRMC) in Kirksville, MO between1998 and early 2008 were reviewed for demographic information, the types of problems for which OMM consultations are obtained, the types of osteopathic techniques commonly used, patient comorbidities, and length of service.

Results: Preliminary results are as follows – Demographic information regarding the patients was as follows: 812 men (37%), 1383 women (63%); 579 Caucasian (98%), 4 Hispanic (1%), 4 African-American (1%), and 2 Asian (<1%); age, mean±SD=61±26 years, range=0-99 years. Chart reviews have been completed on 595 of the 2195 consultations, primarily for admissions during 2005-2008. Within the completed chart reviews, the five most common reasons for the OMM consultation were: (1) pneumonia (adjunctive treatment), (2) nonspecific back pain, (3) neck pain, (4) low back pain, and (5) rib pain. Other nonmusculoskeletal reasons for OMM consultations included bowel ileus and poor feeding in the newborn. The five most common diagnoses at admission were: (1) hypertension, (2) pneumonia, (3) COPD, (4) diabetes, and (5) sepsis. The five most common types of OMT techniques used were (1) myofascial release, (2) indirect technique, (3) soft tissue, (4) muscle energy, and (5) rib raising. The average length of stay was mean±SD=6±6 days, while the average length of the consultation was mean±SD=4±3 days.

Conclusion: With a few exceptions, OMM consultations were primarily for musculoskeletal complaints. A wide variety of OMT techniques were utilized. Demographic distribution of the patients is consistent with the local population. When completed, this study will provide preliminary data that can be used as a baseline for developing prospective hospital-based OMM research studies.

Acknowledgment: This research was supported by a grant from the ATSU Strategic Research Fund.

♦P7

Fibromyalgia Treatment Trial With Gabapentin and Osteopathic Manipulative Medicine

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Hypothesis: Intervention with gabapentin and osteopathic manipulative medicine (OMM) can improve the symptoms of fibromyalgia. Combined therapy can decrease the number and severity of tender points (TPs) and the overall pain level with greater efficacy than monotherapy. Subjects in the combined group will have greater improvement quality of life and function.

Materials and Methods: Subjects between the ages of 18 and 65 years with fibromyalgia symptoms were recruited from Solano, Sonoma, and Contra Costa counties in Calif. Subjects who met inclusion criteria were enrolled, randomized to an arm and monitored at eight weekly intervals, receiving treatment during weeks 2-7. Treatment interventions consisted of OMM only, gabapentin only, or combined therapy of OMM and gabapentin.

Results: 29 subjects completed the study. Subjective ability to perform ADLs (as measured by the Fibromyalgia Impact Questionnaire, FIQ) was significantly improved in the combined treatment group relative to monotherapy groups. Subjects receiving combined therapy had a 12% improvement in FIQ score, versus 8% in the gabapentin group and 2% in the OMM group. Subjects in the combined group also rated themselves with the greatest overall health improvement over the course of their 8-week participation in the study. Subjects in the combined treatment group also had the greatest decrease in number of tender points based on the 18-point American College of Rheumatology exam criteria.

Conclusions: A combined therapy of OMM and gabapentin results in improvement of subjective and objectives measures relative to monotherapy in patients with fibromyalgia. Larger and longer trials are needed to optimize OMM treatment protocols and assess long-term outcomes.

Acknowledgment: Supported by an intramural grant from Touro University.

Clinical Studies

♦C1

Trends in Maternal Group B Streptococcus Colonization in Recurrent Pregnancies: A 10-Year Study

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Hypothesis: The incidence of group B streptococcus (GBS) colonization in pregnancy will be increased in patients colo-

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

nized with GBS in a previous pregnancy and decreased in patients without prior GBS colonization.

Materials and Methods: A retrospective cohort of women having at least two pregnancies between 1997 and 2007 was identified via medical record review at a single institution (N=559). GBS culture results were available on 437 women. Data were categorized according to GBS colonization in the initial pregnancy and every pregnancy thereafter as well as the time interval between pregnancies. Analysis was then conducted to determine the trend of GBS colonization in subsequent pregnancies.

Results: The rate of GBS colonization in a pregnancy subsequent to an initial pregnancy with a positive GBS culture was 53.5% (n=101, 95% CI 43.7-63.2%) whereas the rate of colonization following an initial pregnancy with a negative GBS culture was 20.2% (n=336, 95% CI 15.9-24.5%) ($P<.0001$). Sixty-five patients within the cohort had three pregnancies. After two pregnancies with GBS colonization, the rate of colonization increased to 75.0% (n=12, 95% CI 42.8-94.5%); however, after two pregnancies without GBS colonization, the rate of colonization decreased to 18.9% (n=53, 95% CI 8.3-29.4%) ($P<.0003$). The amount of time that elapsed between pregnancies was found to have no significant association with colonization rate.

Conclusion: GBS colonization in subsequent pregnancies is directly related to colonization in previous pregnancies. A previous colonization dramatically increases the rate of GBS colonization in future pregnancies, while a negative GBS culture protects against future colonization regardless of time interval between pregnancies. Therefore, patients presenting in labor with unknown GBS colonization status should be risk stratified based on culture results from previous pregnancies.

C2

Tuberculosis Screening in HIV-Positive Children: A New Frontier

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Hypothesis: The aim of this study is to determine if a T-cell based, interferon gamma assay (T-SPOT.TB), has the ability to detect infection with *Mycobacterium tuberculosis* in children who are HIV positive. There is presently no large scale published data using the T-SPOT.TB to detect tuberculosis infection in children who are HIV positive in developed countries.

Materials and Methods: Children who were HIV positive were recruited from a local children's hospital HIV clinic. Appropriate IRB approval was obtained and informed consent for these children was obtained from their parents or legal guardians. Children that were enrolled had a standard TST placed and blood was drawn via venipuncture and placed in cell preparation tubes. The specimens were then sent to a reference laboratory and the T-SPOT.TB assay was completed per manufacturer's guidelines. Participants' TST were read after 48 hours. The T-SPOT.TB results where available after 24 hours.

Results: Seven children were enrolled in the study. None of the children had a positive TST. One of the children, an adoptee from South Africa, had a positive T-SPOT.TB result. The child had three previous negative TSTs and had never been treated for tuberculosis. The child had a negative chest x-ray following the positive T-SPOT.TB result and was started on isoniazid (INH).

Conclusion: The T-SPOT.TB test was able to detect tuberculosis infection in a foreign-born HIV-positive child that would have been missed if tuberculosis screening had relied only on the TST. In the future, tests based on gamma interferon release assays such as the T-SPOT.TB may play a greater role in the detection of tuberculosis in children with HIV thereby decreasing mortality from tuberculosis especially in HIV-positive children.

Acknowledgment: This project was funded by an intramural grant from the Western University of Health Sciences where Dr Yang is an associate professor of pediatrics. Dr. Yang does not have any significant financial interest/arrangement with Oxford Immunotech, the makers of the T-SPOT.TB test. Special thanks are given to Dr Antonio Arrieta, Stephanie Wronski, and San San Aye for support.

C3

Changes on the Physiological Lactonase Activity of Serum Paraoxonase 1 in Healthy, Overweight, and Obese Women After Weight Loss

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Introduction: Obesity is a metabolic and cardiovascular risk factor, and a low caloric diet (LCD) is one of the treatment modalities for weight loss. Paraoxonase 1 (PON-1) is associated with the antiatherogenic functions of high-density lipoprotein (HDL). Among limited data on the relationships between obesity and PON-1, there has been no study on the effects of LCD as a stand-alone therapeutic measure on the physiological lactonase activity of PON-1.

Hypothesis: We investigated the prospective effects of a sole LCD intervention for weight loss on serum PON-1 activity (lactonase, arylesterase, and tri-esterase) and HDL cholesterol (HDL-C), and their association with low-density lipoprotein cholesterol (LDL-C), in overweight and nonmorbidly obese but otherwise healthy subjects.

Materials/Methods: A total of 30 Japanese women (mean age, 50.3±8.5 y) with a body mass index (BMI, mean 28.5±3.3 kg/m2) participated in this study. During the intervention period of 2 months, they were placed on a LCD (Diet'sTM,

5023 kJ/day) with meal replacement every dinner. The following data between pre- and postintervention were evaluated: BMI, blood pressure, plasma glucose, and lipids including HDL-C and LDL-C. Serum PON-1 lactonase activity with 5-(thiobutyl)butyrolactone (TBBL), its tri-esterase activity was determined using paraoxon and its mono-esterase activity with phenylacetate as substrates.

Results: All subjects completed the LCD intervention. During the intervention, most variables, except for systolic blood pressure, were significantly reduced. PON-1 lactonase levels decreased by 5.8%. This change was paralleled by its arylesterase (7.2%) and tri-esterase (6.9), $P<.001$. In multiple regression analysis adjusted for age, the percent change of PON-1 was significantly, positively, and independently correlated to that of LDL-C ($\beta=0.51$), HDL-C ($\beta=0.40$), and BMI ($\beta=0.37$).

Conclusions: During a LCD intervention on weight loss, PON-1 lactonase levels were reduced. Our results showed reduced PON-1 activity that correlates significantly with the reduction of LDL-C, plausibly reflecting less need for PON-1 activity to prevent LDL oxidation. This suggests that the decrease in PON-1 lactonase activity as well as HDL-C in response to LCD may not be detrimental.

♦C4

Clinical Spectrum of Type 1 Cryoglobulinemia: Retrospective Analysis of 8-Year Cleveland Clinic Experience

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Hypothesis: Cryoglobulins are immunoglobulins that precipitate from serum at temperatures below 37°C and may be symptomatic or associated with widespread vasculitis. Cryoglobulins are classified into types I, II, and III based on immunochemical composition. Type I cryoglobulinemia is the most clinically rare and has been described in few small-series case reports in the past 25 years. This study was intended to describe a large clinical series of patients with type I cryoglobulinemia from the clinical, pathologic, and etiologic perspectives and to determine the influence of recently introduced biologic therapeutics on clinical outcomes.

Materials and Methods: After receiving IRB approval, the laboratory records of the clinical pathology department were reviewed for the 8-year period starting January 1, 2000, through December 31, 2007. All samples with detected monoclonal cryoglobulins (type I) were included in the analysis. Both print and electronic medical records were reviewed and from them standardized information was extracted on etiology, clinical manifestations, immunologic features, and outcomes.

Results: Over this 8-year period, more than 16,000 clinical samples were analyzed for cryoglobulins. Approximately 400 of these samples were strongly positive (>500 ugr/cc) and screened for immunochemical composition. From these records, a total of 19 patients were found to have type I cryoglobulinemia (freq=1.11/1000 samples). Of these patients, 18 had adequate medical records for examination. The most common clinical manifestations were cutaneous involvement and cold-induced symptoms (61% and 31%, respectively). Renal, hepatic, and articular involvement were also observed (22%, 22%, and 17%, respectively). Overall, 83% had hematologic disorders and only 11% were HCV infected, which is consistent with traditional views of the disease. Eight of our patients were treated with the B cell–depleting agent rituximab with varying responses, and a single patient treated with thalidomide experienced complete remission of his cryoglobulinemia.

Conclusions: (1) Type I cryoglobulinemia is an extremely rare form of blood disorder. (2) The most common etiology appears to be within the spectrum of hematologic disorders and in contrast to previous reports is uncommonly associated with HCV infection. (3) Patients diagnosed with type I cryoglobulinemia since the year 2000 have frequently been treated with newer biologic therapeutic agents with varying responses. Thalidomide was used to induce a complete clinical and laboratory remission in one patient and should be further evaluated in clinical trials.

♦C5

The Use of Paravertebral Blockade to Transition to Ambulatory Bilateral Inferior Pedicle Reduction Mammoplasties: A Retrospective Study of 20 Consecutive Patients

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Hypothesis: Bilateral reduction mammoplasty is associated with severe postoperative pain due to significant soft tissue dissection. The purpose of this retrospective study was to determine the analgesic effect of paravertebral blockade on bilateral inferior pedicle reduction mammoplasties performed under general anesthesia that were transitioned from an inpatient to outpatient procedure. Postoperative charges, total length of stay (h), opiod consumption (in morphine equivalents), and pain scores were compared.

Materials and Methods: Following IRB approval, 20 reduction mammoplasty patient charts were reviewed. Ten who had a 24-hour observation admission for postoperative care were compared to patients undergoing the same procedure in an ambulatory setting with the addition of bilateral paravertebral blockade. The inpatient group (IP) received a PCA and oral opiods for pain control after the procedure. In the outpatient

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group (OP), a bilateral paravertebral blockade was utilized preoperatively as well as oral analgesia postoperatively. The postoperative charges, opiod consumption, total length of stay, and pain scale were compared using a Mann-Whitney nonparametric test.

Results: Twenty charts were reviewed using ten consecutive patients in each group with no exclusions due to demographic variation. Statistical differences were seen in total amount of intraoperative opiod equivalents (IP 15.5 mg vs OP 5.5 mg, $P<.001$), total charges for postoperative care (IP $1,269.50 vs OP $701.80, $P<.001$), total charges of stay in the postoperative care unit (PACU) (IP $373.50 vs OP $322.50, $P=.04$), and total length of stay (IP 23.57 h vs OP 2.77 h, $P<.001$). While the mean pain scores between the inpatient and outpatient groups were not significantly different at any comparative postoperative time, opiod consumption at 4 hours postoperation was less for the outpatients (IP 51.25 vs OP 40.82, $P=.17$).

Conclusions: This data shows in patients undergoing bilateral reduction mammoplasties under general anesthesia the use of bilateral paravertebral blockade decreases the intraoperative narcotic requirements. Total postoperative charges and length of stay were decreased in patients who were outpatients rather than inpatients. The correlation of the effect of paravertebral blockade to these resource reductions will require further controlled study.

C6

Circulating Advanced Glycation Peptides Are Higher in Patients With Chronic Liver Disease

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Background: High molecular weight proteins modified by advanced glycation (AGE) produced by unavoidable glycoxidation reactions are metabolized by macrophages, including Kupffer cells, and have been shown earlier by us and others to circulate as partially digested AGE-peptides. Liver is not only a target organ for AGEs, but also an important site for clearance and catabolism of circulating AGEs.

Hypothesis: Circulating AGE-peptides increase in chronic liver disease.

Methods: We performed a nested case-control study with 35 chronic liver disease patients (aged 45–73 y; 20 alcohol-related, 8 terminal cirrhosis, 2 fatty livers, 5 hepatocellular carcinomas) and 40 age-matched controls. Determination of AGEs is based on the spectrofluorimetric detection. Fluorescence intensity is recorded at the emission maximum (440 nm) on excitation at 350 nm and expressed in arbitrary units (AU). We employ a SPECTRAmax Gemini XPS spectrofluorometer with SOFTmax PRO software (Molecular Devices, Sunnyvale, CA, USA). For low molecular weight AGEs, serum was diluted 1/2 with PBS, ultrafiltered through 10 Kda cut-off Amicon filters, and read without further dilution.

Results: AGE-peptides were higher in liver disease patients than in control subjects: 101 ± 70 vs 50 ± 35 AU ($P<.05$). AGE-peptide levels negative and significantly correlate with serum albumin levels: $r=0.59$, $P=.05$.

Conclusions: We show that AGE-peptide levels are 100% higher in chronic liver disease patients and that this increase strongly correlates with liver function as measured by albumin concentration. Our data support an important role of the liver in AGE disposal.

♦C7

Oral Habits in Individuals With and Without Headaches

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Hypothesis: Patients with temporomandibular disorders (TMD) frequently complain of headache, but the degree of overlap between the two conditions is not well understood. This study tested the hypothesis that individuals who complain of common, chronic headaches would also show symptoms of TMD.

Materials and Methods: In phase I of the study, 20 individuals with self-described chronic headaches were compared to 14 individuals with no complaints of headache. All were assessed using the Research Diagnostic Criteria for TMD. A trained, blinded examiner palpated 16 extraoral and four intraoral muscle sites using RDC protocol. Pain and reproducible clicking or crepitus of the temporomandibular joint was determined by palpation. All individuals completed the National Headache Society's Headache Assessment Questionnaire, and a medical and dental history questionnaire. Headache patients also participated in a structured interview. Experience sampling methodology (ESM) was used to obtain repeated assessment of subjects in their natural environment. A custom-made computer program placed calls to pagers for 7 days. The mean time between calls was 120 minutes, with a 40-minute window of variability when could be placed. Subjects were instructed to fill out a preprinted 3×5 card when they were paged. They reported on pain in the jaw, face or head; the presence and intensity of tooth contact; tension in the jaw, face, or head; mood; and stress.

Results: The RDC diagnostic data was analyzed via a Fisher exact test for 2×2 tables. Analysis of ESM data by one-way analyses of variance showed that individuals in the headache group reported significantly more frequent and more intense clenching, more pain elsewhere in the body, more emotional distress, and greater stress.

Conclusion: There is considerable behavioral overlap between common, chronic headache and TMD patients. The overlap may reflect an underlying similarity in etiology, a common response to pain, or variations in help-seeking by patients. The

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results may point to a simple behavioral approach for treating pain in headache patients.

♦C8

How Well Are Healthcare Providers Educating Pregnant Patients on Key Issues?

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Hypothesis: To describe the perspective of women patients and the perspective of healthcare providers on the education of healthcare issues to women from their healthcare providers during their pregnancy, including oral health, exercise, caffeine consumption, drug use, and alcohol use.

Method and Materials: Twelve-point, forced-choice, questionnaire randomly distributed via postal mail to 52 Kansas City University of Medicine and Biosciences Obstetrics and Gynecology alumni nationwide from August 2008 to present. Each alumnus received 25 patient questionnaires and ten staff questionnaires to be completed and returned in a self-addressed envelope. To qualify for the patient questionnaire, women needed to be either pregnant or had given birth within the past year. A reminder letter was sent to alumni 1 month after the questionnaire was distributed.

Results: 236 patient surveys returned from 11 practices and 31 provider surveys returned from ten practices:

- Age: 17.3% - <20 y, 61.3% - 20-29 y, 19.6% - 30-39 y, 1.7% - >39 y
- Race/ethnicity: 1.7% - American Indian/Alaska Native, 2.1% - Asian, 15.6% - black, 7.2% - Hispanic/Latina, 73.4% - white
- Education: 15.7% - <high school, 25.4% - high school graduate, 26.7% - some college, 22% - college graduate, .4% - trade school, 3.8% - graduate school
- Insurance: 40.5% - private, 55.2% - Medicaid, 3.6% - no insurance
- Number of pregnancies: 41.1% - first, 28.8% - second, 16.4% - third, 7.3% - fourth, 3.7% - fifth, 2.8% - >five
- Length of pregnancy: 9.6% - 1st trimester, 24.9% - 2nd trimester, 62.9% 3rd trimester, 2.6% - after pregnancy
- Number of visits to office: 8.4% - first visit, 17.2% - 1 to 4 visits, 24.6% - 5 to10 visits, 49.8% - >10 visits

Conclusion: According to patient survey responses:

- Physicians discussed dental care less often than other health issues.
- Half of patients have been to or plan to visit a dentist during her pregnancy.
- 70% paid more attention to oral health during pregnancy.
- Over half of pregnant women saw a dentist in the last year.
- One-fourth of pregnant women last saw a dentist for a dental problem.

The key differences in the study were variable and element dependent. Additional research in this area is recommended.

C9

The Effect of Acute Fructose Infusion on Hepatic Glucose Metabolism in Humans

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Background and Hypothesis: Fructose is a gluconeogenic (GNG) substrate that has a minimal impact on glycemia. Based on animal studies that have shown that fructose is a preferred substrate for glycogen synthesis, we hypothesized that fructose is diverted to hepatic glycogen in order to prevent surges in glucose production.

Methods: Five lean nondiabetic and six obese hyperinsulinemic men were admitted to San Francisco General Hospital for a 1-week inpatient stay, during which they were fed a high carbohydrate diet. On the last day of the study, subjects underwent stable isotope tracer and acetaminophen infusions to measure hepatic UDP-glucose flux in the fasting state and during a 20% fructose infusion. Plasma and urine samples were collected throughout the tracer study. D-galactose-1d was used to label UDP-glucose, which in turn was sampled by acetaminophen. Acetaminophen is conjugated with UDP-glucose in the liver to form acetaminophen glucuronide (GlcUA), which is excreted in the urine. Labeled urinary GlcUA was isolated by HPLC, and derivatized for GC/MS analysis. The UDP-glucose flux was calculated by the tracer dilution method.

Results: It was previously documented that fructose infusion significantly increased triglycerides and fractional de novo lipogenesis (DNL). When monitoring the changes of UDP-glucose fluxes during fasting and during an 8-hour fructose infusion, we observed a significant increase in hepatic glycogen flux in both lean (1.00 ± 0.18 vs 2.53 ± 0.34 mg/kg*min, $P < .0001$) and obese (0.89 ± 0.15 vs 2.48 ± 0.40 mg/kg*min, $P < .0001$) subjects, which appears to reach storage 4 to 6 hours after the start of the infusion. Overall hepatic glucose production was not significantly increased by the fructose infusion in either group of patients. However, the contribution of GNG to glucose production was significantly increased (lean: 0.63 ± 0.24 vs 1.92 ± 0.26 mg/kg*min, $P < .0001$; obese: 0.44 ± 0.05 vs 1.46 ± 0.16 mg/kg*min, $P < .0001$) with a corresponding decrease in glycogen breakdown (lean: 1.53 ± 0.25 vs 0.41 ± 0.15 mg/kg*min, $P < .0001$; obese: 1.41 ± 0.15 vs 0.42 ± 0.09 mg/kg*min, $P < .0001$).

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Conclusions: Hepatic glycogen appeared to reach storage capacity 4-6 hours post–fructose infusion and was associated with a concomitant increase in DNL. This suggests that GNG substrates are diverted to hepatic glycogen and DNL to prevent surges in glucose production. In the presence of high GNG flux, tight regulation of glucose production has deleterious consequences on lipid metabolism.

♦C10

Genomic Copy Number Variation in Parkinson Disease

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Hypothesis: It is hypothesized that Parkinson disease (PD) may be caused by previously unrecognized genomic copy number variations (CNVs). The aim of this study is to identify and catalog pathologic CNVs in a cohort of PD subjects in order to identify novel PD candidate genes, provide a more accurate means for diagnosis and risk stratification, and allow rational therapeutic development. This will be accomplished by genomewide and PD pathway–focused CNV analysis, using array comparative genomic hybridization (aCGH).

Materials and Methods: With IRB approval, aCGH is performed on 96 anonymous PD subject DNA samples (Coriell Cell Repository) using standard fluorescence-based protocols on all PD subject DNA samples (versus normal control DNA) utilizing a customized aCGH platform (Agilent). Data is generated by Agilent feature extraction software after scanning with a 2 μm-resolution microarray scanner. Significant CNVs are compared to existing data from control experiments and publicly available CNV databases (TCAG) to ascertain population frequencies, followed by candidate gene identification using the UCSC Genome Browser. Significant CNVs are ranked on a 6-point scoring system to prioritize validation and further study. Points are assigned to CNVs in two categories: (1) significance of genes to PD pathogenesis, and (2) CNV frequency within normal population (maximum score of 3, greatest potential significance in each category; highest possible priority score, 6).

Results: A number of significant CNVs have been identified in PD subjects, receiving priority scores of 5 or greater, including CNVs containing the following PD candidate genes: carboxypeptidase E preproprotein, COP9 constitutive photomorphogenic homolog, alcohol dehydrogenase 4 & 6. CNV analysis and validation is ongoing and identification of a number of additional high-priority CNVs is anticipated based on the degree of already-identified genetic heterogeneity.

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

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♦C11

Dysregulation of the Th17 Pathway in Alopecia Areata

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Background: Alopecia Areata (AA) is a complex autoimmune hair loss condition in which genetic and environmental factors contribute to immune dysregulation. Histologically, AA is characterized by a perifollicular T helper (T_H) 1 lymphocyte infiltrate. Recently, a new subset of IL-17 secreting T helper cells, T_H17, has been shown to be important in certain autoimmune conditions. To date, there have been no studies exploring the T_H17 pathway in AA.

Objective: We investigated the extent to which cytokines associated with the T_H17 pathway are differentially expressed in patients with AA and its clinical subtypes in comparison to healthy relatives and unrelated controls.

Materials and Methods: Serum samples were collected from 64 AA patients, 16 healthy relatives, and 16 healthy blood donors with no family history of autoimmune disease. AA patients were divided into disease subtypes based on the National Alopecia Areata Foundation classification: AA transitory, AA persistent, Alopecia totalis, and Alopecia universalis. A comprehensive set of T_H17 related cytokines was evaluated by ELISA: (1) cytokines that promote T_H17 differentiation in naive T cells (IL-1β, IL-6, IL-21, IL-23, and TGFβ1), and (2) cytokines produced by T_H17 cells (IL-6, IL-10, IL-17A, IL-21, IL-22, and TNFα.). In addition, we evaluated the T_H1 hallmark cytokine IFNγ. Differences between individual groups were assessed by Kruskal-Wallis test (significance level $P<.05$).

Results: We found a significant elevation in the T_H17 products, IL-17A and TNFα, in AA patients regardless of disease subtype compared to controls. Of note, healthy family members grouped together with patients in terms of elevated T_H17 products, distinct from unrelated controls. The same trend was seen for the T_H17-promoting cytokine IL-23. There was no significant difference between subgroups of disease or controls for any of the other T_H17 related cytokines. IFNγ also showed an inheritance-specific regulation.

Conclusion: We see evidence for a dysregulation of the T_H17 pathway in AA. The elevation of T_H17 cytokines in healthy controls that are related to AA patients indicates that T_H17 dysregulation in AA may be genetically based. However, the T_H17 pathway does not seem to be involved in regulating disease severity.

C12

Add-On Nebivolol Produces Significant Additional BP Reductions, Regardless of Background Therapy (ACEI, ARB, and/or Diuretic)

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Hypothesis: Most hypertensive patients require combination therapy to achieve BP goals. We hypothesized that the addition of nebivolol, a cardioselective β1-blocker with vasodilatory effects, to other antihypertensives would provide significant additional BP reductions, regardless of the background therapy (ACEI, ARB,± diuretic).

Materials and Methods: Adults with uncontrolled stage I and II hypertension (mean sitting office diastolic BP [SiDBP] 90 mm Hg and 109 mm Hg, respectively) while taking =1 but =2 of an ACEI, ARB, or diuretic were randomized to once-daily nebivolol (5, 10, or 20 mg) or PBO in a 12-week, double-blind, multicenter trial. The study protocol received central IRB approval. The efficacy endpoint used in this analysis was change from baseline in mean sitting systolic BP (SiSBP) and SiDBP at peak (2–3 hr post-dosing).

Results: 669 patients comprised the intent-to-treat population; 486 (72.6%) were taking one agent and 176 patients (26.3%) were taking two (mostly ACEI or ARB+ diuretic). For patients receiving one-agent background therapy, baseline mean BP was 141/90 mm Hg for PBO (n=122) and 137/88 mm Hg for the combined nebivolol dose groups (n=364). Mean changes from baseline were −8.6/−8.5 mm Hg for PBO vs −14.0/−12.9 mm Hg for nebivolol added to ACEI, −13.8/−12.1 mm Hg for nebivolol added to ARB, and −14.2/−13.3 mm Hg for nebivolol added to diuretic ($P<.001$ vs baseline for all). Differences from PBO were statistically significant ($P=.05$) for all nebivolol values, except for SiSBP for nebivolol added to ARB. For patients receiving two-agent background therapy, baseline mean BP was 140/88 mm Hg for PBO (n=45) and 137/88 mm Hg for the combined nebivolol dose groups (n=128). Mean changes from baseline were −7.6/−10.2 mm Hg for PBO vs −12.0/−11.4 mm Hg for nebivolol added to ACEI+ diuretic and −14.8/−13.1 mm Hg for nebivolol added to ARB+ diuretic, ($P<.001$ vs baseline for all). Reductions from baseline with the nebivolol 5 mg dose were consistent with the results observed in the combined-dose groups and statistically significant ($P=.001$) vs baseline.

Conclusion: Nebivolol 5-20 mg once daily as add-on therapy produced a robust BP-lowering effect, regardless of background therapy, confirming the utility of this agent in patients who require combination therapy to achieve BP goals.

C13

Nebivolol Added to Ongoing Antihypertensive Therapy Produces Significant Systolic BP Reductions, Regardless of Baseline Severity

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Hypothesis: Adding nebivolol (NEB), a cardioselective β1-blocker with vasodilatory effects, to ongoing antihypertensive therapy in patients with uncontrolled hypertension produces significant additional reductions in BP (Neutel et al, in press). Since suboptimal BP control is frequently related to continued elevations in systolic BP despite antihypertensive treatment, it is important to confirm that add-on NEB is effective across a range of baseline systolic values. We therefore undertook a post hoc subgroup analysis by baseline sitting systolic BP (SiSBP).

Materials and Methods: Adults with uncontrolled stage I or II hypertension (mean sitting office diastolic BP [SiDBP] 90 mm Hg and 109 mm Hg, respectively) while taking =1 but =2 of an ACEI, ARB, or diuretic were randomized to once-daily NEB (5, 10, or 20 mg) or PBO in a 12-week, double-blind, multicenter trial. The study protocol received central IRB approval. For this analysis, reductions from baseline to week 12 in mean SiSBP at trough (24±3 h post-previous morning's dose) were analyzed for subgroups with a range of baseline SiSBP values: 140-149 mm Hg, 150-159 mm Hg, and 160-169 mm Hg.

Results: 669 patients comprised the intent-to-treat population; 486 (72.6%) were taking one agent and 176 patients (26.3%) were taking two (mostly ACEI or ARB+ diuretic). Of these, 57% had baseline SiSBP between 140-169 mm Hg (103 PBO-treated and 277 NEB-treated patients). SiSBP reductions from baseline by initial SiSBP values were: in the 140-149 mm Hg group, −5.3 mm Hg to −10.6 mm Hg for NEB doses (n=138) vs −2.7 mm Hg for PBO (n=47); in the 150-159 mm Hg group, −10.5 mm Hg to −11.2 mm Hg for NEB doses (n=91) vs −6.5 mm Hg for PBO (n=41); and in the 160-169 mm Hg group, −9.6 mm Hg to −14.3 mm Hg for NEB doses (n=48) vs −3.1 mm Hg for PBO (n=15). All reductions from baseline for NEB 5 mg to 20 mg were statistically significant across starting SiSBP values of 140-169 mm Hg (from $P<.001$ to $P<.03$).

Conclusion: In patients receiving ongoing antihypertensive therapy (25% of whom were on two-drug therapy at baseline and most of whom had only modest elevations in baseline SiSBP [140-159 mm Hg]), the addition of NEB 5-20 mg once daily produced statistically significant and clinically relevant reductions in SiSBP across a spectrum of baseline SiSBP values.

(continued)

C14

BP Response Rates in a Pooled Analysis of Three Nebivolol US Registration Trials

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Hypothesis: Nebivolol (NEB), a β1-selective blocker with vasodilatory properties, has demonstrated BP-lowering effects in a broad population of hypertensive patients, including obese (body mass index [BMI]=30 kg/m2), elderly (age=65 years), black, and diabetic patients. Assessment of efficacy by BP response rates is clinically important; thus, response to nebivolol was evaluated using pooled data from three registration trials, conducted in general hypertensive populations and in black hypertensive patients.

Materials and Methods: Pooled response rate data on NEB are available from three similarly designed, 12-week multicenter, double-blind, PBO-controlled registration trials in patients aged 18 years with stage I or II hypertension (sitting diastolic BP [SiDBP] 95 mm Hg and 109 mm Hg, respectively): two conducted in general hypertension populations and one in self-identified black patients only. Response was defined as an average trough SiDBP <90 mm Hg or decrease of 10 mm Hg from baseline at study end. The study protocols received central IRB approval.

Results: 2016 patients comprised the intent-to-treat population: 205 treated with PBO and 1811 with NEB at doses of 1.25–40 mg. The median age was 51 years (range: 24–80 y) for PBO and 53 years (range: 22–84 y) for NEB. For the PBO and NEB groups, respectively, the majority of patients were nonblack (65.4% and 74.3%) and <65 years of age (85.4% and 80.9%). Roughly 44% had a baseline BMI=30 kg/m2. Response rates for nonblack patients receiving 5 mg, 10 mg, and 20 mg NEB (the doses commonly used in clinical practice) were 61.7%, 64.7%, and 70.1%, respectively, vs 38.1% for PBO ($P<.001$ for each NEB dose). For black patients, response rates were 51.9%, 49.5%, and 47.6% for NEB 5 mg, 10 mg, and 20 mg, respectively, vs 26.8% for PBO ($P<.05$ for each NEB dose). Comparison of black to nonblack patients was not significant, indicating that the proportion of responders did not differ between racial groups.

Conclusion: Pooled data from rigorously designed US studies showed that once-daily NEB monotherapy effectively lowered BP regardless of race, as assessed using conventional response criteria. BP response rates with NEB monotherapy are similar to those observed with other antihypertensives (~60%); though response rates were slightly lower in black patients, they were not significantly different from rates in nonblacks.

♦C15

Osteopathic Physicians and HIV/STI Prevention: Are HIV Testing and Sexual History–Taking Part of Routine Patient Care?

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Objective: The Centers for Disease Control and Prevention (CDC) has revised guidelines for HIV testing in healthcare settings. The guidelines include recommendations for frequency of HIV testing and "opt out" consent. This study was conducted to understand the behaviors and attitudes of osteopathic physicians toward HIV testing and sexual history–taking as well as examine barriers to both.

Methods: With IRB approval, an anonymous cross-sectional survey was conducted at the 2009 Annual FOMA Convention. Survey participants were asked questions regarding sociodemographics, attitudes, and office practices regarding sexually transmitted infection (STI)/HIV testing, and sexual history–taking. A total of 233 attendees completed the survey and 160 qualified for this study based on entry criteria. Univariate statistics were explored via SPSS 16.0.

Results: Although 65% of physicians provide HIV testing in the office, 77% do not recommend testing at patient's initial visit. Fifty-eight percent of the study participants who obtain a general consent do not include permission for HIV testing on the general consent form. When an HIV test is performed, 87% of physicians precede it with a separate consent form, and 89% used an ELISA test. Furthermore, 81% agreed or strongly agreed that it is very important to obtain a separate consent form before an HIV test. Sixty-three percent recommended an annual HIV test for gay men, 85% recommended an HIV test for patients with a new STI, and 59% recommended an annual STI screening for patients with a history of STIs. Nearly 80% had a positive attitude toward sexual history being part of the initial patient visit. Thirty-one percent of physicians updated a patient's sexual history only when pertinent information is provided by the patient. Regarding sexual history–taking, 84% asked about marital status, 76% asked about history of STIs, 60% asked about gender of sex partners, 53% asked about number of sex partners, and 19% asked about sexual satisfaction.

Conclusion: This study suggests that many Florida osteopathic physicians have not yet adopted CDC recommendations regarding routine HIV testing in all healthcare settings. Although most physicians recommend annual testing for patients in high-risk groups (ie, gay men), more concerted efforts are needed to help physicians incorporate HIV testing as part of routine care for all of their patients.

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

C16

Triptan Refilling Behavior of Triptan-Naive Patients

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Hypothesis: Migraine is threefold more prevalent in women and is ranked the 12th leading cause of female disability by the World Health Organization. This analysis evaluated triptan refilling among primarily female triptan-naive migraineurs. We hypothesized that refilling behavior would differ based on patients' clinical characteristics.

Materials and Methods: Continuation-ratio logistic regression analysis of claims data for triptan-naive migraineurs who initiated therapy with sumatriptan as their first/initial triptan therapy (Jan 2004–Dec 2006) from MarketScan (Thomson Healthcare, Ann Arbor, Mich).

Results: Among 29,009 triptan-naive patients (79.1% women) who filled one sumatriptan prescription, 11,036 (38.0%) refilled ≥one additional sumatriptan prescription, 1275 (4.4%) switched triptans (eletriptan, 46.1%; rizatriptan, 38.5%; naratriptan, 7.7%; frovatriptan, 7.7%), and 16,698 (57.6%) did not refill with any triptan after the initial sumatriptan prescription. Patients who did not refill or who switched triptans were younger (≤45 y; 67.6% and 69.2%, respectively) than those staying on sumatriptan (57.5%). Migraineurs who were <38 years were more likely to refill with frovatriptan (odds ratios: 1.03–1.97 vs 1.02–1.06, eletriptan; 0.96–1.09, rizatriptan; 0.45–0.75, naratriptan).

Conclusion: In this predominantly female migraineur population, those who switched to another triptan or did not refill with any triptan were similarly aged and younger than those who continued using sumatriptan. For younger patients (<38 y) who switched, frovatriptan demonstrated the highest refill probability, suggesting good effectiveness and tolerability within this age group. Most migraineurs did not refill any triptan after the initial single sumatriptan prescription, suggesting an opportunity to educate patients about interpatient variability in response to individual triptans. Physicians should consider a different triptan if the first triptan lacks acceptable efficacy or tolerability.

C17

Efficacy and Tolerability of Migraine Therapy With Frovatriptan in a Predominantly Female Primary Care Population

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Hypothesis: Migraineurs with unsatisfactory response to existing therapy might benefit from switching to frovatriptan. This postmarketing study evaluated the effectiveness and tolerability of frovatriptan for migraine in a predominantly female primary care population.

Materials and Methods: 8603 German migraineurs were prescribed frovatriptan 2.5 mg to treat a single migraine attack. Patients recorded headache characteristics, frovatriptan dosage, time to response, recurrence, treatment satisfaction, and adverse reactions (ARs). The study was reviewed and approved by the institutional review board.

Results: Most patients (80.9%, 6963/8603) were women (mean age=42.6 y; 41.5% [2889/6963] were 20–40 y). Previous therapies included analgesics/nonsterioidal anti-inflammatory drugs (46.6%), ergotamines (27.2%), and other (22.2%). 79.5% of patients reported insufficient effectiveness with previous therapy. Time-to-onset of frovatriptan effect was 40 minutes (median); 71.2% of patients required only one tablet (mean, 1.34 tablets). Attack duration was shorter with frovatriptan (<24 h, 84.5%; 24–48 h, 12.6%; 48–72 h, 1.4%) versus previous therapy (<24 h, 43.6%; 24–48 h, 48.3%; 48–72 h, 5.1%); 77.5% reported no migraine recurrence within 24 hours of taking frovatriptan. Most patients rated frovatriptan as better than previous therapy for headache (86.6%), nausea/vomiting (71.3%), and tolerability (70.9%). ARs (n=53) were infrequent (35/8603 patients [0.41%]).

Conclusion: Migraine is reported to be approximately threefold more prevalent in women vs men, with the largest difference occurring during the childbearing years. This postmarketing study showed that most migraineurs receiving treatment in this primary care sample were women between 20 and 60 years old. With frovatriptan, most patients (84.5%) reported attack duration of <24 hours, fast onset of effectiveness, low recurrence, and ≥71% rated frovatriptan as more effective and tolerable than previous therapy.

C18

A Randomized Controlled Trial of Oxymorphone Extended Release in Opioid-Naive Patients With Chronic Low Back Pain Caused by Osteoarthritis

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Hypothesis: To evaluate 12-week efficacy and safety outcomes of oxymorphone extended release (ER) for treatment of chronic low back pain (CLBP) caused by osteoarthritis (OA). Causes of CLBP include degenerative conditions, such as OA or disc disease, osteoporosis, bone diseases, infections, congenital abnormalities, and inflammation of muscles, joints, or discs. Few randomized controlled trials have assessed whether opioid therapy provides durable relief to OA patients with CLBP.

Materials and Methods: Safety and efficacy data from a randomized controlled trial assessing oxymorphone ER were retrospectively summarized for the subpopulation of opioid-naive patients with moderate to severe CLBP resulting from a primary diagnosis of OA. The methodology and results for the overall study population have been described (*Curr Med Res Opin*. 2007;23:117-128). Patients were titrated (for ≤1 mo) to a stabilized oxymorphone ER dose that reduced pain to ≤40 mm on the 100-mm Visual Analog Scale (VAS). Stabilized

patients were randomized to double-blind treatment with placebo or oxymorphone ER for 12 weeks. Informed consent and institutional review board approval were obtained.

Results: 55 (61%) of 90 patients with CLBP from OA completed the titration period (median stabilized dose=40 mg/d) and began double-blind treatment with oxymorphone ER (n=26) or placebo (n=29). Successful titration was associated with a large decrease in the mean VAS from 71.6 mm at screening to 21.1 mm after dose stabilization. After randomization, mean VAS increased significantly with placebo vs oxymorphone ER (+27.4 mm vs +13.8 mm; least squares mean difference between treatments, -22.8; P=.007). During the double-blind period, the most frequent adverse events (No. [%]) were nausea (6 [23.1] oxymorphone ER group, 5 [17.2] placebo group) and diarrhea (3 [11.5] oxymorphone ER group, 4 [13.8] placebo group). Two patients treated with oxymorphone ER and three with placebo discontinued because of adverse events. No discontinuation was due to opioid withdrawal.

Conclusions: Opioid-naive patients with moderate to severe CLBP from OA obtained generally well-tolerated, effective, and durable analgesia following individualized titration with oxymorphone ER.

Acknowledgment: This research was supported by Endo Pharmaceuticals Inc, Chadds Ford, Pa.

♦C19

Static and Dynamic Facial Nerve Reconstruction Following Head and Neck Tumor Resection

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Hypothesis: Facial nerve grafting results in superior outcomes to static facial reconstruction in head and neck tumor patients.

Materials and Methods: IRB approval was obtained. Retrospective chart review of 96 patients who underwent facial nerve reconstruction between March 2000 and January 2009. The majority of patients required facial nerve resection for squamous cell carcinoma (57.5%) of the parotid (46.1%). Patients underwent static (n=66) or facial nerve grafting (n=30) reconstruction. Facial nerve function was measured using the House-Brackmann scale. The modified National Hospital of Norway questionnaire was mailed.

Results: Median follow up was 6.7 months (range, 0-96.1 mo). Patients receiving static reconstruction were, on average, 3.7 years older and had a worse prognosis (survival at 2 y, 80.3% vs 90%). A significant proportion (91.3%) had some recovery of facial nerve movement after facial nerve grafting (median House-Brackmann score=3) after a median 14 months. Patients having static reconstruction were more likely to require revision eye surgeries for exposure keratitis (mean number of procedures=9 vs 2).

Conclusion: Although facial nerve grafting requires a significant time for recovery of function, many patients requiring facial nerve reconstruction for head and neck tumors will survive past 2 years. In head and neck tumor patients, facial nerve grafting often results in return of movement and reduces the number of procedures required for eye protection.

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

C20

Quality of Life Measures in Patients With Chronic Noncancer Pain: Baseline Data From the Opioid Utilization Study (OPUS)

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Hypothesis: To report baseline quality of life (QoL) measures of patients enrolled in the Opioid Utilization Study (OPUS). OPUS will characterize opioid usage patterns in patients with chronic noncancer pain (CNCP), analyzing the economic impact of adding, titrating, and/or rotating opioid analgesics. Secondary objectives include analyzing several QoL measures.

Materials and Methods: OPUS is a 1-year, multicenter, prospective observational study of adult (≥18 years) patients with CNCP receiving opioid therapy. Exclusion criteria are cancer pain, risk or history of drug abuse, or an open workers' compensation claim. QoL assessments include the Brief Pain Inventory (BPI); the Short Form-12 (SF-12) General Health Survey; and the Depression, Anxiety and Positive Outlook Scale (DAPOS).

Results: 1668 out of a cohort of 2003 patients with CNCP completed baseline assessments. The majority were women (992/1668; 59.5%) and white (1444/1631; 88.5%). Most had an annual household income ≤$60,000 (1163/1619; 71.8%), duration of pain >1 year (1313/1409; 93.2%), and had used opioids for >1 year (1095/1406; 77.9%). Mean (SD) BPI scores (0, none; 10, worst/complete) indicate moderate average pain (5.7 [1.9]), interference with daily activities (6.3 [2.5]), and substantial pain relief from opioids (6.2 [2.2]. Mean (SD) SF-12 scores for physical functioning (38.0 [6.4]) and mental functioning (43.6 [12.0]) were lower than estimates for the general population (50 [10] for both measures; P<.001). DAPOS scores (1, almost never; 5, almost all the time) indicate that patients had mild to moderate depression (2.0 [1.0]) and anxiety (2.0 [1.1]) and a moderately positive outlook (3.4 [1.0]). In subgroup analyses, BPI scores did not differ according to gender, ethnicity, income, or duration of pain or opioid use. SF-12 mental functioning scores were lower in women (P<.001) and higher in patients earning >$60,000 ($P$≤.04). DAPOS

scores showed more depression and anxiety in women ($P<.001$ for each) and patients earning ≤$20,000 ($P=.03$, $P<.001$, respectively). Nonwhite patients reported more anxiety ($P=.003$). Patients with duration of pain >1 year ($P=.02$) and income ≤$20,000 ($P=.05$) had a less positive outlook.

Conclusions: Patients enrolled in OPUS will repeat the BPI, SF-12, and DAPOS at 6 and 12 months to assess long-term effects of opioid therapy on QoL in patients with CNCP.

Acknowledgment: This research was supported by Endo Pharmaceuticals Inc, Chadds Ford, Pa.

C21

Efficacy of Oxymorphone Extended Release in Oxycodone-Experienced Patients With Chronic Low Back Pain

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Hypothesis: To assess the safety and efficacy of oxymorphone extended release (ER) for chronic low back pain (CLBP) in opioid-experienced patients previously treated with oxycodone. Opioid therapy for CLBP is becoming more common. Patients unable to tolerate or obtain adequate relief with one opioid often can be switched to another opioid that provides well-tolerated and effective analgesia. However, many patients require trials of several opioids before finding one that is satisfactory.

Materials and Methods: Safety and efficacy data from a randomized controlled trial assessing oxymorphone ER in opioid-experienced patients with moderate to severe CLBP were retrospectively summarized for the subpopulation of patients who were receiving oxycodone at screening. Patients discontinued oxycodone and were titrated (for ≤1 month) to a stabilized oxymorphone ER dose that reduced pain to less than or equal to ≤40 mm on a 100-mm Visual Analog Scale [VAS]) with ≤2 doses/day of rescue medication. Patients were then randomized to double-blind placebo or oxymorphone ER for 12 weeks. The methodology and results for the overall study population have been describe elsewhere (*J Pain*. 2007;8:175-184). Informed consent and institutional review board approval were obtained.

Results: 79 of 250 enrolled patients were receiving oxycodone analgesia at screening. Of these, 46% (n=36) were successfully titrated to a stabilized oxymorphone ER dose (median, 110 mg/d). Median pain on the VAS decreased from 74 mm at screening to 29 mm following titration. After stabilization, all patients began double-blind oxymorphone ER (n=18) or placebo (n=18) treatment. During double-blind treatment, mean VAS increased 33.0 mm with placebo vs 5.2 mm with oxymorphone ER (least squares mean difference, -27.0; $P=.009$). Fewer oxymorphone ER patients than placebo patients discontinued owing to lack of efficacy (n=2, 11% vs n=12, 67%, respectively). Two oxymorphone ER patients and three placebo patients discontinued owing to adverse events.

Conclusions: Approximately half of oxycodone-experienced patients were successfully converted and titrated to a twice-daily dose of oxymorphone ER that provided effective, durable, and generally well-tolerated analgesia for moderate to severe CLBP.

Acknowledgment: This research was supported by Endo Pharmaceuticals Inc, Chadds Ford, Pa.

C22

An Open-Label Long-Term Safety Trial of Diclofenac Sodium 1% Gel in Patients With Osteoarthritis of the Knee

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Hypothesis: To assess long-term outcomes with topical diclofenac sodium 1% gel (DSG) in patients with knee osteoarthritis (OA) treated for up to 1 year. Nonsteroidal anti-inflammatory drugs (NSAIDs) can relieve symptoms of OA but may cause dose-related adverse events (AEs), including gastrointestinal (GI) bleeding and ulcers. Topical NSAIDs limit systemic drug concentrations and have shown good efficacy and tolerability in trials lasting up to 12 weeks.

Materials and Methods: This open-label trial included 583 adults aged ≥35 years with a ≥6-month history of symptomatic mild to moderate knee OA. Half of the patients had completed previous 12-week trials of DSG (n=291) and half were DSG-naive (n=292). Patients applied 4 g of DSG 4 times daily to one (n=355) or both knees (n=228). Rescue acetaminophen (≤4 g/d) was allowed. Efficacy outcomes were the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain, stiffness, and physical function subscales measured at 3-, 6-, 9-, and 12-months post-baseline. Efficacy results from the first ≥250 patients followed up for 1 year and safety results in all patients receiving ≥1 DSG dose are reported here.

Results: At each assessment, WOMAC pain, stiffness, and physical function scores were substantially lower, with improvements from baseline of 39.8%, 33.4%, and 36.9%, respectively, after 1 year (n=268). Patients treating one knee (n=146) had greater improvement than patients treating two knees (n=122) in WOMAC pain (45.4% vs 32.2%, respectively), WOMAC stiffness (37.9% vs 27.8%), and WOMAC physical function (41.6% vs 30.8%). Patients treating one knee and those treating two knees were equally likely to report any AE (75% each), but fewer patients treating one knee reported treatment-related AEs (16.9% vs 22.8%) or application-site dermatitis (12.4% vs 14.9%). One patient discontinued owing to a GI AE (pancreatitis, not treatment related). No patient experienced a serious AE related to treatment. Increased alanine aminotransferase was reported in 1.4% of the total population (one knee, 0.7%; two knees, 2.2%).

(continued)

Abnormal laboratory values were suspected to be treatment related in three patients (0.5%). Physical examinations were unremarkable.

Conclusions: DSG was well tolerated and substantially improved OA symptoms vs baseline over 12 months in patients treating one or both knees.

Acknowledgment: This research was supported by Novartis Consumer Health, Parsippany, NJ, and Endo Pharmaceuticals Inc, Chadds Ford, Pa.

♦ C23

The Prevalence of Dermatological Conditions Seen During a Medical Mission to Guatemala

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Hypothesis: We conducted a medical mission in February 2009, in conjunction with DOCARE International, in Guatemala. Prior missions resulted in clinical impressions that the most common dermatological diagnoses were verrucae, scabies, psoriasis, and photdermatoses. Based on past experience, we also felt it likely that most patients would be women and children. Our goal in this year's project was to continue to provide screening and educational services and to assess the accuracy of prior clinical impressions using a structured, data collection form.

Materials and Methods: With IRB approval, patients seen during the 2-week mission had their nonidentifiable data gathered in a computer-readable sheet that was used as the encounter form. All individuals screened during this visit lived in rural areas several hours distant from the capital, Guatemala City.

Results: Proportions of valid, nonmissing data included 2129 patients, 69% female, and 31% male. Age ranges most represented were <1 year of age (3% of total patients), 1-4 years (10%), 5-11 years (13%), 18-39 years (31%), 40-64 years (25%), and 65+ years (12%).The dermatologic diagnoses most often made from all presenting patients were dermatitis (4.4%), eczema (3.4%), fungal infections (1.9%), and pediculosis (1.4%). The most commonly dispensed agents were topical corticosteroids (7.2% of visits), topical moisturizing lotions (5.4%), sunscreen (3.6%), topical antifungals (2.5%), and permethrin (1.5%).

Conclusions: The most commonly seen dermatologic diagnoses were dermatitis, eczema, fungal infections, and pediculosis. The majority of patients were women and adults 18-39 years of age. We don't have data on why the proportion of men was so low. We will utilize this data to assist in preparing the formulary for future mission, and better educate participants prior to the mission. Some data may be skewed as medication supplies affect dispensing habits, and we often deplete the entire stock of medication. This year, we depleted stores of topical steroids, permethrin, and sunscreen, so we will increase quantities of those products next year. The international rotation served to improve health of Guatemalans, and served as an eye-opening experience for medical students, faculty, and staff, with extremely positive subjective feedback from participants and patients. The data will be used to better understand the population served and provide an empirical basis for planning for future missions.

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

Basic Sciences

B1

Chlorogenic Acid Inhibits Alpha-Dicarbonyl Glycation and Peroxidation of Human Low Density Lipoprotein

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Introduction: Oxidation of the lipid component of LDL leads to scavenger receptor uptake and inflammation, key initiators/perpetuators of atherogenesis. It is well known that glycation reactions initiate oxidative modifications that start with the nonenzymatic addition of sugars or carbonyl radicals to the primary amino groups of proteins. Ilex paraguariensis extracts have shown in vitro antiglycation activity in our previous studies using other model systems. Methylglyoxal is a potent dicarbonyl that mediates many of the glycation reactions, and is formed at increased concentrations as a side product of the glycolytic and other pathways when the flux is increased at the triose level (diabetes, fatty acid overflow in the metabolic syndrome).

Hypothesis: Chlorogenic acid, 5-CAQ (5-caffeoylquinic acid), one of the main phenolic compounds in yerba-maté beverages protects LDL from glycation by alpha-dicarbonyls and prevents its oxidation.

Materials/Methods: Human LDL (d=1.063 g/ml) prepared by sequential flotation ultracentrifugation was incubated in the presence of oxygen or nitrogen with methylglyoxal (2.0 mmol/L) in the presence and absence of 5-CQA at different concentrations (0.5 to 2.0 mmol/L) and also with aminoguanidine (2.0 mmol/L). The LDL fraction was incubated for 48 h at 37°C. Advanced glycation endproducts (AGEs) were measured by fluorescence (λexc 340; λemis 440) and the peroxidation was evaluated by measuring the peroxides formed after this period by the iodine method of El-Saadani.

Results: Chlorogenic acid markedly inhibited AGEs generation (up to 80%) more efficiently than aminoguanidine (70% inhibition at the same concentration) and was able to inhibit peroxide generation by 80%, at the lower concentration, while aminoguanidine was not effective. Incubation either in the presence of oxygen or nitrogen lead to the same results.

Conclusions: 5-CQA exhibits a very potent anti-AGE and

antiperoxidation activity vis-à-vis LDL, while aminoguanidine protects against glycation but not against peroxidation. This data confirm our previous report using the crude maté extract, at the same time that suggest 5-CQA is a strong candidate to explain its antiglycation effect. Further studies on the other constituents are warranted and are in course.

B2

In Vitro Antiglycation Effects of Chlorogenic Acid: Preliminary Results

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Introduction: Glycation is one key molecular basis of diabetic complications due to hyperglycemia. Therapeutic approaches against glycation, including the development of new drugs or the use of herbal extracts have been a focus of several research publications.

Hypothesis: Since phenolic acids, saponins, and even xantines show antioxidant activity, our goal is to identify which of these classes of substances would contribute to the antiglycation activity previously observed for yerba maté aqueous extracts—but not for green tea (*Camelia sinensis*), which has strong antioxidant activity and high polyphenols content.

Materials/Methods: Bovine serum albumin and histones were incubated with methylglyoxal (MG) (5.0 and 1.0 mmol/L respectively) in the presence and absence of 5-caffeoylquinic acid (5-CQA) at different concentrations (0.5 to 10.0 mmol/L) and also with aminoguanidine (1.0 mmol/L). Proteins were incubated up to 7 days at 37°C. Advanced glycation endproducts (AGEs) were measured by fluorescence (λexc 340; λemis 440). We evaluated the protein cross-linking using sodium dodecyl-sulfate polyacrilamide gel (SDS) electrophoresis.

Results: In both protein models, chlorogenic acid displayed a concentration-dependent inhibition of AGEs generation (up to 60-89%) values much higher than those observed for aminoguanidine (35% inhibition at the same concentration). Histone cross-linking by MG was also inhibited by the addition of 5-CQA in a concentration-dependent manner. High molecular weight polymers (over 500 KDa) were almost absent at 10 mmol/L 5-CQA as compared to the control. MG also produced a crosslink band at 90 KDa in BSA incubations, which was blocked by 5-CQA.

Conclusions: 5-CQA, one of the major components of *Ilex paraguariensis* extracts, exhibits a very potent anti-AGE formation action in two protein models, a concentration-dependent effect that is even stronger than that produced by equimolar concentrations of aminoguanidine, the prototype AGE inhibitor. This data confirm our previous report using the crude extract—at the same time suggesting that 5-CQA is a strong candidate to explain the antiglycation effect of the popular beverage. Further studies on the other constituents are warranted and are in course.

♦B3

Structural Changes of the Muscularis Propia in the Distal Versus the Proximal Diverticula

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Background: Diverticulosis of the colon is a common problem in the older population, usually more prominent in the sigmoid colon in the United States. Its development is correlated with age and declining colonic wall mechanical strength. Studies have shown that increased collagen (fibrosis) and decreased neuronal numbers in the elderly colon may result in a slower colon transit time, allowing for an increased tendency for mucosal herniations. Other contributing factors include increased intraluminal pressure in older people secondary to changes in peristaltic contractions. Our research involves two aspects of diverticulosis and their relationship to each other; the amount of collagen (fibrosis) present in the diverticula and the significance of its distribution.

Hypothesis: Our working hypothesis is based on the knowledge of diverticular distribution in the West, and the varying known contributions to the development of diverticulosis. Therefore, we hypothesize that the colonic wall in diverticula will show more collagen (increased fibrosis) distally rather than proximally or in a random distribution when compared within each cadaver.

Materials and Methods: Colonic specimens were obtained from cadavers at NYCOM. Several diverticula from each of three to four defined regions along the colon—distally to proximally—were sampled. After slides were cut and mounted, we examined the tissue with H&E and Trichrome stains. The amount of collagen was quantified with digital analysis. Finally, we compared the amount of fibrosis present at each diverticular site in each cadaver.

Results: Preliminary results indicate a trend of increased muscularis propria fibrosis distally. For example, we saw proximally a ratio of 89%:11% muscle to fibrosis, compared to distally 26%:73% muscle to fibrosis.

Conclusion: Collagen in the colon is mostly located in the submucosal layer, thus making this the most important layer for structural integrity and mechanical strength. Concurrently, smooth muscle in the muscularis propria allows for peristalsis. The increased intraluminal pressure and occurrence of nonperistaltic contractions in an already-compromised colonic wall, due to a decreased calcium release and reuptake, induce reactive fibrosis. Our findings support the theory that structural changes in the muscularis propria, particularly changes in collagen amount and distribution contribute to the development of diverticulosis. Statistical analysis will be performed.

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

B4

3-Dimensional Modeling of Two Injury Mechanisms Resulting From Whiplash-Type Accidents

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Hypothesis: Atrophic changes in rectus capitis posterior minor (RCPMi) muscles have been reported in some patients who have headaches resulting from whiplash-type injuries (*Spine*. 2006;31:E847-E855) that are not seen in either female control subjects (*Clinical Rad*. 2005;60:355-363) or patients with chronic, insidious-onset pain (*Clinical Rad*. 2008;52:273-277). We hypothesize that the biomechanical response of the upper cervical spine to whiplash-type distortions puts RCPMi muscles at risk for two types of injury, both of which may result in muscle atrophy.

Methods and Materials: We used 3D Studio Max (Autodesk Inc) and commercial, three-dimensional datasets to generate accurate and realistic renderings for the skull, the atlas, and the axis. Published morphometric data (*Spine*. 2008;33:1503-1508) were used to position these three structures in a neutral position. Published kinematic data (*Spine*. 1999;24:240-247) were then used to model the biomechanical response to retraction of the head in the sagittal plane (the "chin-tuck" position). Rear-impact loading of the cervical spine (*Spine*. 2001;26:1252-1258) forces the head and neck into an S-shaped curvature (*Spine*. 1997;21:2489-2494) during the retraction phase of a whiplash-type accident. Our biomechanical model extends the physiologic chin-tuck position to the nonphysiologic position that occurs during some whiplash-type accidents.

Results: Our model shows that the RCPMi muscles are at risk for a tearing injury at the musculoskeletal junction due to high levels of strain caused by eccentric lengthening of these muscles during the retraction phase of a rear-end automobile accident (*Spine*. 2007;32:756-765). There is also the potential for entrapment of, and injury to, the C1 dorsal ramus by rectus capitis posterior major (RCPMa) muscles (*Spine*. 1982;7:319-330).

Conclusion: We have demonstrated that there are two injury mechanisms that may result in atrophic changes in the RCPMi muscles as a result of whiplash-type distortions. It is proposed that atrophy of these muscles may result in postural compensation that will place abnormal stresses on cervical structures (*Arch Phys Med Rehabil*. 2000;81:62-66), and may also result in abnormal levels of tension being placed on the spinal dura via the tissue bridge that interconnects the two structures (*Spine*. 1995;20:2484-2486). Both have the potential to result in the type of head and neck pain that is seen in some groups of patients with whiplash-associated disorders.

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

♦ B5

Effect of Glyphosate on *Escherichia Coli:* Is Development of Resistance to Glyphosate Accompanied by Resistance to Antibiotics?

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Background and Significance: Glyphosate is the most broadly used herbicide in the world and is the active ingredient in Roundup, an extremely popular herbicide manufactured by Monsanto. Glyphosate kills plants by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase, encoded by the gene *aroA*. EPSP synthase is very important in aromatic amino acid biosynthesis, a pathway present in both plants and bacteria. One bacterium of significance is *Escherichia coli* (*E coli*), whose lifecycle includes the mammalian gastrointestinal tract as well as the environment. Since the major source of pathogenic *E coli* is contamination of meat, it is reasonable to conclude that *E coli* has exposure to glyphosate in the environment where animals graze.

Hypothesis: Bacteria that develop resistance to the ubiquitous herbicide glyphosate also develop resistance to antibiotics.

Materials and Methods: *E coli* strains MG1655 and BW25113 lacking resistance to glyphosate were grown in minimal medium with increasing concentrations of glyphosate. When a high level of resistance was achieved, the resistant strain and its isogenic parent were tested for resistance by microdilution growth experiments in minimal medium containing varying concentrations of different antibiotics. We measured both the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Determinations were performed at least twice, using triplicate samples.

Results: Strains with demonstrated resistance to glyphosate showed nonsignificant variations in resistance as compared to the parent strain when MIC values were compared. In contrast, strains with resistance to glyphosate showed significantly increased MBC values to certain antibiotics.

Conclusion: Exposure to environmental glyphosate, and development of resistance to this compound, can cause development of resistance to antibiotics. This may lead to treatment failure and recrudescence of human bacterial infections.

♦ B6

Interaction of Bupropion With Muscle Nicotinic Acetylcholine Receptors

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Hypothesis: Bupropion interacts with different potencies and affinities with the AChR ion channel in distinct conformational states.
Materials and Methods: To characterize the binding sites and the mechanisms of inhibition of bupropion on muscle-type nicotinic acetylcholine receptors (AChRs), structural and functional approaches were used including radioligand competition binding assays, Ca^{2+} influx and macroscopic current recordings, thermodynamic and kinetic measurements, and molecular docking and dynamics studies.
Results: The results established that bupropion: (1) inhibits epibatidine-induced Ca^{2+} influx in embryonic muscle AChRs, (2) inhibits adult muscle AChR macroscopic currents in the resting/activatable state with ~100-fold higher potency compared to that in the open state, (3) increases desensitization rate of adult muscle AChRs from the open state and impairs channel opening from the resting state, (4) inhibits [^{3}H]TCP and [^{3}H]imipramine binding to the desensitized *Torpedo* AChR with higher affinity compared to the resting AChR, (5) binds to the *Torpedo* AChR in either state by an entropy-driven process, and (6) interacts with a binding domain located between the serine (position 6′) and valine (position 13′) rings, by a network of van der Waals and polar interactions.
Conclusion: Collectively our data indicate that bupropion first binds to the resting AChR, decreasing the probability of ion channel opening. The remnant fraction of open ion channels is subsequently decreased by accelerating the desensitization process. Bupropion interacts with a luminal binding domain shared with phencyclidine that is located between the serine and valine rings, mainly byan entropy-driven process.

♦B7

Can a Novel Ultrasound Method Evaluate Vascular Function in Antihypertensive Therapy Independent of Pressure Reduction?

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Background: Hypertension represents not only an increased stress on the heart and vessels but also modulation of the pulse waveform and transmission through the vasculature. The shape of the pulse waveform is dependant on cardiac dynamics and vascular properties including compliance and resistance. Here, we examine the morphology of the pulse wave via Fourier analysis of aortic blood flow in spontaneously hypertensive rats (SHR) during baseline and therapy. We hypothesize that Fourier analysis can be used to demonstrate that the reduction of blood pressure in hypertension is associated with an altered pulse waveform unique from the normotensive state.
Methods: The SHR is treated with either Captopril (CAP) (n=6) or sodium nitroprusside (SNP) (n=6) and compared to the control group (n=12), the normotensive Wister-Kyoto rat (WKY). Under approval of the IACUC, animals are instrumented with electrocardiogram, intra-arterial catheter and ultrasonic flow through the abdominal aorta. Mathematical analysis extrapolates pertinent pressure and flow biomarkers as well as the aortic blood-flow spectrum.
Results: SHR and WKY groups are differentiated by systolic, mean and diastolic pressures, heart rates, and flow volumes ($P<.001$). However, parameters such as cardiac output, maximum flow velocity, and acceleration of flow were not found to be different. Spectral differences between the groups are identified at low range harmonics ($P<.04$). During treatment with SNP, SHR pressure equilibrates with the WKY group while heart rate and flow volume remain different ($P<.02$). The pulse spectrum reveals SNP increases the lowest harmonic while decreasing upper range harmonics ($P<.03$), indicating a shift to low frequency flow. CAP treatment also equilibrates to WKY hemodynamics, except heart rate. In addition, CAP increases aortic compliance ($P<.02$) and produces a shift to low frequency flow by reducing upper range harmonic magnitudes ($P<.04$).
Conclusions: The analysis demonstrates that acute reduction of blood pressure in the hypertensive vasculature leads to an altered flow waveform that deviates from both the hypertensive and normotensive vascular states. This suggests that vascular function in hypertension is an important and identifiable target of therapy. Furthermore, this method permits pulse wave analysis that is independent of changes in blood pressure. Application in clinical ultrasound may provide utility for both drug development and appropriate patient care.

♦B8

Targeting the Lipogenic Pathway in Lung Cancer: Effects of Novel Fatty Acid Synthase Inhibitors

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Background and Hypothesis: Recent studies have demonstrated that unregulated lipogenesis is a common feature of many cancer cells. Fatty acid synthase (FAS), the rate-limiting enzyme in the lipogenic pathway, is overexpressed in breast, lung, and other cancers. Drugs targeting this enzyme were shown to have some anticancer activity in animal models. However, FAS inhibitors identified to date have suffered from a limited therapeutic index. The discovery of novel FAS inhibitors may provide additional treatment options for patients with tumors that overexpress this enzyme. In addition, FAS inhibitors may potentiate the activity of drugs targeting other metabolic pathways. It has been proposed that the inhibition of lipogenesis alters purine biosynthetic pathways and lowers ribonucleotide reductase (RR) activity. Because

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decreased RR activity would enhance the efficacy of gemcitabine, a drug used to treat lung cancer, using gemcitabine in combination with a FAS inhibitor may be a beneficial therapeutic strategy. The goals of this work were to investigate the anticancer activity of novel natural product FAS inhibitors alone and in combination with gemcitabine in lung cancer cells. It was hypothesized that gemcitabine would be more effective at inhibiting cell growth when used with a FAS inhibitor.

Methods: A549 and Lewis Lung cancer cell lines were used in this study. The anticancer activity of the synthetic FAS inhibitor C75 was compared to that of extracts from three different plants used in traditional Chinese medicine. These extracts were recently shown to inhibit FAS catalytic activity using purified enzyme. Growth inhibition studies were done using the CellTiter proliferation assay kit from Promega in 96-well format.

Results and Conclusions: C75 weakly inhibited cell growth with IC_{50} values of 105 and 62.9 μM in A549 and Lewis Lung cells respectively. One of the extracts demonstrated limited activity against Lewis Lung cells (IC_{50}=33.6 μg/mL). The efficacy of gemcitabine was not affected by the addition of C75 or the herbal extracts. These results suggest that inhibition of FAS by C75 does not result in downstream metabolic changes that enhance the efficacy of gemcitabine. They also reinforce previous findings suggesting that more potent and specific FAS inhibitors are needed. Isolation of the active molecules responsible for FAS inhibition from the extracts studied here would be a step forward in that direction.

♦ B9

Establishment of the VPA-Rat Model as a Tool for Studying the Auditory Deficits Associated With Autism

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Introduction: Autism is a complex neurologic disorder that affects social development and is associated with auditory deficits including deafness, increased thresholds to tones, intolerance for ordinary sound levels, and difficulty hearing in the presence of background noise. A general disorganization in the auditory brainstem nuclei has been described in the medial superior olive in postmortem human autistic specimens. Taken together, these observations provide the foundation for a systematic and thorough evaluation of all the components of the auditory system in a controlled model for autism. Researchers have hypothesized that early gestational injury may provide a starting point for examining the effects of autism and prenatal exposure to valproic acid [VPA] in rodents yields morphologic and behavioral changes similar to those observed in autism.

Hypothesis: Components of the brainstem in rats exposed to VPA will differ from a control group in neuronal morphology and number.

Materials and Methods: Our examination has focused on the components of the superior olivary complex (SOC). Specifically, we have examined the medial superior olivary nucleus, the lateral superior olivary nucleus, and the medial nucleus of the trapezoid body. Our morphometric study includes examination of neuron number, neuron size, circularity, and orientation. Animals were divided into two groups: prenatal exposure to VPA (n=8) or control (n=4). On embryonic day 12.5, dams were given an injection of VPA or normal saline. Adult rats were anesthetized. Brains were dissected, incubated, and sectioned at a thickness of 40 μm. Specimens were mounted on glass slides, and stained for Nissl substance or for myelin and counterstained with neutral red. For morphometric analyses, sections were randomly (but systematically) selected and neurons were sampled throughout the rostrocaudal extent of each nucleus. Cell bodies were traced while focusing with the aid of a camera lucida attachment to a microscope. Grayscale tracings were analyzed using ImageJ software for neuron size, circularity, and orientation.

Results: Quantitative analysis of neuronal morphology and number revealed highly significant differences between control and experimental animals. These findings are in line with descriptions of the auditory brainstem in autistic individuals.

Conclusion: The VPA-rat model is a useful research tool for investigations into the auditory deficits observed in autistic individuals.

♦ B10

Analysis of the Role of AbgR in *Escherichia Coli* Using Growth Curves

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Hypothesis: In *E coli* the *abg* operon enables utilization of *p*-aminobenzoyl-glutamate (PABA-GLU), a product of folic acid catabolism. This operon is present in nonpathogenic *E coli*, *E coli* 0157:H7, and *Shigella*. *E coli* 0157:H7 lacks a functional *abgT*. We are interested in determining whether differences in sequence of this region correlate with pathogenicity. AbgT catalyzes import of PABA-GLU, while AbgA and AbgB together cleave PABA-GLU. Studies of the DNA sequence suggest that a fourth gene, *abgR*, is a transcriptional regulator. This project was designed to identify conditions under which *abgR* might be important for cellular growth. The regulator protein AbgR is needed for growth under specific conditions. These conditions can be identified by comparing growth properties of a strain lacking *abgR* to its isogenic parent. We predict that PABA-GLU may bind AbgR and induce or suppress transcription of the *abg* region.

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Materials and Methods: Growth of *E coli* JW1333, which lacks the *abgR* gene (AbgR−), and its isogenic parent (AbgR+), BW25113, were compared in various growth environments. Strains were grown in different media using a BioscreenC analyzer; this instrument incubates cells in liquid culture at 37°C, shaking. This device measured and recorded cell turbidity every 15 minutes for 25 hours. Analysis was performed by averaging five trials for each experiment, and quantifying pattern of growth via doubling time and lag time. Growth conditions included: rich medium (LB), and minimal medium containing varying concentrations of folic acid, PABA-GLU, amino acids, and purine and pyrimidine bases.
Results: AbgR− and AbgR+ strains exhibit similar growth under the following growth conditions: Luria Broth and minimal media (MM) containing all amino acids. AbgR− exhibited delayed growth in comparison to AbgR+ strains under the following growth conditions in MM: folate, PABA-GLU, purines, and pyrimidines. AbgR− and AbgR+ showed similar growth patterns under conditions containing certain amino acids, particularly isoleucine, leucine, valine, cysteine, and methionine.
Conclusion: AbgR is a transcriptional regulator that seems to be involved in the biosynthesis of certain amino acids.

♦B11

Juxtapositions Between the Catecholaminergic and Somatostatin-Immunoreactive Elements in the Human Hypothalamus

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Hypothesis: Previous studies revealed that stress suppresses growth. Numerous data suggested that stress hormone neuropeptide Y (NPY) may suppress growth hormone (GH) release via the hypothalamic growth hormone–releasing hormone (GHRH) system, involving direct synaptic mechanisms (DelTondo et al, 2008). Recent studies also indicated that catecholamines may be involved in the stress-suppressed GH secretion via influencing the hypothalamic somatostatin system—that in turn suppresses GHRH release in the human hypothalamus. However, the exact mechanism of this phenomenon has not been elucidated yet. In the present study, we hypothesized that catecholaminergic axonal varicosities influence somatostatinergic elements via direct synaptic connections.
Materials and Methods: Since the utilization of electron microscopy combined with immunohistochemistry is virtually impossible in the human brain due to the long postmortem period, double label immunohistochemistry evaluated with oil immersion light microscopy was used to reveal the catecholaminergic-somatostatinergic associations. The catecholaminergic elements were identified using antibody against the key enzyme of the catecholamine synthesis, tyrosine hydroxylase.
Results: The catecholaminergic-somatostatinergic juxtapositions were most numerous in the infundibular area/median eminence and in the periventricular zone, where catecholaminergic, tyrosine hydroxylase-IR axon varicosities abutted somatostatinergic perikarya. No gaps were revealed between the contacting elements during the examination of these associations with high magnification oil immersion light microscopy.
Conclusion: The catecholaminergic-somatostatinergic juxtapositions we described in the present study may be functional synapses and may represent the morphologic basis of the stress-influenced GHRH release in humans. The present results may open an entirely new avenue in understanding the mechanism of numerous growth disorders including psychosocial dwarfism.

♦B12

Distribution and Morphology of the Juxtapositions Between the Growth Hormone–Releasing Hormone (GHRH) Immunoreactive Neuronal Elements

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Hypothesis: Previous studies revealed that growth hormone–releasing hormone (GHRH)-IR perikarya are located in the basal infundibulum/median eminence of the human hypothalamus (DelTondo et al, 2008). Here, GHRH is released to the hypothalamo-hypophyseal portal system and influences the release of growth hormone (GH) of the pituitary gland. Moreover, as we previously described, the majority of the GHRH-IR neurons receive abutting fiber varicosities of various neurotransmitter systems, including the neuropeptide Y (NPY) system (DelTondo et al, 2008) and the catecholaminergic (tyrosine hydroxylase-IR) system (unpublished data). These juxtapositions may be functional synapses, and may represent the morphologic substrate of the impact of stress and growth. Physiologic data raised the possibility that in the basal hypothalamus, GHRH-IR neuronal elements directly influence the activity of GHRH neurons, via synapse-like mechanisms. However, the morphologic substrate of this GHRH-GHRH juxtaposition has not been elucidated yet.
Materials and Methods: Since the utilization of electron microscopy combined with immunohistochemistry is virtually impossible in the human brain due to the long postmortem period, single-label immunohistochemistry was utilized to reveal the associations between the GHRH elements. The juxtapositions were evaluated with light microscopy using oil immersion objective.
Results: GHRH-GHRH juxtapositions have been detected in the infundibular area/median eminence, where GHRH-IR axonal varicosities often formed multiple contacts with GHRH-IR perikarya. Examination of these associations with high magnification oil immersion light microscopy revealed no gaps between the contacting elements.

(continued)

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Conclusion: The GHRH-GHRH juxtapositions we have described in the present study may represent the morphologic basis of the GHRH-influenced GHRH release and may result in synchronized activity of GHRH-IR neuronal subgroups in humans.

B13

Sympathetic Nerve Expression in the Uterine Vasculature in Unilateral Oviduct Ligated Pregnant Rats by the Glyoxylic Acid Whole Mount Method

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Hypothesis: During pregnancy, there is a reduction in the sympathetic innervation to the uterine myometrium, but the degree to which the denervation process extends to the uterine vasculature is not clear. The mechanisms for the myometrial sympathetic denervation are thought to be related to changes in the hormonal milieu and physical stretching of the uterine wall by the growing fetus. We asked whether perivascular innervation of extrauterine vasculature was affected in rat pregnancy, and whether the presence of a fetus affected the denervation process.

Materials and Methods: In 16 female Sprague-Dawley rats, a unilateral oviduct ligation was performed. After at least a 14-day recovery period, four rats were bred and five rats served as nonpregnant controls. The arterial supply to the uterine wall at the junction of the (larger) uterine artery and the (smaller) arcuate arteries was stained and visualized for catecholamine-containing (sympathetic) nerves by the glyoxylic acid whole mount method and fluorescence microscopy, respectively. Vasculature from the nongravid (ligated) horn and gravid (nonligated) horns were examined from the nonpregnant and pregnant groups.

Results: Using a grid system, the number of nerve fibers was counted in a given mount. The relative sympathetic innervation of the ligated and nonligated horns in the nonpregnant rats (32 [SD17] vs 36 [SD13], nonligated vs ligated, P=.3) was not significantly different by the Wilcoxon signed rank test. In the pregnant rats, there was a clear trend of lower innervation in the gravid horn (18 [SD5]) compared to the nongravid horn (26 [SD12]) (P=.068) that was exposed to circulating hormonal factors associated with pregnancy but did not contain fetuses. Combined data from the two horns in the nonpregnant group compared to the gravid and nongravid horns in the pregnant group indicated no statistical difference between the nonpregnant and pregnant-nongravid (ligated) horn (P=.18) by the Mann-Whitney test. However, it was clear that the gravid horn had a less dense sympathetic innervation compared to uterine horns in the nonpregnant rats (P=.014).

Conclusion: These preliminary data indicate that while sympathetic innervation is present at late pregnancy in the larger arteries supplying the uterine horns, its relative density is reduced. In addition, the presence of growing fetuses is an important factor that determines the extent of vascular sympathetic denervation in the rat.

♦B14

Detection of *Chlamydia Pneumoniae* in the Alzheimer Disease Brain and the CNS Tissue of BALB¢ mice Following Experimental Infection

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Background and Hypothesis: Previous findings from this laboratory indicate that *Chlamydia pneumoniae* (Cpn) infection may be associated with sporadic/late onset Alzheimer disease (AD). In addition to identifying Cpn AD brain tissue, nontransgenic BALB/c mice were inoculated with Cpn to model AD-like pathology. This experimental system presents a potentially exemplary mode of pathogenesis implicated in AD. Our hypothesis is that Cpn can be consistently detected using nested PCR and Western analysis from both human AD brain tissues and mouse tissues in or near regions typically affected in AD.

Materials and Methods: BALB/c mice were experimentally infected, either intranasally or by direct intracranial injection with a respiratory isolate of Cpn (AR-39). These brains, and CNS tissues obtained at autopsy from individuals with confirmed AD, were analyzed via nested PCR and Western analysis with probes specific for Cpn.

Results: *Chlamydia* omp A-specific PCR products were noted from the mouse cerebrum (via intranasal inoculation), and this detection was prior to substantial amyloid deposition. CNS tissue, analyzed by nested PCR, displayed a 207 bp Cpn-specific product, and Western analysis with two commercially available Cpn-specific monoclonal antibodies, consistently revealed a single band between 50 and 75 kDa.

Conclusions: PCR products and analysis of immunoblots detected Cpn from clinical brain samples demonstrating that these techniques are valid in analyzing Cpn in CNS tissues. Thus, our animal models are useful for the evaluation of Cpn infection–induced pathology in the CNS as it correlates to Alzheimer disease.

B15

In Vitro Human Fibroblast (HF) Injury Repair in Response to Modeled Repetitive Motion Strain (RMS) and Myofascial Release (MFR)

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Background: Despite clinical efficacy, the cellular basis for osteopathic manipulative treatment (OMT) is not well understood. By utilizing various in vitro strain profiles, we investigated commercially available HF cellular responses to modeled RMS and MFR. We focused here on wound closure rate of HF in response to mechanical strain.
Hypothesis: We hypothesize that (1) RMS will delay HF wound closure and (2) these effects are mediated by signal transduction dependant on HF secretions in response to strain and that they are reversed by MFR treatment and/or inhibition of nitric oxide synthase (NOS).
Methods: A modeled scratch-wound approximately 2 mm wide was applied to subconfluent monolayers of cultured HF. Cells were then strained with 8 hours RMS, 60 seconds MFR, or a combined RMS followed by MFR. Each treatment was conducted in the presence and absence of the NOS inhibitor L-NMMA. In addition, unstrained HF were inoculated with condition media (CM) from these strain groups and assessed for wound closure rates microscopically at 0, 24, and 48 hours postinjury.
Results: At 48 hours postinjury, RMS treated HF responded with a 79.5% ($P<.0001$, n=9) reduction in wound closure when compared to nonstrained control HF. CM derived from RMS-treated HF also impaired wound closure by 30.5% ($P<0.05$, n=4) when compared to nonstrained CM. This phenomenon was not observed in HF treated with CM from MFR, nor combined RMS+MFR strained groups. MFR treatment alone improved the rate of wound closure by 138.5% ($P<0.01$, n=6) in HF subjected to RMS. L-NMMA treatment had no significant effect on wound closure in nonstrained HF. However, equivalent NOS inhibition resulted in wound closure improvement in RMS and RMS+MFR treated HF as compared to RMS treatment alone (191.0%, $P=.004$, n=6; 194.8%, $P=.009$, n=3, respectively).
Conclusion: These data suggest that injurious strain results in significant impairment of wound closure and that CM from strained fibroblasts is sufficient to mimic this impairment. These results support the stated hypothesis; that impairment of wound closure is induced by both biomechanical strain and soluble mediators secreted by HF in response to strain. The reduced wound closure rate caused by RMS can be normalized by treatment with modeled MFR and/or inhibition of NOS. Taken together, these in vitro studies suggest additional cellular evidence that may explain the clinical efficacy of OMT postinjury.

♦B16

Expression Profile of Genes Associated With Excessive Serotonin Autoinhibition

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Introduction: Serotonin (5-HT) is implicated in mood regulation and drugs that act on this system are effective in treating anxiety and depression. In particular, the 5-HT 1A receptor (5-HT1AR) appears to play a key role in disease states. However, the genes targeted through 5-HT1AR activation are not yet known.
Hypothesis: Through a hypothesis-driven approach, we have characterized the effects of WAY100635 (an antagonist of the 5-HT1AR) on behavior and brain gene expression in zebrafish in order to use this genetically tractable animal for understanding the actions of psychiatric drugs.
Materials and Methods: Adult zebrafish (*Danio rerio*) were used for all experiments described herein. Zebrafish (n=6 fish/group) were exposed to WAY100635 (200 μL in 100 mL of aquaria water; equivalent to a 0.1 M solution of WAY100635). Control fish were included in this experimental design (n=6 fish/group) but did not receive WAY100635. Following control conditions or drug exposure, innate fish behavior was digitally recorded for 15 minutes to characterize the behavioral effects of WAY100635 exposure. Fish brains were collected 90 minutes after each experimental condition. QPCR was used to measure Phox2b and DISC2 expression using gene-specific primers. Behavioral data and gene expression profiles were analyzed by Student's t-test and/or ANOVA with a statistically significant P value of .05.
Results: Following 15 minutes of drug exposure, experimental zebrafish were less active ($P\leq.05$) relative to controls (8.2±0.92 grid passes vs 148.3±24.1 grid passes, respectively). In addition, gill movements were significantly increased ($P\leq.05$) in animals treated with WAY100635 than control fish. However, when WAY100635-treated fish were active, they exhibited a right-sided swimming preference on an anteroposterior axis. When zebrafish brains were analyzed for gene expression, we found that the Phox2b and DISC2 genes were inversely upregulated by WAY100635 exposure. Phox2b gene expression was downregulated, whereas DISC2 was upregulated in zebrafish brain ($P\leq.05$) relative to control gene values.
Conclusion: The objective of the present work is to utilize the zebrafish as an animal model for understanding the actions of psychiatric drugs on certain genes involved in breathing automaticity. We have found that the Phox2b and DISC2 genes are targeted by the actions of WAY100635, thus suggesting that autoreceptors on 5-HT–containing neurons of the brainstem may be involved in disease states.

♦B17

Role of Transforming Growth Factor-β in Development of Oviduct Pathology in Chlamydial Genital Infection

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We evaluated the role of transforming growth factor-β (TGF-β) in fibrosis and oviduct occlusion associated with murine

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chlamydial infection. Upper genital tract pathology appears to result from the immune response to infection which influences increased fibrosis and eventually occlusion of the oviducts. Because TGF-β signaling pathways play a key role in fibrosis resulting in various disease states, we administered monoclonal antibody (MoAb) against TGF-β at specific time points during the course of infection and assessed scar formation, TGF-β levels and collagen content. Control animals received mouse IgG only. We hypothesized that neutralization of TGF-β during chlamydial infection course would reduce or eliminate fibrosis and oviduct occlusion. When administering this treatment to a susceptible strain of mice (BALB/c), we found that the amount of TGF-β was significantly decreased at all time points postinfection. The rate of chronic sequelae observed on day 56 postinfection was also significantly decreased in MoAb-treated BALB/c mice compared to the control group. We found no change in collagen levels between control and treatment groups in these mice. Therefore, although the treatment was effective in reducing TGF-β levels, we determined that a change in the time course of MoAb administration may be necessary to effect collagen levels as well as further diminish the chronic outcomes of infection.

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♦B18

Environmental Impact on Insulin Modulation of *Escherichia Coli* Biofilm Formation

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Hypothesis: *E coli* contamination poses a hazard to the safety of the food supply and causes biofouling and corrosion in agricultural and industrial settings. *E coli* association with food and other substrates relates to its ability to form biofilms. Inhibition of biofilm formation could increase food safety. The phenotypic alterations required for biofilm formation are regulated by quorum signals. Human insulin has been demonstrated to function as a mimic for microbial insulin as a quorum-signaling molecule. We hypothesize that the ability of *E coli* to form food-associated biofilms can be affected by microbial insulin. The focus of this study is to determine the effect of available food substrates and aeration on insulin-modulation of biofilm formation.

Materials and Methods: Biofilm formation was measured using pipette tips as the platform. Preweighed 200 μL sterile tips (n=12) in Mueller-Hinton broth (MHB) alone or containing various concentrations of glucose, lactose, or galactose with and without insulin (Humulin; 20 mU and 200 mU) were inoculated with 100 mL of 10^5 CFU/mL *E coli* ATCC 25923. After incubation (24 h; 25°C; static or shaking) half of the tips were dried prior to reweighing; the remainder were sonicated (7 min). The CFU/mL of sonicate was determined by standard plate count. Biofilm production (mg/CFU) was calculated.

Results: Biofilm formation was significantly ($P<.05$) decreased in shaking conditions vs static growth conditions with the exception of galactose where there was significantly ($P<.05$) more biofilm production (0.1% and 20 mU insulin) under shaking conditions than static growth conditions (threefold increase). The relative amounts of biofilm production were highest in glucose >galactose>>lactose. The presence of insulin (20 mU and 200 mU significantly ($P<.05$) increased biofilm formation two- and sixfold, respectively. This increase in biofilm formation regardless of condition tested was not related to changes in *E coli* adherence to the tips (CFU/tip), which was unaffected by the static conditions tested.

Conclusion: Production of biofilms at environmental temperature is significantly impacted by the presence of insulin. However, the effect of insulin correlates more with alterations in glycocalyx production, not alterations in bacterial adherence properties.

♦B19

Modulation of *Escherichia Coli* Biofilm Formation by Insulin

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Hypothesis: *Escherichia coli* causes several nosocomial infections including bacteremia and catheter-associated urinary tract infections. The ability to form biofilms, which enhance colonization and resistance to antibiotics and host factors, is an important virulence-associated behavior. Human insulin has been demonstrated to act as a quorum-like chemical signal that in vitro affects biofilm formation. We hypothesize that the effect of insulin on biofilm formation is affected by environmental conditions (eg, aeration and available sugar).

Materials and Methods: Biofilm formation was measured using pipette tips as the platform. Preweighed 200 μL sterile tips (n=12) in Mueller-Hinton broth (MHB) alone or containing various concentrations of glucose, lactose, or galactose with and without insulin (Humulin-R; 20 μU and 200 μU) were inoculated with 100 μL of 10^5 CFU/mL *E coli* ATCC 25923. After incubation (24 h; 37°C; static or shaking) half of the tips were dried prior to reweighing; the remainder were sonicated (7 min). The CFU/mL of sonicate was determined by standard plate count. Biofilm production (mg/CFU) was calculated.

Results: Biofilm formation in response to galactose and insulin >>glucose and insulin >> lactose and insulin as compared to sugar alone and insulin alone controls. Biofilm production

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in response to galactose (0.1%), static growth and insulin (200 mU) was approximately 1000-fold more than analogous conditions with glucose as the test sugar and more than 10,000-fold more than bacteria grown in the presence of lactose. These differences were a reflection of overall differences in amount of bacterial load (CFU/tip) (ie, the maximal numbers of CFY/tip was measured for lactose thus the bacteria adhere to the plastic more avidly when grown in the presence of lactose; however, the amount of glycocalyx produced is less per cell).

Conclusion: Human insulin affects biofilm formation on hydrophobic surfaces is affected by both aeration/shear forces and available sugars.

♦B20

The Effects of Perinatal Exposure to DDE on Immune Cell Populations

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The pesticide DDT was banned in 1973 in the United States for adverse effects on wildlife, but its major metabolite p, p'-DDE remains in the environment. It accumulates in adipose tissue and acts as an androgen receptor antagonist. We found previously that splenocytes from 3-week-old mice exposed to DDE in utero produced significantly lower levels of cytokines interleukin-2 and interferon-g, possibly indicating an alteration of immune cell types. The **hypothesis** of the present study predicts that perinatal exposure to DDE will alter the phenotypes of immune cells. Our **materials and methods** involved examining male and female offspring of DDE and control-treated pregnant female Swiss ICR mice at 3, 6, 9, and 12 weeks after birth (n=3 per group). Following mating, pregnancy was confirmed by vaginal plug and vehicle (cottonseed oil) or DDE (100 mg/kg) was injected subcutaneously on gestational days 17 and 19, with delivery on day 21. We used flow cytometry to examine T and B cell populations in thymuses and spleens. WinMDI software was used to evaluate cell populations and data were analyzed for significance using GraphPad software. One-way ANOVA tests with Bonferroni post hoc analysis were done to determine significance. **Results** include significant differences in cell populations, particularly within 3-week-old mice. In the thymuses, more CD4 cells were present in mice from DDE-treated mothers (6.53% control versus 21.60% DDE in males and 5.51 % control versus 22.17% DDE in females, $P<.0001$ for both). 3-week-old male offspring of DDE-treated mothers had fewer CD8 single-positive cells (5.69% control versus 0.54% DDE, $P<.01$). In spleens, mice from DDE-treated mothers had fewer mature lymphocytes overall. Total B cells measured were significantly less in mice from DDE-treated mothers (72.34% control versus 55.16% DDE in males and 66.80% control versus 36.37% DDE in females, $P<.01$ for both). Total T cells were also fewer in spleens of mice from DDE-treated mothers (46.33% control versus 16.88% DDE for males and 39.94% control versus 18.60% DDE for females, $P<.001$) as were CD8 positive cells (5.05% control versus 1.06% DDE for males and 5.79% control versus 1/52% females, $P<0.001$ for both). In **conclusion**, early exposure to DDE does influence immune development and may have functional consequences. Whether these or other changes related to early DDE exposure have long-lasting consequences on the immune system is the subject of further investigation.

♦B21

The Impact of Short and Long-Acting Anti-Androgens on Lymphocyte Populations

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Flutamide is a short-acting antiandrogen used as a prostate anticancer agent. DDE is a long-lasting environmental antiandrogen that accumulates within adipose tissue. Since androgens may protect from autoimmunity, our **hypothesis** predicts that these antiandrogens will impact the immune system and that short- and long-acting antiandrogens will have differential effects. For **materials and methods**, both flutamide and DDE were given to normal male mice of the C57 Bl/6 strain. Flutamide has been show in previous studies to cause maximum changes in splenocyte development and B cell populations 2 days after injection. Therefore, we injected C57BL/six male mice at 6 weeks of age with flutamide (0.5 mg) (n=3) or vehicle control (n=3) on two daily occasions and sacrificed them 2 days after the last injection. A second group was treated at 12 weeks of age and sacrificed 2 days after injection. DDE, however, has been shown to alter the immune system up to 6 months after administration. Therefore, we treated C57BL/six mice at 6 weeks with DDE (200 mg/kg) (n=3) or with vehicle control (n=3) and sacrificed them at 6 months of age. We then analyzed the differences in spleen and thymus weights and also assessed lymphocyte populations using flow cytometry. **Results** showed that when mice were given flutamide at 6 weeks of age, there was a statistically significant decrease in both B cells and T cells as well as a decrease in spleen weights. The B cell (B220) population in spleen was 70.8% for control and 65.53% for the flutamide-treated group ($P<.05$). The total T cell population (CD3) population was 45.15% for control and 38.07% for the treated group ($P<.05$). In addition, for mice treated at 12 weeks of age, the mean control spleen weights were 94 mg while the mean for the flutamide-treated group was 64 mg ($P<.05$). The longer acting antiandrogen, DDE, however, did not cause changes in total B or T cells but in subpopulations of T cells. The T helper (CD4) population increased with DDE treatment from 10.9% for the control group to 17.2% for the DDE-treated group ($P<.05$). The CD8 population increased from 6.6% for the

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control group to 10.1% for the treated group ($P<.05$). In **conclusion**, we saw decreases in total B cell and total (CD3) T cell populations in mice treated with the short-acting antiandrogen, flutamide. In contrast, we saw increases in specific T-cell populations CD4 and CD8 after exposure to DDE, a longer-acting anti-androgen.

♦ B22

The Role of SIRT1 in the Modulation of Presenilin- 1 Activity and Notch-Mediated Neurogenesis

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Hypothesis: Presenilin 1 (PS1) plays an essential catalytic role in the gamma-secretase/PS1 complex which, if malfunctioning, is responsible for the accumulation of extracellular beta amyloid (BA) plaques associated with Alzheimer disease (AD). The gamma secretase/PS1 complex also modulates the proteolytic cleavage of the Notch transmembrane receptor, which is involved in the proliferation and differentiation of adult neural stem cells (NSC). Resveratrol (RES), a potent phytochemical, has unique anti-aging and neuroprotective qualities, most of which are mediated by an NAD-dependent protein deacetylase, Sirtuin 1 (SIRT1). RES-dependent SIRT1 activation is known to mimic the effects of a caloric restriction (CR) diet, which has been demonstrated to slow signs and symptoms of AD in cellular and animal models of AD. We therefore hypothesize that, on RES administration, SIRT1 activation results in a measurable augmentation of PS1 function and Notch-mediated NSC proliferation.

Materials and Methods: SIRT1 DNA targets were determined by chromatin immunoprecipitation (ChIP) followed by 293 HEK geonomic DNA sequencing. PS1 expression was measured in vitro after 48 hours of resveratrol exposure by quantitative PCR (qPCR) compared to the control. PS1 expression in adult rat hippocampus and prefrontal cortex were measured in vivo after a 2-week dietary resveratrol regimen (70 g/d) by qPCR compared to the control.

Results: SIRT1 was found to associate with the promoter region of PS1. PS1 expression was significantly increased twofold in vitro compared to control. In vivo, PS1 expression was significantly increased in rat hippocampus fourfold, and in rat prefrontal cortex twofold, compared to control. All results were analyzed using a Student *t* test/ANOVA, with a *P* value $<.05$.

Conclusions: Resveratrol-dependent SIRT1 activation resulted in a significant increase of PS1 expression in vitro and in vivo. While mutant PS1 is directly involved in the altered processing of BA, knockout PS1 has been shown to produce AD-like neurodegeneration in the absence of cerebral BA plaques. Hence, upregulation of PS1 via resveratrol may hold unique therapeutic potential for the treatment of AD. Furthermore, to assess SIRT1's effect on Notch-mediated NSC proliferation, in vivo immunohistochemistry with Bromodeoxyuridine (BrDU), Sox2, Nestin, and doublecortin will reveal mitotic activity and cell identity in the rat dentate gyrus (DG) and subventricular zone (SV).

♦ B23

Quantitative Real-Time Polymerase Chain Reaction as a Sensitive Diagnostic Methodology for Detecting Chlamydial Infection

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Hypothesis: *Chlamydia* is a bacterial genus with great medical significance for humans, as it is the leading cause of sexually transmitted disease and infectious blindness worldwide. As many as 80% of infections are asymptomatic and *Chlamydia* are thought to persist in a latent, nonculturable form in vivo. Historically, the "gold standard" for the diagnosis of *Chlamydia*, an obligate intracellular pathogen, has been via culture within mammalian cells. More recently, nucleic acid amplification tests (NAATs) have been developed and exceed culture sensitivity, but problems exist in discrimination between active infection versus residual, nonviable pathogen or pathogen remnants. Our goal is to develop a more efficient, reliable, and reproducible method with which to diagnose both symptomatic and asymptomatic infections of *Chlamydia*.

Materials and Methods: BALB/c mice were infected with *Chlamydia muridarum* and cervical-vaginal swabs were taken at specific days postinfection. Swabs were used to isolate *C muridarum* via culture with subsequent enumeration of inclusion forming units (IFUs). Total RNA extraction from the same swab was performed and treated overnight with DNase. Using primers for chlamydial 16S ribosomal RNA, a marker of chlamydial metabolic activity, we performed a reverse transcriptase PCR (RT-PCR) reaction with subsequent agarose gel analysis. The remaining RNA was then converted to cDNA and run on a real-time PCR machine with Taqman 16S rRNA chlamydial primers.

Results: A majority of mice were culture positive on day 21 but none had viable, recoverable IFU on day 56. The results from RT-PCR show that samples with a high IFU count via culture exhibit visible amplification via 16S chlamydial primers. Real-time PCR demonstrated a specific chlamydial 16S rRNA copy number within each sample. In addition, this copy number correlated well with IFU count in all samples (ie, a high IFU count from culture has a high copy number and vice versa). Also, day 56 mice, despite having no detectable infection via culture, exhibited measurable levels of amplified, metabolically active *Chlamydia* in their genital tract via real-time PCR.

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Conclusion: Real-time PCR shows a greater sensitivity for the ability to not only detect persistent, viable *Chlamydia* below the level of culture and RT-PCR detection, but also for the ability to quantify the specific copy number of chlamydial 16S ribosomal RNA present.

B24

Human Fibroblast (HF) Model of Repetitive Motion Strain (RMS) and Myofascial Release (MFR): Potential Roles in Muscle Development

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Background: Guided fascia manipulation is an osteopathic technique capable of alleviating pain and accelerating muscle trauma recovery. The fundamental cell type of fascia, HF, is known to secrete numerous cytokines with roles in muscle development (eg, FGF-2, TGF-β, IGF-1, IL-6, and IL-1α). When HFs are strained in manners modeling RMS, MFR, and RMS followed by MFR, cytokine release is altered, suggesting mechanisms for clinical efficacy.

Hypothesis: Modeled RMS and MFR differentially regulate HF release of signaling molecules involved in myoblast differentiation into functional myotubes.

Methods: To test for myotube differentiation, HFs were plated onto flexible collagen-coated membranes and subjected to the following four strain paradigms: 8 hours, 10% cyclic RMS; 60 seconds, 6% MFR acyclic strain; RMS followed 3 hours later by MFR; and no strain. At 24-hours poststrain, HF conditioned media (CM) from all groups were collected, and aliquots used to culture C_2C_{12} myoblasts. Differentiation media containing horse serum and untreated media served as positive and negative controls, respectively. Differentiation of myoblast to myotubes was assessed by cellular elongation, fluorescent labeling of acetylcholine receptors via α-bungarotoxin, and hematoxylin eosin staining to document multinucleation. Each experiment was performed three times, with duplicate measures in each. Photomicrographs were obtained from each well at 24-hour intervals yielding N=18 per time point.

Results: Although CM from all groups induced differentiation to some degree, the differentiation index (DI; represented in myotubes per cm^2), varied among treatment groups. MFR resulted in the earliest myotube formation at times 72 and 88 hours (DI=35.6±9.9 N=18, 71.5±28.6 N=6), leveling to 80.9±25.4 (N=18) at 96 hours. RMS DI was delayed at 72 and 88 hours (28.5±11.5 N=18, 14.3±9.1 N=6), however at 96 hours RMS DI was the greatest among all groups (114.3±30.42 N=18). RMS+MFR DI was also delayed at 72 and 88 hours (28.6±11.0 N=18, 28.5±14.2 N=6), and was attenuated vs RMS DI at 96 hours (47.6±14.7 N=18). Nonstrained CM had the lowest DI (9.6±7.4 N=18, 38.1±16.6 N=18) at 72 and 96 hours.

Conclusion: These data suggest that HFs secrete soluble mediators of myoblast differentiation, and that biomechanical forces modeling RMS and MFR differentially regulate muscle development. Ongoing research is currently investigating the involvement of candidate cytokines and mechanisms associated with differentiation.

B25

Intracellular Carbonic Anhydrase Isozymes Are a Potential New Therapeutic Target for Renal Cell Carcinoma

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Introduction: Renal cell carcinoma accounts for about 85% of all renal cancers and more than 30,000 new cases are diagnosed annually in the United States. The disease commonly presents at the metastatic stage, when it is notably refractory to currently available therapies. Carbonic anhydrase (CA) catalyses the reversible hydration of carbon dioxide to bicarbonate and a proton, and is present in several human isozyme forms. Our previous data indicate the potential efficacy of highly specific sulfonamide inhibitors of CA in the treatment of renal carcinoma. In the kidney, both intracellular and cell-surface isozymes are expressed. Intracellular CA activity appears to be essential for cancer cell growth. Both intracellular and cell-surface CA isozymes may be required for the elimination of acid generated by hypoxia in cancer cells, thus promoting cell growth and reducing extracellular pH which may promote cell invasion. The CA IX isozyme is virtually specific to cancer cells, and is down-regulated by the von Hippel-Lindau (VHL) tumor-suppressor gene. It is strongly expressed on the surface of most renal cell carcinomas, and is considered to be diagnostic.

Hypothesis: Specific CA isozyme(s) involved in the growth of renal carcinoma cells may be identified in order to enhance targeting of future therapy.

Methods: We employed two renal cancer cell lines: 786-O cells which strongly express the extracellular CA IX isozyme, and Caki-1 cells which do not. The effect of CA inhibition on the growth of each cell line was investigated using the classical, highly specific CA inhibitor acetazolamide (DIAMOX), which is cell-permeant, and benzolamide, an even more effective CA inhibitor, which is not cell-permeant and so would inhibit only cell-surface CA isozymes.

Results: Acetazolamide strongly inhibited the growth of both 786-O and Caki-1 cell lines in culture (GI_{50}~0.4 mM), but benzolamide had negligible effect on either, though a slight inhibition was achieved with 786-O cells. These data indicate that the inhibition of renal carcinoma cell growth is not principally attributable to inhibition of the tumor-specific isozyme CA IX, which may explain why preliminary attempts at immunotherapy, targeting CA IX, have achieved only limited success.

Conclusion: Intracellular carbonic anhydrase isozymes are a potential new target of future therapy for renal cell carcinoma.

(continued)

♦B26

Long-Term Effects of Common Antiepileptic Drugs on Glutamate Receptors, Cell Fate, and Memory During a Critical Growth Period

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Hypothesis: Antiepileptic drugs (AEDs) can cause cognitive impairment during a critical growth period, possibly due to proapoptotic effects. In adult rats, certain AEDs are ineffective at blocking seizures; however, some afford neuroprotection. We hypothesized that certain alterations of glutamate receptor subunit stochiometry secondary to AED treatment may underlie the learning deficits, cell death, and cell damage associated with seizures.

Materials and Methods: Lamotrigine (LTG), carbamazepine (CBZ), phenytoin (PHT), valproate (VPA), and topiramate (TPM) were administered (ip) on P14 and continued daily for 7 days before status epilepticus was induced with Kainic acid (KA) on P21. Seizure severity, memory retention, histologic outcome, and glutamate receptor expression were determined with electrograph (EEG), Morris Water Maze testing, Nissl staining, and immunohistochemistry, respectively.

Results: None of the four AEDs tested efficiently attenuated KA-induced seizures. Only PHT increased mortality, identifying an adverse effect of this drug. Despite poor antiepileptic activity, LTG, VPA, and TPM protected CA1 but not CA3 hippocampal neurons from seizure-induced injury. TPM was less protective if seizures were severe. Chronic LTG, VPA, PHT, or TPM in the absence of KA did not affect memory, but CBZ diminished performance in the water maze. CBZ and PHT did not prevent KA-induced cell death or memory deficits. In contrast, KA animals pretreated with LTG, VPA, and TPM had improved memory performance, compared to KA animals that were unable to find the platform. None of the AEDs had effects on the maturational differences in expression of AMPA proteins after KA; however, LTG, VPA, and TPM prevented seizure-induced AMPA alterations in protected areas of the hippocampus. The expression of NR1 was significantly increased in the CA1 but decreased in the DG after TPM in the absence or presence of KA seizures (ANOVA, $P<.01$). Interestingly, mGluR1a, an age-specific interneuronal marker, was prematurely elevated in CA3 pyramidal cells after KA seizures and chronic LTG or VPA treatments.

Conclusion: Beneficial effects of LTG, VPA, and TPM on memory may be due to the neuroprotection associated with these AEDs. Preservation of GluR1 and NR1 subunits and elevations of mGluR1a-type glutamate receptors in an age-dependent manner may contribute to the underlying mechanisms of neuroprotection of AEDs with poor antiepileptic activity in developmental epilepsy.

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♦B27

The Development of a Rodent Model to Study the Effects of Lymphatic Pump Treatment on the Lymphatic and Immune System

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Hypothesis: Osteopathic physicians have long recognized the importance of the lymphatic system in the maintenance of health and have included treatments such as the lymphatic pump technique (LPT) in clinical trials studying the effects of manipulative medicine on infectious disease with promising results. However, there has been a lack of animal research in this field. Previously our laboratory developed a large animal model to study LPT in healthy subjects. In the canine model, LPT increased lymph flow, leukocyte concentrations, and cytokine release from both the thoracic and intestinal lymph duct. In order to study the effects of LPT in a disease model, we have developed a rodent model of LPT, which allows us to use better characterized disease models, have access to a wider range of reagents and biomarkers, and minimize cost.

Methods: In order to demonstrate that LPT in our rodent model is comparable to the canine model in an acute setting, a catheter was inserted into the thoracic duct of the rats and lymph was collected during baseline, 4 minutes of LPT, and recovery. In addition, we collected blood following a single application of LPT. In contrast to our large animal model, the repeated application of LPT requires the use of anesthesia. Due to this difference, we have characterized the use of volatile and nonvolatile anesthetics in healthy animals in order to perform repeated treatments over several days.

Results: In the thoracic duct lymph, both lymph flow and leukocyte counts were increased, resulting in a greater than threefold increase in leukocyte flux through the thoracic duct. An increase of 4,000,000 leukocytes was seen in the jugular venous blood approximately 45 minutes following treatment. We also found that the daily application of the inhaled volatile anesthetic isoflurane caused a significant increase in lung leukocytes after 6 days, regardless of disease status. We replaced isoflurane with propofol, a nonvolatile, shorter acting sedative in order to minimize immune modulation in the tissue sites of our disease models.

Conclusion: Following the development and characterization of the rodent model of LPT, we will be able to study the effects of LPT on both a pneumonia and metastatic cancer, both of which have significant clinical relevance in the osteopathic community.

♦B28

The Effects of Lymphatic Pump Manipulation on Tumor Development and Metastasis

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Hypothesis: It is well recognized in the osteopathic community that metastatic cancer or cancer with metastatic potential is considered a relative or absolute contraindication for certain osteopathic manipulative treatments (OMT), including high velocity low amplitude (HVLA) and lymphatic pump techniques (LPT). However, studies from our laboratory demonstrate that the application of LPT to the rat increases thoracic duct lymph flow and leukocyte numbers, and enhances survival and reduces pulmonary bacteria during pneumonia. In addition to the direct effects of increased numbers of circulating leukocytes produced by LPT, it seems likely that these cells improve immune surveillance, which may enhance protection against tumor development and metastasis.

Methods: To test the hypothesis that LPT enhances antitumor immunity, F344 rats were injected intravenously with MADB106 tumors. Twenty four hours following tumor injection, rats received: (1) no treatment (control), (2) 4 minutes of light touch under anesthesia (sham), or (3) 4 minutes of LPT under anesthesia for 5 consecutive days. Seven days after tumor injection, lungs were removed for assessment of tumor metastasis and leukocyte populations.

Results: The application of sham and LPT reduced pulmonary tumors by approximately 50% compared to control. In addition, sham and LPT increased pulmonary leukocytes approximately twofold compared to control. This finding suggests that the administration of isoflurane anesthesia during either sham or LPT increases leukocyte trafficking into lungs and subsequent tumor killing. Therefore, to ascertain if the application of sham or LPT under isoflurane anesthesia would increase the pulmonary trafficking of leukocytes in the absence of tumors, healthy rats were given control, sham, or LPT as described. The administration of isoflurane increased pulmonary leukocyte numbers approximately twofold, suggesting that pulmonary tumors are not necessary to increase leukocyte trafficking into lungs in response to isoflurane anesthesia.

Conclusion: The results from our preliminary studies demonstrate that the administration of isoflurane gas (during either sham or LPT) increases leukocyte trafficking into healthy lungs and lungs burdened with tumors. In addition, the use isoflurane gas during sham or LPT enhanced killing of tumors in the lung. In our ongoing studies, we are exploring new anesthetics to elucidate the effects of LPT on tumor development and metastasis.

♦B29

Anti-Inflammatory Effects of Ethanol Include Modifications of BK Channels in Macrophages

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Introduction: Ethanol is a potent immunomodulatory agent, yet the mechanisms of its effects remain elusive. It is possible that ethanol may nonspecifically modify the cell membrane fluidity, thus changing interactions between cell membrane proteins. However ethanol may also target the large conductance calcium-activated potassium (BK) channels, which have been extensively studied in neuronal cells where ethanol was shown to target the pore-forming subunit of the channel. BK channels are also active in other cell types, including macrophages. It was shown that BK channels function in TLR and IL-1 receptor–mediated macrophage inflammatory responses.

Hypothesis: The immunomodulatory action of ethanol involves its effects on the function of BK channels expressed by the cells of the immune system.

Methods: This study compared the effects of BK channel–modifying agents paxilline and BMS 191011 on LPS-induced TNFα production in human monocytic cell line THP-1. The cells were cultured in the presence or absence of ethanol.

Results: Paxilline decreased TNFα secretion in both control and ethanol-exposed cells. The rate of inhibition by 10μM paxilline was higher in the ethanol-treated THP-1 cells (92%) than in the control cells (38%). BMS 191011 reduced TNFα levels in the control (25%, 10μM BMS), but not in the ethanol-exposed cells. These results suggest that ethanol increased blocking activity of paxilline and reduced sensitivity to BMS 191011 in THP-1 cells. We showed previously that ethanol inhibited MAPK p38 signaling pathway. In this study, we demonstrated that blocking BK channels with paxilline attenuated activation of p38 in the control, but not in the ethanol-treated cells. To examine whether ethanol treatment modified the effect of Ca^{2+} availability on inflammatory responses, EGTA and BAPTA-AM (chelators of extracellular and intracellular Ca^{2+}, respectively) were used. The production of TNFα was diminished in the presence of EGTA in the control cells but not in the ethanol-treated cells. BAPTA-AM reduced TNFα levels in both control and ethanol-exposed cells.

Conclusion: This study indicated that immunomodulatory action of ethanol may include targeting BK channels. The blockade of BK channels on macrophages by paxilline potentiated the inhibitory effect of ethanol on TNFα production, though it had no additional effect on already-reduced (ie, due to ethanol) MAPK p38 activity. In addition, ethanol may affect inflammatory responses by modifying the availability of Ca^{2+}.

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♦B30

Expression of Mucins in Rat Adjuvant-Induced Arthritis

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Hypothesis: This project aimed to elucidate the various mucin proteins that are expressed within the synovium of arthritic joints by performing immunohistochemistry on paraffin sections of ankle tissue from an adjuvant-induced arthritis rat model. Previously, we have identified the presence of mucin protein, MUC3, in the synovium of arthritic rat joints using immunohistochemistry on frozen cryosections. In addition, we have reported expression of MUC3 and MUC5AC in arthritic human joint tissues and synovial fibroblasts. We hypothesize that several different mucins may be expressed in synovial tissues during the peak of arthritis inflammation. In this work, we report the results of a survey study of the protein expression of multiple mucins in paraffin-embedded sections of rat ankles in order to ascertain which mucins are potentially involved in the pathogenesis of arthritis.

Materials and Methods: This project used the rat adjuvant-induced arthritis model as a model of human rheumatoid arthritis. The study utilized paraffin-embedded sections of arthritic rat ankles, which maintain the joint structure and composition better than frozen sections. Immunohistochemistry was performed on the paraffin ankle sections using antibodies to 5 mucins (MUC1, MUC3, MUC5AC, MUC12, and MUC16) or control IgG and then examined under the microscope for staining.

Results: Staining for MUC3 was pronounced in 7 of the 8 ankles examined which confirmed the previous report using frozen sections. A novel finding showed that 7 of the 8 rat ankles expressed MUC12. MUC1, MUC5AC, and MUC16 did not produce positive staining in rat sections (n=8).

Conclusion: The staining confirmed the presence of MUC3 and also identified the presence of MUC12, suggesting these membrane-bound mucins may be involved in the pathogenesis of arthritis. Mucin proteins MUC1, MUC5AC, and MUC 16 were not detectable in paraffin-embedded rat ankles, suggesting that they are not expressed or that these antigens are masked in formalin-fixed paraffin-embedded ankles.

♦B31

Lymphatic Pump Manipulation Mobilizes Inflammatory Mediators into Lymphatic Circulation

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Hypothesis: Lymph stasis can result in edema and accumulation of particulate matter, exudates, toxins, and bacteria. This can lead to inflammation, impaired immune trafficking, tissue hypoxia, tissue fibrosis, and a variety of diseases. In earlier studies, using a canine model, we demonstrated that lymphatic pump treatment (LPT) significantly increased thoracic and intestinal duct lymph flow and leukocyte concentrations. It is likely that this increase in lymphatic flow during LPT facilitates the release of inflammatory mediators from tissues into circulation.

Methods: To determine the acute effects of LPT on lymphatic cytokine and chemokine concentrations, a catheter was inserted into either the thoracic (n=6) or intestinal (n=6) lymph ducts of mongrel dogs. Lymph was collected during 4 minutes of resting (baseline), during 4 minutes of LPT, and during 10 minutes following LPT. The concentrations of IL-4, IL-6, IL-8, IL-10, IL-15, MCP-1, TNF-a, INF-y, KC (CXCL1), and MCP-1 were measured in both thoracic and intestinal duct lymph using a multiplex assay.

Results: On average, LPT increased thoracic duct lymph cytokine/chemokine concentrations approximately tenfold, and intestinal lymph cytokines/chemokine concentrations approximately fivefold compared to baseline. Furthermore, by 10 minutes following cessation of LPT (recovery), thoracic and intestinal lymph cytokine/chemokine concentrations were similar to baseline, suggesting their release is transient. There was no preferential release of either pro- or anti-inflammatory cytokines/chemokines during LPT; however, the greatest increases were seen in IL-2, IL-6, IL-8, IL-10, KC, and MCP-1.

Conclusion: The results from this study demonstrate that, in addition to enhancing lymph flow and lymphatic leukocyte concentrations, LPT is able to mobilize inflammatory mediators into lymphatic circulation. This redistribution of inflammatory mediators during LPT may provide scientific rationale for the clinical use of LPT to enhance immunity and treat infection.

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♦B32

Phorbol Ester-Induced Monocytic Differentiation of HL-60 Leukemia Cells is Associated With Alterations in Cul5 Protein Expression

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Background and Significance: The HL-60 myeloid leukemia cell line originated from a patient with acute promyelocytic leukemia (APL), and has been extensively used as a research model for studying both granulocytic and monocytic differentiation. Treatment of HL-60 cells with all-*trans* retinoic acid promotes granulocytic differentiation to neutrophil-like cells and treatment with an active phorbol ester promotes mono-

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cytic differentiation to macrophage-like cells. Previously, we demonstrated that granulocytic differentiation of HL-60 cells is associated with an increase in the expression of Cullin-5 (Cul5) mRNA and protein. Cul5 functions as a scaffold within E3 ubiquitin ligase complexes to target cellular proteins for ubiquitin-mediated degradation by the 26S proteasome.

Hypothesis: The goal of the present research was to determine if Cul5 protein expression increases during monocytic differentiation of HL-60 cells.

Materials and Methods: HL-60 cells were treated with 16.2 nM phorbol 12-myristate 13-acetate (PMA) for 48 hours to promote monocytic differentiation. Differentiation was monitored by observing the expected change from suspension cells to adherent cells. Cells were also treated with a phorbol ester that is more biologically active than PMA (Phorbol 12,13-didecanoate), and with an inactive phorbol (4α-Phorbol 12,13-didecanoate). Changes in Cul5 protein expression were monitored by Western blot analysis.

Results: Treatment with PMA resulted in the appearance of Cul5 immunoreactive bands (~45, 58, 88 kDa) which were absent in the nontreated and vehicle control groups. In contrast, Cul5 immunoreactive bands with smaller molecular weights (~19, 24, 25 kDa) were present in the untreated and vehicle control groups, but were absent in the PMA-treated cells. Phorbol 12,13-didecanoate promoted differentiation and demonstrated a greater increase in the expression of Cul5 immunoreactive proteins (~45, 58, 88 kDa) versus PMA. 4α-Phorbol 12,13-didecanoate did not promote monocytic differentiation, and acted in a similar fashion to the untreated and vehicle control groups with regard to Cul5 expression.

Conclusion: The finding of altered Cul5 expression with monocytic differentiation may be significant since full-length Cul5 has been shown to be antiproliferative and truncated Cul5 has been shown to be proliferative.

♦B33

The Effects of N-Acetylaspartic Acid on Stem Cell–Derived Oligodendrocytes In Vitro

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Hypothesis: Green fluorescent protein (GFP) Lewis rat pups are an appropriate model for isolating neural stem cells (NSC) to grow oligodendrocytes in vitro; N-acetylaspartic acid (NAA) is toxic to oligodendrocyte development.

Materials and Methods: NSC were dissected from cerebral cortices of 3-day-old GFP or wild-type Lewis rat and suspended in DMEM–F12 media. Round phase-light neurospheres (oligodendrocyte precursors) appear after 7 days and aspirated to retain the cells for growth in suspension. Add isolated neurospheres to uncultured dish with new media: neurobasal media (NB+B27+FBS) in the presence of PDGF-A to ensure growth into oligodendrocytes. Cultures were stained for DAPI, GFP, and O4 antibody to calculate gliogenic potential and show genuine differentiation of NSC into oligodendrocytes. NAA was added to half of the wells and incubated for 6 days, then stained for oligodendrocyte markers. Cells were viewed and quantified with Olympus BX51F upright microscope with Stereologer software.

Results: 56.5% total viable cells were GFP+/DAPI+. 68.4% of oligodendrocytes were GFP+/O4+. The total number of cells cultured with NAA does not differ from the total number of cells cultured without NAA. A 65% decrease in O4+ cells is found when oligo progenitors are cultured in 5mM NAA (Student *t* test, $P<.008$).

Conclusions: Neural stem cells isolated from GFP Lewis rats differentiated into oligoprogenitors when cultured in NBM/FBS+PDGF-A and displayed a gliogenic potential of 68.4% in vitro. These results suggest that GFP – Lewis rat pups are a viable source of neural stem cells. Neural stem cells isolated from wild-type Lewis rat pups also differentiated into oligodendrocyte progenitors and showed a 65% reduction in O4+ cells when 5mM NAA was added to the NBM/FBS+PDGF-A medium. The toxic effects of NAA in vitro suggest that oligodendrogenesis is drastically inhibited by high NAA. This project accomplished two goals: it showed that the stem cell cultures proliferated and differentiated appropriately in NBM/FBS with PDGF-A and that high NAA concentration in the medium resulted in an inhibitory effect on oligodendrocyte differentiation. These results support a toxic role of NAA during oligodendrocyte development.

♦B34

Regulation of Blimp-1 Nuclear Localization in Drosophila Melanogaster

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B lymphocyte–induced maturation protein-1 (Blimp-1) is a critical regulator for the differentiation of B cells into antibody-secreting plasma cells. In addition, it has been shown to be an important regulator for the development of multiple tissues in many different vertebrates. Recently, Blimp-1 has been shown to be vital in the development of *Drosophila melanogaster*. The goal of this project was to characterize Blimp-1 protein expression in developing *Drosophila*. Since it has been shown that Blimp-1 is expressed in response to the molting hormone, ecdysone, we hypothesized that Blimp-1 protein expression would be uniform in all cells during early pupariation, which occurs right after a large ecdysone surge. A number of *Drosophila* organs from the early pupa were stained with a Blimp-1 antibody and visualized with immunofluorescence. Guinea pig serum that contains an antibody raised against *Drosophila* was preabsorbed with <1-hour-old fixed *Drosophila*

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embryos overnight at 4°C to decrease background staining. This antibody was used in a standard immunofluorescent staining protocol at a final dilution of 1:500. We found that Blimp-1 is expressed in many tissues, but in most cases it was found only to be in the cytoplasm. This finding was unexpected considering that Blimp-1 is a transcription factor with five DNA-binding zinc fingers and a putative nuclear localization signal. Intriguingly, Blimp-1 staining colocalized in some tissues where there was an abundance of DAPI-positive molecules in the cytoplasm, presumably mRNA. This indicates that *Drosophila* Blimp-1 does indeed associate with nucleic acids. Interestingly, some salivary gland cells had Blimp-1 staining that was exclusively nuclear, while neighboring cells had cytoplasmic staining with no staining in the nucleus. We conclude that Blimp-1 is not expressed in every cell and that even if it is expressed within a cell, the regulated nuclear localization is another mechanism controlling the action of Blimp-1.

♦B35

Effect of Age on Severity and Extent of Infection With *Chlamydophila Pneumoniae* and the Efficacy of a Heptaepitope Minigene Vaccine in C57BL/6 Mice

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Background and Hypothesis: The intracellular bacterium *Chlamydophila* (*Chlamydia*) *pneumoniae* has been linked etiologically to several respiratory diseases and may also have a causative role in the pathogenesis of several chronic diseases of aging, including atherosclerosis and Alzheimer disease. We assessed whether advanced age correlates with increased burden of infection in C57BL/6 mice after intranasal inoculation with *C pneumoniae* and also whether a heptaepitope minigene vaccine would induce protective immunity against *C pneumoniae* in aged mice as well as in young mice. Our hypothesis is that aged C57BL/6 mice will have an impaired ability to clear *C pneumoniae* respiratory infection, and that the heptaepitope minigene vaccine will be less protective in the aged mice, as compared to young counterparts.

Materials and Methods: For the first experiment, 6- and 20-month-old female C57BL/6 mice were infected intranasally with 5×10^5 inclusion forming units (IFU) of *C pneumoniae*. Fourteen and 28 days after infection, lung tissue was analyzed for infectious *C pneumoniae* by immunofluorescence. For the second experiment, female C57BL/6 mice were immunized beginning at the age of 2-3 months or 16-17 months. The vaccine was delivered in three 33 μl injections at 3-week intervals. Twelve days after the third dose, when the mice were 6- and 20-months of age, they were infected intranasally with 5×10^5 IFU of *C pneumoniae*. As in the first experiment, lung tissue was analyzed 14 and 28 days postinfection by immunofluorescence.

Results: In the first experiment, we found that advanced age was associated with a decrease in the proportion of animals able to completely clear the infection, with 71% (5 of 7) of young mice, but only 50% (4 of 8) of aged mice, resolved by day 28 pi. An increase in the burden of infection in the lungs was also noted in aged mice. In the second experiment, at day 14, all of the immunized mice had *C pneumoniae* titers below detection, while the unvaccinated mice showed considerable lung bacterial recoveries. At day 28, approximately 50% of the vaccinated aged mice were still completely protected from *C pneumoniae* infection and the remaining 50% were partially protected, when compared to unvaccinated aged mice.

Conclusions: Our results suggest that although aged mice experience a greater burden of infection (measured as lung bacterial load), they appear to be equally protected by the vaccine as the young mice.

♦B36

MDM2 Function in Osteoblasts and Its Upregulation by 1,25 Dihydroxyvitamin D3

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Hypothesis: Amplification of Mdm2 gene has been found to be related to a subset of osteosarcomas, whereas targeted deletion of this gene in bone produces an osteoporotic phenotype. Having shown a role of p53 gene in osteoblast differentiation, we worked on the hypothesis that Mdm2 function is required for proper bone differentiation. Our goal with this study was to analyze regulation of the Mdm2 gene to gain better understanding of its p53 dependent and independent roles in osteoblast functioning.

Materials and Methods: Regulation of MDM2 gene is mediated through P1 and P2 promoters. The P2 promoter region we analyzed was a 500bp region of the first intron which harbors two copies of the p53 response element and is therefore p53 responsive, whereas the P1 region is not. We used reporter luciferase assays and Reverse Transcriptase PCR to demonstrate changes in Mdm2 gene expression during vitamin D treatment.

Results: Bone cells showed higher basal levels of MDM2-P2 activity in osteoblasts when compared to P1. Treatment with 1,25 dihydroxyvitamin D3 (Vitamin D) an important bone anabolic agent produced activation of the MDM2-P2 but not P1. Activation of the Mdm2 gene through P2 required the presence of wild type p53 as no activation was observed in p53 null osteoblasts and very little was observed in cell lines with temperature-sensitive p53 expression. When MDM2 expression was analyzed by real-time PCR we found that activation of transcription of MDM2 by vitamin D occurred early and

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proceeded without any antecedent increase in p53 expression. We also tested the effect of transient knock down of MDM2 on bone marker gene expression. MDM2shRNAs were used to reduce the MDM2 levels. We found a dramatic decrease in Cbfa1 activity, a master regulator and a transcription factor for osteoblast differentiation.

Conclusions: The results from these studies suggest that proper osteoblast differentiation and function may depend on both basal activity of p53 and MDM2 and its induction by vitamin D.

♦ B37

Perineuronal Nets Are Restricted to Periolivary Nuclei in the Human Superior Olive

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Introduction: Perineuronal nets [PNN] are specialized constructs of the extracellular matrix associated with distinct neuronal populations in the human central nervous system. PNN form a glove-like covering over the soma, dendrites, and initial axon segment and have been specifically associated with fast-spiking neurons and function in plasticity and maintaining the local ionic environment.

Hypothesis: Based on our description of PNN in the human cochlear nucleus [Wagoner and Kulesza, 2009] we expect PNN to be preferentially associated with principal neurons involved in low frequency hearing and sound localization.

Materials and Methods: This study is based on analysis of five human brainstems (74 to 94 years of age). Tissue was obtained through the HGR and was only included if the tissue could be preserved within 12 hours of death, the cause of death was nonneurologic, and there was no evidence of neurologic disease. IRB approval was obtained for all procedures. For staining of PNN, free-floating sections were processed for wisteria floribunda histochemistry with biotinylated-wisteria floribunda agglutinin at a concentration of 10 mg/mL overnight. Sites of wisteria binding were identified by reacting the tissue in a solution of diaminobenzidine, hydrogen peroxide, and NiCl. Sections were mounted onto glass slides from cresyl gelatin, dehydrated, cleared in xylene, and coverslipped or counterstained for Nissl substance with neutral red. Tissue sections were examined using an Olympus BX45 microscope and photographed with an Olympus DP12 digital camera. Tracings of the superior olivary complex (SOC) nuclei were made with a camera lucida attachment and the locations of perineuronal nets were plotted directly on these tracings.

Results: Within the human SOC, perineuronal nets are exclusive to the nuclei of the trapezoid body (medial and ventral) and posterior tier. In these nuclei, perineuronal nets surround a fairly high percentage of neurons. Perineuronal nets are also found occasionally in the lateral nucleus of the trapezoid body, but never in the medial or lateral superior olives.

Conclusion: These preliminary data provide evidence of a limited, but highly specific, distribution of perineuronal nets within the human superior olive that is largely outside the principal nuclei.

B38

Presence of the Functional Caspase-12 Allele in Indian Subpopulations

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Hypothesis: In rodents, caspase-12 (casp12) is a negative regulator of interleukin-1 production. Most humans lack a functional CASP12 gene, with a nonfunctional variant (CASP12p1) found in 100% of the Caucasian and East Asian population and approximately 80% of people of African descent. However, 20% of sub-Saharan Africans carry an intact allele of CASP12, which produces a full-length noncatalytic proenzyme and is associated with an increased risk of sepsis. As only persons from East and Central Asia have been genotyped for *CASP12*, we examined *CASP12* allele distribution in individuals from Southern Asia, specifically from the Indian subcontinent.

Materials and Methods: Subject DNA was collected via buccal swabs and PCR-based single nucleotide polymorphism analysis was used to genetoype CASP12 alleles. DNA from persons who genotyped as CASP12+ was sequenced to verify results.

Results: As compared to members of the Indo-European language group, CASP12 was significantly present in members of the Dravidian language group (the predominant ethnic and language group of southern India), particularly in persons from Tamil Nadu. The CASP12 allele also occurs at a higher frequency in individuals with the Australoid morphotype that also predominates in southern India, as compared to those with the Caucasoid morphotype prevalent in central and northern India.

Conclusions: This is the first description of the presence of the CASP12 allele in south Asia. The data suggest that there is a north-to-south gradient of increasing prevalence for the CASP12 allele. As the CASP12p1 allele has been rigorously selected for in populations outside of Africa and southern Asia, we hypothesize that a pathogen exploiting the inflammatory immune response in Africa and southern India may be exerting selective pressure for the maintenance of CASP12.

♦ B39

(continued)

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Cellular Distribution of Calsequestrin and SERCA2a in Ventricular Myocytes From Neonate Rat

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Hypothesis: In the mammalian heart, the mechanism of excitation-contraction coupling (ECC) changes dramatically during postnatal development. While the adult myocyte contraction depends on ryanodine receptors (RyR)-mediated Ca^{2+} induced Ca^{2+} release (CICR) from the sarcoplasmic reticulum (SR), neonate myocyte contraction mainly relies on Ca^{2+} influx across the sarcolemma through voltage-gated Ca^{2+} channels. In neonate, the specific contribution of RYR to ECC is significantly less than what could be anticipated based on its density and functional properties. Recent studies showed a fraction of RyRs located in the center of the cell that are not activated during a normal action potential (AP) despite their mature functionality. We have recently found that the transverse tubes containing these central RyRs are not connected yet to the surface membrane, explaining their apparent quiescence during the AP. To maintain robust CICR responses by the Ca^{2+} release site, it is necessary the presence of other crucial SR proteins, such as Ca^{2+}-ATPase (SERCA2a) and calsequestrin (CSQ). Thus, the specific hypothesis tested in this work was the quiescence of the central Ca^{2+} release sites in the NB cardiomyocyte results from transverse heterogeneities in the expression levels of key Ca^{2+} handling proteins in the SR; namely, SERCA2a and CSQ.
Materials and Methods: After isolating neonate and adult myocytes, immunolabeling was used in conjunction with linescan confocal microscopy to evaluate distribution heterogeneities of both SERCA2a and CSQ.
Results: Our results indicate that the fluorescence associated with SERCA2a and CSQ localization exhibits a homogeneous pattern of intensity across the cell. This finding suggests that these proteins exhibit similar density between the center of the cell and the periphery, contradicting our working hypothesis.
Conclusion: This work has provides additional evidence to the mechanism of membrane discontinuity as primarily responsible for the lack of contribution of RyRs to the intracellular Ca^{2+} transient in the neonate stage.

Medical Education

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♦ME1

Use and Spread of Osteopathic Medical Literature and Research Findings

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Background: Evaluating how information about the history, practices, and science of osteopathy is disseminated and used by the scientific and medical community is an important and ongoing challenge. One way to achieve this goal is by measuring the citation history of articles related to osteopathy, as this is one index of an article's impact and contribution to its field of study.
Hypothesis: We hypothesized that performing a citation analysis of articles relating to osteopathy would help determine the spread and influence of osteopathic studies and publications within the scientific community.
Methods: Using the Web of Science and Scopus databases, we performed a citation analysis of the articles published with "osteopathic" in the title. From these data we evaluated the citation trends and timelines of these articles and identified a number of important indices of the spread and use of osteopathic related literature.
Results: Of the primary articles that were evaluated, a limited number of articles were cited, and of those that were cited, the vast majority was cited only once. We found that the citation rates are highest within the first 5-10 years of the original publication date, but have occurred as much as 45 years later. Searches of primary articles published in *JAOA—The Journal of the American Osteopathic Association* also revealed limited citation number. We also found that the number of articles with "osteopathic" in the title has increased from 78 published between 1960 and 1989 to 339 published between 1990 and 2008.
Conclusion: The greatest expansion of osteopathic literature has occurred within the past 18 years. However, these data indicate an area for continued expansion of osteopathic related literature as well as need to develop methods to increase the dissemination of results from osteopathic research.

♦ME2

Physician Familiarity With the Five Most Common Misdiagnosis

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Hypothesis: In the United States, there are, on average, 110,000 malpractice claims filed per year, and as many as 98,000 preventable deaths from medical errors. The cost of medical failures is estimated to be between $17 billion and $29 billion. In

2004, a total of $4.2 billion dollars was paid in malpractice lawsuits. The highest payouts (and the most common type of lawsuits) were related to misdiagnosis, failure to diagnose, or delayed diagnosis. These payouts were even higher than other lawsuit causes such as surgical errors. In a previous publication, the authors presented the five most commonly misdiagnosed conditions (as confirmed at autopsy and malpractice proceedings) with the hope of aiding medical personnel and ultimately contributing toward better patient care. Since the state of Florida requires all physicians to take at least two continuing medical education credit hours for both licensure and renewal, it is important to gauge the effect of such programs.

Materials and Methods: This study surveyed a random sample of Florida physicians in order to assess their knowledge of the most common misdiagnosis and the important process errors that lead to misdiagnosis.

Results: Of the 175 Florida physicians who completed the survey, only 40% correctly identified pulmonary embolism as the most commonly diagnosed condition (in terms of relative incidence). Moreover, only 10% of physicians correctly identified infections as the most common misdiagnosis (in terms of total incidence). On the other hand, 58% of physicians understood that breast cancer is a common misdiagnosis, which leads to malpractice.

Conclusion: It is clear from the results, that most physicians are not aware of the most common misdiagnosis, nor are they aware of the most common process errors, which lead to misdiagnoses. The data collected in this study should be used to improve continuing medical education programs, and in the larger scope, prevent thousands of misdiagnosis.

ME3

Making a Difference: Results of Medical Teaching in Kabul, Afghanistan

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Hypothesis: Fourth- and fifth-year medical students at the Kabul Medical Institute in Afghanistan would improve their understanding of the seriousness, diagnosis and treatment of diarrheal, dermatologic and pediatric cardiac disorders after didactic sessions on these topics.

Materials and Methods: A questionnaire for both a pre- and a postevaluation was developed. The instrument included the demographics of the medical students in attendance and their understanding before and after the presentations concerning diarrheal, dermatological, and pediatric cardiac disorders.

Results: Sixty-three (63) pre- and postinstruments were matched. All participants were male and the majority (61.9%) was between 25-30 years of age. Their primary language was Dari. The mean comfort level with English was 5.53 on a scale of 7, where 7 indicated full understanding. The majority of the medical students were into their fifth year in medical school in a five-year curriculum. Most of the students were not initially comfortable treating the targeted medical conditions prior to the presentations but postevaluation indicted an improved understanding of the concepts discussed.

Conclusions: Outreach programs which allow western medical personnel to instruct Afghanistan medical students would improve medical education in Afghanistan.

ME4

Spiritual and Religious Characteristics of Osteopathic Medical Students

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Hypothesis: Osteopathic medicine has recognized the importance of teaching medical students about spirituality and religion (McClain et al, 2008). A recent survey of physicians indicates that 55% of the participants acknowledged that their religious beliefs influence their clinical engagements (Curlin et al, 2005). However, little is known about medical students' religious values and how they shape the clinical encounter. This study assessed which osteopathic medical students are most likely to address patient spirituality and religiosity. This would help educators to (1) clarify the nature of medical students' attitudes about spiritual discussion with patients, (2) identify potential gaps in the existing medical curriculum, and (3) develop a model for predicting which medical students are likely to incorporate spiritual concerns into patient care.

Methods: Participants included 222 students enrolled in an osteopathic medical school. A survey was completed at baseline to assess their attitudes regarding the integration of patient spirituality in the clinical encounter. Medical students also rated the importance of individual spirituality and communal religiosity in their lives using the Religious and Spirituality Experiences scales (Smith and Orlinsky, 2004).

Results: Students who rated individual spiritual experiences as more important in their lives currently also rated themselves as more likely to complete spiritual assessments with their patients (r=.32, P<.001). Students who rated religious experiences as more important in their lives currently also rated themselves as more likely to complete a spiritual assessment with their patients (r=.21, P<.001). Though medical students' religious characteristics are diverse, they generally endorsed a commitment to both personal spirituality and communal religiosity.

Conclusion: These findings suggest that the importance medical students place on their own spiritual and religious experiences is positively related to the likelihood that they will integrate spirituality with patients in clinical scenarios. Results

highlight the importance of medical educators being sensitive to individual variability in osteopathic medical students' own spiritual/religious values prior to engaging in spiritual diversity training. Medical students would benefit from curricular components that facilitate a deeper self-awareness of how their personal religious worldviews may shape their care of patients.

ME5

Teaching Psychiatry in Osteopathic Medical Schools: A Survey of Current Curriculum and a Suggested Primary Care Approach

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Hypothesis: The current research analyzes the prevalence of dedicated psychiatry instruction in the curricula of the current osteopathic medical schools to show that psychiatry is often underemphasized given its prevalence in primary care practice.

Materials and Methods: The curricula of the current osteopathic medical schools were obtained from the Web sites of the schools that have online access available for incoming students. The preclinical and clinical curricula were analyzed separately with attention to the psychiatry and behavioral health courses offered. Data was separately obtained via literature search and tabulated to find the most common complaints and disorders seen in primary care.

Results: The curricular analysis showed a lack of dedicated psychiatry training in preclinical courses for the majority of the current osteopathic medical schools. However, all current osteopathic medical schools require a clinical rotation in psychiatry or behavioral health. The data from the most commonly seen primary care disorders shows depression and anxiety near the top of family practice encounters while attention-deficit hyperactivity disorder is near the top of pediatric encounters.

Conclusions: Based on the current curricular offerings and the most commonly diagnosed and treated disorders in primary care, a proposed primary care psychiatry curriculum was constructed and is introduced here. This proposed curriculum will aid osteopathic medical schools in deciding which psychiatric disorders should be emphasized to help their students—the majority of whom pursue careers in primary care—prepare for medical practice. A psychiatry curriculum that includes these disorders, and others seen commonly in primary care, should help prepare osteopathic medical students to treat psychiatric disorders more effectively in the future.

ME6

Prevention in Osteopathic Undergraduate Medical Education: A Survey of the Core Competencies in Disease Prevention and Health Promotion

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Background: Preventive medicine and public health have been established as integral parts of medical education. The purpose of this study is to survey undergraduate osteopathic medical school programs to identify the degree to which curricula include the four core competencies in health promotion/disease prevention, the associated evaluation methods, the barriers to such a curriculum, and the future plans for implementation.

Methods: Eighteen faculty or deans from the 26 osteopathic medical schools (69%) responded to a survey. The questionnaire explored information about the school, the curriculum and the four core competencies in health promotion/disease prevention, evaluation procedures, barriers to implementation, and future plans. Responses were anonymously recorded. Descriptive statistics were used to summarize the findings.

Results: The schools ranged in size from 88-1040 students; 72% have a required prevention curriculum, with an average instruction time of 49 hours (sd=50.3). Most schools teach preventive medicine in a required course plus other settings; 6 schools teach the curriculum as part of a course or clinical rotation. Of the 13 schools that have a required Preventive Medicine curriculum, 84.6% teach all aspects of Clinical Prevention and all teach all or almost all core competencies of Quantitative Skills. Fewer (53.8%) teach all competencies of Health Services Organization and Delivery; no schools teach all, but 69.2% teach almost all competencies of Community Dimensions of Medical Practice Topics. All 13 schools use written evaluation to assess performance, the majority also uses observation, oral presentations, and computer-assisted simulations. The most common barrier to teaching prevention is other curricular demands.

Conclusion: A response rate on the questionnaire was similar to the allopathic medical school response and better than the osteopathic medical school response rate to the Prevention Self-Assessment Analysis (PSAA) in 1997. As in the PSAA, there was acknowledgment of the importance of Clinical Prevention and Quantitative Skill and less emphasis in Health Services Organization and Delivery and in Community Dimensions of Medical Practice. Greater attention in the Prevention curriculum must be placed on these latter two areas to be consistent with current recommendations of the Institute of Medicine that all medical students receive basic public health training in population-based prevention approaches to health.

ME7

Simulation of Gross Motion Testing in Palpatory Diagnosis

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Introduction: The Virtual Haptic Back, a virtual reality simulation of the feel of the human back using haptic interfaces, has been used by students at Ohio University College of Osteopathic Medicine as part of their OMM training in palpatory diagnosis for 3 years (*J Am Osteopath Assoc*. 2008;108:29-36). The purpose of the present project was to extend the simulation to include gross motion of the simulated patient being examined, to more fully reflect the processes of the osteopathic palpatory examination.
Hypothesis: The hypothesis was that such simulation is feasible with existing haptic interfaces.
Materials and Methods: The simulation was programmed on a Microsoft Windows computer in Visual C++ with an OMNI haptic interface for palpation and a Sidewinder haptic interface for motion inputs.
Results: The simulation is now capable of permitting the user to impose movements (side bending a rotation in either direction, flexion, and extension) by moving the handle of a flightstick–type of haptic device in the appropriate direction with one hand. This is done while the user holds a finger of the other hand on the virtual back palpating the changes in tissue texture caused by the various movements. Through the incorporation of a speech recognition system, movements can also be done actively by the virtual patient in response to verbal instruction by the user. The tissue texture responses can be programmed to simulate clinically determined patterns, such as those known as Fryette's Principles and tissue responses described by Johnston in *Functional Methods*. Restrictions of vertebral motion in which the motion is restricted to a subset of vertebrae can also be illustrated. The virtual back of the patient can be made to appear on the monitor clothed or unclothed with a transparency function activated which makes underlying skeletal structures visible.
Conclusion: The creation of a virtual haptic human back that permits simulation of clinically observed tissue texture changes in response to gross motion of the simulated patient is feasible. Further development will include testing with medical students in the context of palpatory diagnosis training.
Acknowledgment: Supported by American Osteopathic Association Research Grant #08-08-563.

♦ME8

Commercial Board Review Courses and COMLEX-USA Level 1 Performance

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Background: The COMLEX-USA Level 1 is a high-stakes exam for osteopathic medical students. Several companies create and market board review courses with the purpose of increasing COMLEX-USA Level 1 scores, and increasing pass rates. Some previous research has failed to demonstrate an association between professional board review courses and improved COMLEX-USA Level 1 scores. In spite of this, due to anecdotal evidence supporting their effectiveness and COM encouragement or financial support, many students still spend a significant amount of time and money taking such courses.
Hypothesis: Utilizing commercial board review courses will be associated with better performance on the COMLEX-USA Level 1.
Methods: An online survey was administered through AACOM's COSGP Research Committee. Student government presidents were asked to forward the online survey to their respective third-year classes. The survey asked students who had taken their first attempt at the COMLEX-USA Level 1 test between May 15, 2007, and August 31, 2007, to respond to a 22-item survey regarding their COMLEX-USA Level 1 score and their primary method of board preparation, including utilization of a commercial board review course or other board review method, as well as other demographic and academic variables. Descriptive statistics were calculated. Odds ratios were also calculated for the relationship between passing the COMLEX Level 1 and the utilization of a commercial board review course.
Results: 593 Students from 10 COMs completed the survey. 90 students reported that their primary board preparation method was a commercial board review course. The odds ratio for passing the COMLEX Level 1 exam having used a commercial board review course was 0.67.
Conclusion: Based on this self-report survey, it does not appear that utilizing a commercial board review course as a primary method or preparation is associated with better performance on the COMLEX-USA Level 1 exam. Further research in this area should control for confounding variables, such as medical school academic performance and MCAT score, and seek to identify subgroups of students who may benefit from commercial board review courses. At this point, we cannot recommend that osteopathic medical stu-

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

dents rely on commercial board review courses as their primary method of board preparation.

Health Policy

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The Osteopathic Physician and End-of-Life Care

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Hypothesis: As modern medicine discovers more ways of prolonging life, Americans are indeed living longer, but there is a high price for longevity. In the United States, 41% of people die in hospitals and perhaps as many as 40% die in pain. As a result, end-of-life (EOL) care has become crucial to the care of millions of Americans. Because palliative care requires treatment of the whole patient, the osteopathic physician is uniquely poised to be invaluable to these efforts.

Materials and Methods: An extensive search of the literature was performed for previous publications with data on osteopathic physicians and end-of-life care. After considering study characteristics (ie, location, setting, and sample size) for each paper, a multiplier was assigned to the results in order to allocate more weight to heterogeneous sample studies performed in the United States. These methods served to augment the data sets, which best represent the population of the United States as a whole.

Results: The average US-trained osteopathic physician is more than twice as likely to enter a geriatrics fellowship program than the average US-trained allopathic physician. The osteopathic advantage in end-of-life care can be largely attributed to OMT (osteopathic manipulative treatment). Osteopathic manipulative treatment has been shown effective at improving quality of life in patients suffering from cancer, disability, heart disease, stroke, neuropathy, vascular dementia, stroke, myopathy, and Parkinson disease. When OMT is not a part of these patients' treatment, their plans often include additional pharmacologic agents that usually cause unwanted side effects.

Conclusion: With the hands-on care that osteopathic physicians provide and the unique benefits that OMT offers the patient, this osteopathic brand of medicine holds a unique and vital role in this developing arena. Therefore, the American Osteopathic Association and other osteopathic organizations must collaborate with other professional organizations and state governments in order to educate health professionals and the public, with the goal of investigating the best practices, and incorporating to EOL the highest standard of quality care that modern medicine has to offer.

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The doctor of the future will give no medicine, but will interest his patients in the care of the human frame, in diet, and in the cause and prevention of disease.

Thomas Alva Edison, 1847–1931