55th Annual AOA Research Conference—Abstracts, 2011

This issue of JAOA—The Journal of the American Osteopathic Association *features abstracts from the posters that will be presented at the 55th 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 6 groups:

- *series F—fellowships (see below)*
- *series P—osteopathic manipulative medicine/osteopathic principles and practice (see page 488)*
- *series C—clinical studies (see page 491)*
- *series B—basic sciences (see page 499)*
- *series ME—medical education (see page 510)*
- *series HP—health policy (see page 511)*

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

This year's AOA Research Conference, "The Science Supporting the Impact of OMT on the Human Condition: The Structure-Function Relationship and Mechanisms of Action for Self-Regulatory and Healing Processes," will take place in Orlando, Florida, from Sunday, October 30, to Tuesday, November 1, during the AOA's 116th Annual Osteopathic Medical Conference and Exposition (OMED 2011), "The Future of Medicine Is in Your Hands."

For more information on the AOA Research Conference or other programs taking place during OMED 2011, access the conference's Web site at http://www.osteopathic.org/omed. The AOA Research Conference program can be accessed at http://www.osteopathic.org/inside-aoa/events/annual-aoa-research-conference/Pages/presentation-program-and-schedule.aspx.

AOA Research Fellowship

♦F1

Evaluation of Osteopathic Manipulative Medicine Pretreatment for the Prevention of Acute Mountain Sickness

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Context: Acute mountain sickness (AMS) is seen in unacclimatized persons shortly after ascent to high altitude. Its pathogenesis is still unclear but appears multifactoral, arising from inadequate response to hypoxia.

Hypothesis: We hypothesized that somatic dysfunction (SD) via increased allostatic load hampers the body's ability to respond, and that osteopathic manipulative treatment (OMT) would improve it.

Methods: The TUCCOM institutional review board approved this double-blind, randomized, sham-controlled trial. Sixteen healthy volunteers were randomly assigned to receive OMT or sham therapy. Four subjects served as their own control (half received sham first). Heart rate (HR), respiratory frequency (f), tidal volume (Vt), and hemoglobin O_2 saturation (SaO_2) were monitored pre- and post-OMT/Sham 48 hours before acclimatization. Subjects were evaluated for location and severity of SD (utilizing the American Academy of Osteopathy's SOAP note), received sham or OMT, and then was reevaluated by 1 DO. No one in the sham group had improvement in their SD. During a 2-day/2-night stay at 12,500 feet (White Mountain Research Station, California); HR, f, Vt, and SaO_2 were monitored, and AMS symptoms were evaluated using the Lake Louise Scoring system (LLS).

Results: At high altitude, no significant differences were seen in HR, f, Vt, or SaO_2 between groups at any time point. At day 2, LLS scores were higher ($P<.05$) in the OMT group vs sham group (mainly headache and difficulty sleeping). Separate analysis for the 4 repeating subjects showed no significant difference in any parameters, but a trend was noted toward more rapid acclimatization after OMT as measured by the

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rate of increase in SaO_2 (P=.068, day 3).

Conclusions: The data obtained from the 4 repeat subjects support our hypothesis that by treating SD, OMT might better prepare the body to adjust to this hypoxic environment. However when compiled with the other subjects, the positive effect of OMT became questionable. Possible explanations include: The inequality in SD severity between groups, while there was no significant difference in SD severity for each of the 4 compared to themselves, the mean SD severity score was significantly higher in the OMT group compared to sham (P<.05); 48 hours may be an insufficient length of recovery time after OMT. There is large individual variability in high altitude response and AMS susceptibility. Further study and a larger number of repeating subjects are needed to evaluate the use of OMT for prevention of AMS and to better understand its effects on the ventilatory response to high altitude.

♦F2

What Drew You to Osteopathic Medicine? A Survey of Osteopathic Medical Students From 10 Schools

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Hypothesis: The motive behind students choosing osteopathic medicine can predict the students' attitudes toward osteopathic principles including osteopathic manipulative treatment (OMT).

Materials: A self-administered, 25-item electronic questionnaire was distributed to matriculating, first- and second-year medical students at 10 osteopathic medical schools (n=3091). The questionnaire contained items addressing student attitudes toward osteopathic principles, including OMT; perspectives toward OMT education; and plans for integrating OMT into future practice. The final question was open-ended, asking students to identify "what drew you to osteopathic medicine?"

Methods: The responses were categorized into 6 broad groups: osteopathic philosophy, OMT, factors related to a specific school (including location, curriculum), desire to serve (including practicing in a rural or underserved area), desire to be a doctor regardless of degree, and other influence (including family, spiritual guidance). Chi-square and Mann-Whitney tests were used to examine the relationship between these responses and students' attitudes.

Results: The overall response rate was 31% (n=973). A majority of students stated that osteopathic philosophy and/or OMT is what drew them to osteopathic medicine with 56% and 26%, respectively. Students who reported they were drawn by osteopathic philosophy and/or OMT had more favorable attitudes toward osteopathic principles, OMT, and intention to use OMT, while students drawn by a specific school or who reported the desire to be a doctor as what drew them to osteopathic medicine had less favorable attitudes toward osteopathic principles, OMT, and intention to use OMT.

Conclusion: The motive behind a student choosing osteopathic medicine appears to influence the student's level of agreement with osteopathic philosophy and their intention to use OMT. Students who were motivated by osteopathic philosophy and/or OMT were more favorably disposed toward osteopathic principles and OMT, had more positive educational perspectives toward OMT, and intended to use OMT more frequently in their future practice compared to those who did not indicate these reasons.

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Osteopathic Manipulative Medicine/Osteopathic Principles and Practice

P1

Effects of Osteopathic Cranial Manipulation on Biogenic Amine Levels, Heart Rate, and Blood Pressure in Adult Humans

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Cranial manipulation had been known to affect the function of the autonomic nervous system. It has been proposed that these effects are mediated be cerebrospinal fluid (CSF) movement and intracranial pressure. Studies suggest possible alteration of CSF flow by compressing the third ventricle, constituting a CSF pump. Indeed, previous data suggest that cranial manipulation may have a sedating or relaxing effect, most likely by decreasing sympathetic tone. In the current study, we expected to see a change in levels of biogenic amines, dopamine (DA), norepinephrine (NE), epinephrine (E) and Serotonin (5-HT) measured with HPLC, and consequent blood pressure and heart rate decrease in response to cranial manipulation. We also expected to see suppression in sympathetic function with decreased levels of NE and E as well as increased 5-HT levels and a change in the mood in individuals after the CV4 technique. Metabolites of 5-HT (5-HIAA), those of NE and E (VMA and DHMA), and those of DA (DOPAC, HVA, 3-MT) were also tested. Results suggest that the CV4 technique may actually lower the pulse rate by about 10%. It also appears that the CV4 technique increases average NE level by about 25%. No statistically significant changes in blood, pulse rate, respiration rate, NE concentration, or E con-

centration were noted. In addition, there was a trend for lower blood pressure, heart rate, and respiratory rate after the CV4 technique and after the sham technique. However, these differences were not statistically significant. These findings suggest that cranial manipulation, whether the CV4 or sham therapy may affect blood pressure, respiratory rate, and heart rate. Results also showed that levels of E were decreased after CV4 but increased after sham. The opposite was observed with NE levels, suggesting that the effects of cranial manipulation on biogenic amine levels are dependent on the type of cranial manipulation used. The alteration of E levels in response to the CV4 technique indicates that cranial manipulation may affect the mood, particularly anxiety states. It appears, however, that cranial manipulation did not affect the levels of the other biogenic amines tested. Since the present data were obtained over a relatively short period after the procedure, more studies are needed on the long-term effects of the CV4 technique, including the assessment of mood before and after cranial manipulation.

P2

Effects of Short-Term Examiner Training Upon Interexaminer Reliability of Diagnostic Palpation for the Thoracic and Lumbar Spine

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Hypothesis: Osteopathic physicians commonly use diagnostic palpation to determine the presence of somatic dysfunction of the spine and resolution of the finding posttreatment. However, the reliability of this primary diagnostic measure has not been established.

Objective: To determine if short-term examiner training by a specialist on palpation methodology has an impact on observer concordance and if interrater agreement differs by anatomic location.

Methods: The thoracic and lumbar spine subgroup sessions of this study were conducted during separate timeframes, had different subgroup sets of examiners, and followed the same methods. Subjects (thoracic, n=60; lumbar, n=60; total, N=120) recruited for this study were asymptomatic. In the pretraining sessions, 4 board examiners independently evaluated T3-T7 (n=30) and L1-L5 (n=30) spinal segments for rotational asymmetry and rated the severity of the finding. Examiners underwent 2 training sessions to standardize the examination method and held 3 rehearsal sessions. In the posttraining sessions, the examiners used the standardized methods to evaluate the same thoracic (n=30) and lumbar (n=30) spinal segments as in the pretraining sessions. Kappa (κ) statistics were used to compare pre- and posttraining interexaminer reliability results.

Results: Poor to fair interexaminer agreement was demonstrated in the pretraining sessions with κ scores of 0.067 to 0.325 (thoracic) and from 0.00 to 0.300 (lumbar). In contrast, moderate to substantial interexaminer concordance was obtained following the posttraining sessions with κ scores of 0.467 to 0.650 (thoracic) and 0.458 to 0.650 (lumbar). No statistically significant difference was found on observer agreement by anatomic location (pretraining, P=.168; posttraining, P=.287).

Conclusion: The results of this study suggest that short-term training with standardization of the palpation methods used by the examiners improves interexaminer agreement and that examiner concordance does not differ by anatomic location.

P3

Osteopathic Medical Students' Attitude Toward Complementary and Alternative Medicine

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Introduction: Complementary and alternative medicine (CAM) has grown into a huge industry and source of healthcare in the United States. Many medical schools have responded to the public's interest in CAM by developing integrative medicine clinics and research centers and increasing the amount of CAM in medical curricula. Given the expansion of CAM education and its increased use, understanding the baseline attitudes and beliefs of osteopathic medical students toward CAM and the factors that may have formed them is important.

Hypothesis: Osteopathic medical students will be more positive (open) toward CAM compared to the allopathic medical students.

Methods: A previously validated 29-item Integrative Medicine Attitude Questionnaire (IMAQ) and 10-item CAM Health Belief Questionnaire (CHBQ) was administered to medical students. Respondents were 803 osteopathic medical students from 7 osteopathic medical schools in the US and 95 medical students from 2 allopathic medical schools. Demographic and other data were collected on students' use of CAM modalities and their awareness and use of primary CAM information resources.

Results: Osteopathic medical students demonstrated positive attitudes and beliefs toward CAM use. Osteopathic medical students' attitudes toward CAM were statistically significantly more positive (open) than the allopathic medical students (IMAQ Score difference, t=4.173, df=28, P<.00001). Students showed high levels of self-reported CAM use.

Eighty-eight percent of osteopathic medical students receive information about CAM from the Internet.

Conclusion: Medical students showed positive attitudes toward CAM and high self-reported use of CAM. When compared to allopathic medical students, osteopathic medical students viewed CAM more favorably. Knowledge and understanding of CAM may allow osteopathic physicians to provide care that is in line with popular demand.

♦P4

Using Palpation Technology Feedback to Improve Interexaminer Reliability

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Background: Diagnostic palpation is a vital skill for manual practitioners. Interexaminer reliability studies using the kappa (κ) statistic to help identify useful palpatory tests and how they are taught. Interexaminer reliability studies of the right Anterior Superior Iliac Spine (ASIS) Compression Test (Test) were previously reported. Using data from 330 subjects gathered by 2 osteopathic medical students, the κ statistic was applied to prevalence index controlled subgroups. Performed from the subject's right side, the Test rated "excellent" (κ=0.80) in female subjects, "fair" overall (κ=0.43), but "poor" for male subjects and for any left-sided assessment.

Hypothesis: Studies of the ASIS Compression Test in both genders will benefit from feedback monitors to insure bilaterally consistent engagement pressures; that without feedback, examiners misjudge right-left pressure. Feedback will improve agreement and increase κ values even in a population where prevalence index is not controlled.

Methods: The same 2 examiners from our prior study replicated the Test on 6 men and 6 women. Pressure sensitive material over finger pads and thenar eminences (IsoTOUCH Palpation Monitors; Neuromuscular Technologies: Nashville, TN) sent instantaneous data to a computer display screen. Two separate trials conducted on different days used the same 12 subjects. From the supine subject's right side, 2 sequential pulsatile ASIS compressions were transmitted posteromedially (60° from horizontal) towards S3; the side of greatest end-feel resistance denoted "positive." In trial 1, both examiners were blinded to pressure data. In trial 2, they were directed to use 6 to 8 lbs bilateral engagement pressures using live monitoring feedback.

Results: In the same healthy population (without prevalence index controls), feedback improved the κ statistic from 0.059 to 0.571 (left positivity) and -0.05 to 0.47 (right positivity). In females, κ values improved from 0.08 to 0.67 (left) and from 0.36 to 0.67 (right). Feedback did not improve reliability in male subjects.

Conclusion: Using objective pressure feedback during the ASIS Compression Test greatly increases interexaminer reliability; such technology could enhance efforts to document and improve manual skills. Pressure feedback improved right-left issues without changing sides to perform the Test. It did not resolve male-female κ differences where variables such as Test pressure application angles may need study.

P5

Documenting and Comparing Frequencies and Forces Used to Perform Lymphatic Pump OMT Manually and With Use of a Machine

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Background: The Dalrymple pedal lymphatic pump (DPLP) is a common osteopathic manipulative treatment (OMT) technique used clinically to increase lymphatic circulation and fight infection. As traditionally taught, "correctly performed" DPLP involves oscillating force applied to the patient's feet at an individualized rate and force combination that produces a visible "abdominal sloshing" motion at the level of the diaphragm. This force has never been measured, but an average DPLP rate of 120 per minute (2.00 Hz) is suggested. Machines such as the AT101 (CIS, Florida) may be capable of replicating DPLP OMT and its physiologic effects for potential use in research and clinical arenas.

Hypothesis: Manual and AT101 frequency and force (F&F) can be replicated interchangeably as can the visual ("sloshing") OMT titration point.

Methods: Fifty-one subjects were randomly assigned to 2 groups; an accelerometer measuring F&F in all subjects. Group 1 subjects (n=25) received manual DPLP titrated to create an "abdominal slosh." They were then placed on the AT101 where machine settings were adjusted attempting to replicate identical F&F measurements recorded during manual DPLP. Finally, the examiner blindly modified machine settings to attempt to recreate the "OMT slosh" on the AT101. Group 2 subjects (n=26) all received an AT101-induced motion using predetermined settings and then underwent a manual attempt to replicate machine-induced F&F. Each was then re-evaluated while receiving traditional DPLP.

Results: In group 1, manual DPLP frequency and force averaged 2.23 Hz and 0.27 G, respectively, with successful F&F

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replication on the AT101 (average= 2.20 Hz at 0.26 G). On the AT101 a visible "slosh" in the same subjects was re-created using an average F&F of 2.18 Hz at 0.29 G. In group 2 subjects, optimal manual DPLP averaged 2.34 Hz at 0.29 G. Machine presettings in group 2 subjects created higher F&F averages (2.47 Hz at 0.42 G); manual F&F replication attempts averaging 2.35 Hz at 0.43 G.

Conclusion: While individualized, a traditional DPLP can be performed using an average 0.28 G force at a rate of 135 per minute (2.25 Hz). An experienced operator using an AT101 machine is able to reproduce the F&F used in the manual DPLP as well as its traditional "slosh." Frequencies and forces in manual- and machine-induced pedal pump are reproducible. Factory settings on the AT101 machine create only a few F&F combinations that were unable to be fully reproduced or sustained by hand.

Clinical Studies

♦C1

Depression, Anxiety, Pain, and Disability Correlates in Postamputee Pain

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Background: In amputees, depression is the most common comorbidity of anxiety, which has been implicated in exacerbating postamputee pain. Comorbidity anxiety and depression may amplify pain and disability. Though amputees commonly suffer depression, no studies have investigated how depression may relate to anxiety, pain, and disability in this population.

Hypothesis: Depression will be higher in postamputee pain subjects vs controls, and this depression will relate to pain, anxiety, and disability.

Methods: The study protocol was approved by the Northwestern University institutional review board. Twenty-six amputees were recruited to participate in the study. Sixteen subjects reported postamputee pain, while 10 subjects without postamputee pain were selected as controls. Anxiety related to pain was measured by the Pain and Anxiety Symptoms Scale-20 (PASS). Current pain was rated with the Visual Analog Scale (VAS) and the McGill Pain Questionnaire Short Form (MPQ). Depression was evaluated using the Pain Disability Index (PDI). Mean BDI scores were compared in postamputee pain subjects vs controls using 2-tailed nonparametric *t* tests. Relationships between depression, anxiety, pain, and disability in postamputee pain subjects were assessed using 2-tailed Pearson correlations.

Results: BDI scores were significantly higher in post-amputee pain subjects versus controls. BDI correlated with only 1of 3 PASS subscores. BDI did not correlate with VAS, MPQ, or PDI scores.

Conclusion: Greater levels of depression in subjects with postamputee pain reflects previous findings in other chronic pain patients. However, our finding that pain levels do not correlate with depression contrasts with that reported elsewhere in the literature. Our findings further marginalize relationships between depression, disability, and anxiety. This may be explained by anxiety and depression moderators GABA and glutamate becoming unbalanced after amputation and affecting expected relationships between psychological symptoms.

C3

Validation of Emergency Department Triage Classification on a National Scale

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Background: Nurse-driven triage is standard of care in emergency departments. Mis-triage can potentially prevent timely care from reaching a patient and therefore presents a danger to patient safety. But in the context of emergency department overcrowding, when a patient is over-triaged, it may also use resources unnecessarily. Nurse triage has been validated in specific contexts, but not on a national scale previously.

Hypothesis: Nurse driven triage is an accurate predictor of patient morbidity and mortality as patients present to emergency departments nationally.

Methods: Data were drawn from the 2008 National Hospital Ambulatory Medical Care Survey (NHAMCS), excluding patients with no triage data. Demographic factors were analyzed. The following outcomes, broken down by triage class, were analyzed: mortality, admission, treatment time (length of visit minus waiting time), diagnostics orders, and medications given.

Results: A total of 28,484 patients had triage data recorded, as shown on page 492.

Conclusion: When examined on a cross-section of patients presenting to emergency departments nationally, nurse triage is a good predictor of severity of illness and intensity of service.

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Class	n	Percent ± CI95 Admit	Mortality	Median (Q1 - Q3) Mins Treated	Total Tests	Meds Given
I	1369	43 ±1.5%	2.4 ± 0.5%	180 (91-284)	6 (2-9)	1 (0-3)
II	3975	32.2 ± 0.8%	0.1 ± 0.1%	161 (85-270)	5 (1-8)	1 (0-3)
III	13535	16.3 ± 0.9%	0 ± 0%	119 (59-215)	2 (1-6)	1 (0-2)
IV	6906	5.7 ± 0.9%	0 ± 0%	66 (31-129)	1 (0-2)	1 (0-2)
V	2699	4.9 ± 1.1%	0 ± 0%	60 (30-127)	0 (0-2)	0 (0-1)
P Value		<.001	<.001	<.001	<.001	<.001

Results for abstract C3.

C4

INSPIRE DIABETES: Basal Bolus Insulin as the First Treatment of T2DM

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Background: The progressive nature of type 2 diabetes mellitus (T2DM) continues to be an area that has been elusive to intervention. Most people who have diabetes need increasing numbers of medications over time to control glycemia. Insulin, when used early in patients with T2DM, may exert different effects than later in disease.

Hypothesis: The INSPIRE DIABETES clinical trial is testing the benefit of a 12-week pulse of basal bolus insulin as the initial management of T2DM in terms of long-term glucose control and preserving β-cell function.

Methods: Sixty adults with T2DM will be randomly assigned to routine care (as described by the 2009 American Diabetes Association treatment recommendations) vs a pulse of basal-bolus insulin. The insulin regimen is a weight-based pulse of insulin for up to 12 weeks with a forced up and down titration. Routine care will be ongoing and start with metformin, followed by glimepiride, and then pioglitazone. Oral medications will be up-titrated to goal and will be continued throughout the trial. Primary outcomes include time to rescue therapy and need for rescue therapy. Secondary outcomes will be HbA_{1c}, hypoglycemia, and other metabolic parameters: lipids, weight, c-peptide, HOMA-I, and HOMA-R.

Results: To date, 10 patients have been randomly assigned. Two are in active treatment time and 8 are in the postintervention period. Three patients (2 routine care and 1 intensive insulin) have needed rescue therapy at this time.

Conclusions: If successful, INSPIRE DIABETES may have a profound impact on the treatment algorithm for T2DM.

C5

A Possible Contribution of Osteopathic Medicine in the Management of Children Affected by ADD/ADHD

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Background: Attention-deficit/hyperactivity disorder (ADHD) is a neurobehavioral developmental disorder affecting children and teenagers, characterized by the co-existence of attentional problems and impulsivity/hyperactivity.

Hypothesis: Aim of the study was to evaluate the efficacy of osteopathic manipulative treatment (OMT) on children affected by ADHD syndrome. Differences between study and control groups in terms of score from the bells test, including subdomains accuracy (A) and rapidity (R), were detected.

Methods: An exploratory prospective study was performed in a cohort of children consecutively admitted at a single neuropsychiatry unit between 2008 and 2009. Out of 28 subjects, aged 5 to 15 years, enrolled with confirmed diagnosis of ADHD following routine medical/psychological care, 14 received OMT. ADHD was diagnosed through a psychiatric assessment following the criteria in the *Diagnostic and Statistical Manual of Mental Disorders (DSM IV)*. Statistical analyses were based on univariate tests and multivariate linear regression.

Results: Univariate statistical analysis showed no significant imbalances among study and control groups in terms of main characteristics measured at baseline, except for the psychological treatment (study group, N=6; control group, N=12; *P*=.05). At the end of the study, the following characteristics were found to be associated with bells test A: OMT, gender, age, medications. None of the measured variables were associated to the bells test R. After adjusting for all potential confounders, multivariate regression showed an association

between OMT and changes in bells test A [1.256; 95% CI, 0.277-2.235; $P<.01$] but not in bells test R.

Conclusion: According to the bells test, this study showed that OMT may improve the score of the test in children affected by ADHD. However, further studies are needed based on a stronger study design and a broader population.

C6

Bipolar Disorder: Emotions, Attitudes, Perceptions, Self-Concept, and the Parenting Role

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Background: Bipolar disorders exact substantial personal, financial, psychological, and societal costs including an increased mortality rate. Patients with bipolar disorders are prone to recurrences of problematic symptoms showing high rates of relapse and distress.

Objective: This current project expanded the investigation of the costs of bipolar disorder to the realm of parenting.

Hypothesis: It was hypothesized that parents diagnosed with bipolar disorder would show diminished self-concept, problematic attitudes, perceptions, behaviors, and emotions related to the parenting role.

Methods: The participants in this study were men and women with a diagnosis of bipolar disorder parenting at least 1 child and a control group of parents without any psychiatric diagnosis. A structured interview was conducted to assess 5 domains related to behaviors, attitudes, perceptions, and emotions associated with the parenting role: parental guilt/fears, perception of children problems, parental anger, overall parenting problems, and negative/pessimistic commentary concerning parenting. The Tennessee Self-Concept Scale-2 (TSCS2) was used to assess self-concept. The Midwestern University institutional review board approved the research.

Results: Parents with bipolar disorder showed lower family self-concept (t=6.77, $P<.001$), physical self-concept (t=4.68, $P<.001$), moral/ethical self-concept (t=3.31, $P<.003$), personal self-concept (t=5.70, $P<.001$), academic/work self-concept (t=4.18, $P<.001$), identity (t=5.85), and behavioral self-concept (t=4.85, $P<.001$), as well as greater parental guilt (t=4.19, $P<.001$), perception of children problems (t=4.56, $P<.001$), parental anger (t=4.98, $P<.001$), overall parenting problems (t=7.59, $P<.001$), and negative/pessimistic commentary (t=5.94, $P<.001$).

Conclusion: Parents with bipolar disorder showed significantly diminished self-concept and significantly greater guilt, anger, perception of problems in their children, inadequacy as a parent, and negative outlook compared with parents in the control group. Results suggest a need for parenting support services and programs to promote a healthy sense of self-concept among persons with bipolar disorder.

C7

Effectiveness and Safety of Lidocaine 5% Patch Combined With Gabapentin in Patients With Postherpetic Neuralgia, Diabetic Neuropathy, or Low Back Pain: Comparison of Patients With and Without Allodynia

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Hypothesis: The presence or absence of allodynia has an influence on the effectiveness and/or safety of add-on topical treatment with lidocaine 5% patch in patients with neuropathic pain caused by postherpetic neuralgia, diabetic neuropathy, or low back pain.

Methods: This was an open-label, nonrandomized, multicenter trial that took place at 9 centers in the United States with approval by the institutional review board of each center. Adult patients with postherpetic neuralgia (PHN), diabetic neuropathy (DN), and low back pain (LBP) who had a partial response to a gabapentin-containing regimen (pain rating >4 where 0=no pain, 10=most severe pain) were included. Patients received once-daily application of up to 4 lidocaine 5% patches (10 cm by 14 cm; Lidoderm, Endo Pharmaceuticals, Chadds Ford, Pennsylvania) to the area of maximum peripheral pain for 14 days. Patients maintained their gabapentin regimen with no adjustment or additions permitted. This post hoc analysis considered change from baseline to day 14 in Brief Pain Inventory [BPI] scores (0=no pain, 10=most severe pain) for worst pain, least pain, and average pain.

Results: Allodynia was present in 57 of 107 enrolled patients (53.3%), including all 11 patients (100%) with PHN, 27 of 49 (55.1%) with DN, and 19 of 47 (40.4%) with LBP. The baseline mean pain ratings were 6.7 (PHN), 3.5 (DN), and 2.7 (LBP). Patients with allodynia had greater change from baseline in BPI worst (2.0), average (1.5), and least (1.7) pain scores compared with those without allodynia (worst pain, 1.1; average pain, 0.9; least pain, 0.8). Patients with LBP and allodynia had greater change from baseline in BPI worst (1.5), average (2.4), and least (2.0) pain scores compared with LBP patients without allodynia (worst pain, 0.6; average pain, 1.0; least pain, 0.8). Overall, 29% of patients reported at least 1 treatment-emergent adverse event; the most frequent was dermatitis (PHN, 0%; DN, 2%; LBP, 4.3%). Patients with PHN had a total of 4 adverse events in 4 diverse categories.

Conclusion: Addition of topical lidocaine 5% patch to a gabapentin regimen reduced pain intensity in patients with PHN, DN, and LBP. Patients with allodynia obtained relatively greater pain relief than those without allodynia.

Funding: This study was funded by Endo Pharmaceuticals in Chadds Ford, Pennsylvania.

C8

Open-Label, Open-Ended Study of the Safety of Diclofenac Topical Solution for Management of Osteoarthritis in Patients ≥65 Years of Age: Characterization of Gastrointestinal Adverse Events

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Hypothesis: Oral nonsteroidal anti-inflammatory drugs (NSAIDs) are effective for the management of osteoarthritis (OA), but their chronic use is associated with serious gastrointestinal (GI), cardiovascular, and potentially other systemic adverse events, particularly in older individuals. Diclofenac topical solution (TDiclo) has demonstrated efficacy similar to oral diclofenac (ODiclo) in randomized, double-blind, clinical trials in OA of the knee, with fewer GI adverse events. The incidence of GI adverse events occurring with TDiclo in patients aged 65 years or older in an office setting was analyzed using safety data from a compassionate-use treatment program of TDiclo for OA. The incidence of GI adverse events occuring with TDiclo in an office-based practice setting was expected to be lower than in clinical trials.

Methods: In this multicenter, open-label, open-ended, compassionate-use, phase 3 study, patients with physician-diagnosed OA were instructed to apply 5 drops (small joint, eg, knuckle), 20 drops (medium joint, eg, wrist), or 40 drops (large joint, eg, knee) of TDiclo to the affected joint 4 times daily in an uncontrolled, real-world setting. Follow-up safety assessments were scheduled at 1, 3, 6, and 12 months, and yearly thereafter. At each follow-up visit, patients were asked open-ended questions regarding the onset and nature of any adverse event that occurred since the last visit. It was at the patient's discretion whether to contact the investigator at the onset of an adverse event or wait until the next visit.

Results: A total of 1133 patients were aged 65 years or older at baseline. The duration of exposure to TDiclo extended over 6 to 12 months in 12.7% and longer than 12 months in 18.7%. Gastrointestinal adverse events occurred in 24 patients (2.1%); the most frequently reported were dyspepsia (0.8%), abdominal pain (0.5%), and nausea (0.3%). A GI adverse event was listed as reason for discontinuation in 13 patients (1.1%). None of the GI adverse events that occurred during the study was deemed by the investigator to be related to TDiclo treatment; however, specific reasons for adverse events were not recorded.

Conclusion: In an uncontrolled office-practice setting, the occurrence of GI adverse events in patients aged 65 years receiving TDiclo was low. No individual GI adverse events was reported in more than 1% of patients, and few patients discontinued therapy due to these events. The results of this open-label study demonstrate that TDiclo is an option for patients aged 65 years or older who wish to reduce their likelihood of experiencing GI adverse events commonly associated with oral NSAIDs.

C10

Influence of Ezetimibe Monotherapy on Ischemia-Modified Albumin Levels in Hypercholesterolemic Patients

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Background: Ezetimibe is a selective inhibitor of intestinal cholesterol transport, thereby reducing circulating concentrations of low-density lipoprotein cholesterol (LDL-C).

Objectives: To investigate the influence of ezetimibe treatment in patients with hypercholesterolemia on circulating levels of IMA, in addition to other atherosclerotic risk factors used in daily practice.

Methods: The study included 31 hypercholesterolemic patients with serum LDL-C concentrations of 3.64 mmol/L (male/female ratio, 13/18; mean [SD] age, 65.7 [5.6] years), who received 10 mg/d ezetimibe during a 12-week treatment period. Serum IMA was measured by the decrease in cobalt 2+ binding. Paired *t* test was used to compare the pre- and posttreatment levels of respective markers. Simple and multiple linear regression analysis, controlled for age, gender, smoking and all measured markers, were used to observe the correlations between changes in the respective markers' levels (post- minus pre-data).

Results: During the treatment period, the levels of IMA (0.57 ± 0.52 vs 0.55 ± 0.62 AU, P=.036) and LDL-C (3.98 ± 0.58 vs3.18 ± 0.56, P<.0001) were significantly reduced. Simple regression analysis revealed that IMA levels were not significantly correlated with those of the other atherosclerotic risk markers: Multiple regression analysis failed to show significant correlations between IMA levels and those of the other markers: β-coefficient (*P* value), BMI, 0.152 (0.484); MBP, 0.213 (0.299); LDL-C, -0.062 (0.786); HDL-C, -0.178 (0.449); TG, -0.219 (0.332); glucose, -0.039 (0.877).

Conclusion: The new finding of the present study is a significant reduction of IMA levels during a period of ezetimibe treatment on hypercholesterolemic patients. While the present study appears to provide insight into daily lipid management in hypercholesterolemic patients, more studies are necessary to confirm the clinical relevance of the present findings and to verify the mechanisms underlying the relationship.

C11

Factors Affecting Acceptability of Titrated Oxymorphone Extended Release in Chronic Low Back Pain: Diabetes, Hypertension, and Cardiovascular Disease

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Hypothesis: Similar to advanced age (>65 years), the presence of comorbid hypertension, diabetes, or cardiovascular disease may reduce the likelihood of successful titration of patients with chronic low back pain (cLBP) to a well-tolerated and effective dose of oxymorphone extended release (ER).

Methods: This was a post hoc subanalysis of 2 multicenter, randomized-withdrawal trials that took place at multiple multidisciplinary pain centers in the United States. Opioid-naive and opioid-experienced adult patients with CLBP, including subpopulations with comorbid hypertension, diabetes, or cardiovascular disease, were included. Open-label oxymorphone ER was initiated at 10 mg/d (5 mg every 12 hours) in opioid-naive and opioid-experienced patients at a dose equianalgesic to their previous opioid plus 5 mg oxymorphone immediate release rescue medication every 4 to 6 hours, as needed. The protocol did not specify adjustments to titration for comorbid conditions, sex, or age, but investigators could exercise discretion for each individual. Successful titration to oxymorphone ER was defined as establishment of a stable, tolerable dosage that reduced pain to less than 40 mm on a 100-mm Visual Analog Scale within 1 month.

Results: Titration was completed by similar proportions of patients in the total pooled population (348 of 575 [60.5%]) and patients with comorbid hypertension (114 of 204 [55.9%]) or diabetes (37 of 65 [56.9%]); however, titration was successful in fewer patients with cardiovascular disease (39 of 80 [48.8%]). Previously published analyses showed titration was completed by similar proportions of men (63%) and women (59%), and opioid-naive (63%) and opioid-experienced (57%) patients, but by fewer patients aged older than 65 years (45%). Incidence of more than 1 adverse event was similar in patients with and without hypertension, diabetes, and cardiovascular disease. Treatment-related adverse events included nausea, constipation, somnolence, headache, and diarrhea.

Conclusion: Titration of oxymorphone ER using a flexible dosing schedule was successful in patients with hypertension or diabetes at a rate similar to the population without these comorbidities; however, patients with cardiovascular disease had a lower rate of successful titration, similar to patients older than 65 years.

Funding: This research was supported by Endo Pharmaceuticals, Chadds Ford, Pennsylvania.

C12

Influence of Physical Activity Intervention on Circulating Soluble Receptor for Advanced Glycation End Products in Elderly Subjects

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Background: Inflammation caused by advanced glycation end products (AGEs) may be quenched by the soluble receptor for AGEs (sRAGE).

Hypothesis: Physical activity produces changes on circulating sRAGE and paraoxonase 1 (PON1). We aimed at exploring the putative association between changes of circulating sRAGE and PON1 activity (as an antioxidative enzyme) in a physical activity intervention study on an elderly subject cohort.

Methods: Serum sRAGE, PON1 activity and cardiometabolic variables were measured in 30 community-dwelling healthy Japanese volunteers (15 men, 15 women; mean age, 65 years) in the pre- and post-phase of a 6-month interventional program for physical activity increase.

Results: The levels of body mass index and sRAGE (1103 ± 496 to 1030 ± 437 ng/L, $P<.05$) were reduced during the intervention period. In addition, the change of sRAGE was significantly and inversely correlated with that of PON1 activity, independent of the other cardiometabolic variables (β=-0.511, $P<.01$).

Conclusion: The present study showed a reduction of sRAGE levels, and an inverse correlation between sRAGE and PON1 activity, after a 6 month intervention study increasing physical activity on an elderly cohort. These findings may represent an adaptive regulation of sRAGE in this type of exercise intervention, future studies are warranted on the clinical relevance of sRAGE changes in physical activity.

C13

Serum Paraoxonase Esterase and Lactonase Activities Correlate With Intermediate Size HDL Particles

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Background: Paraoxonase 1 (PON1) is a promiscuous esterase carried by high-density lipoprotein (HDL), which protects low-density lipoprotein (LDL) from oxidation and decreases homocysteine-thiolactone damage via its lactonase activity, which is considered the physiologic, cardioprotective function of PON1. Different HDL subclasses have been linked to different degrees of cardioprotection. Little is known about the distribution of PON1 across HDL subclasses.

Hypothesis: We tested the hypothesis that there might be differential distribution of PON1 activity across different HDL subclasses.

Methods: In this cross-sectional study we sequentially enrolled 30 healthy subjects (14 males, 16 females) with HDL-cholesterol ranging from 20-110 mg/dL. Paraoxonase 1 activity was explored with 4 substrates. Paraoxonase 1 triesterase activity was determined using paraoxon as a substrate. Activity of PON1 lactonase was measured with 5 (thiobutyl)butyrolactone (TBBL) as well as dihydrocoumarin. Activity of PON1 mono-esterase was measured using phenylacetate as a substrate. Subfractions of HDL were analyzed in non-denaturing tube gels using the Lipoprint HDL system. Nine classes of HDL sizes can be quantified using this procedure.

Results: HDL subclasses were stratified as high size (HDLa-c, Rf 0-0.15, HDL 2b), intermediate size (HDLd-f, Rf 0.20-0.29, HDL 2a-HDL3a), and low size (HDLg-1, Rf 0.38-0.53, HDL3b-c). All PON1 activities correlated significantly and positively with intermediate size HDL concentrations (both relative and in concentration of cholesterol): lactonase r= 0.72, $P<.0005$; triesterase r= 0.69, $P<.001$; arylesterase r= 0.70, $P<.001$. No correlation was found with high- or low-sized HDL subclasses.

Conclusion: We show that PON1 lactonase and other activities strongly correlate only with a discrete size distribution of HDL, namely particles with intermediate sizes compatible with HDL3 and small HDL2 for the most part. Our data suggest that specific HDL particles are responsible for most PON1 activity and pave the way for future studies on the isolated particles themselves as well as to research on the inter-phase of HDL subclasses and PON1 activity to make better predictions for human disease.

C14

Bioavailability of Crush-Resistant Oxymorphone Extended-Release 40 mg With Ethanol Under Fasted Conditions

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Hypothesis: The bioavailability of a 40 mg tablet of a new formulation of oxymorphone extended release designed to be crush resistant (Oxy-CRF) does not significantly differ when coingested with 240 mL of water, 20% ethanol, or 40% ethanol under fasted conditions.

Methods: This open-label crossover study enrolled healthy adults who were randomly assigned to receive single oral doses of Oxy-CRF 40 mg coadministered with 240 mL of 20% ethanol, 40% ethanol, and water in 3 periods separated by 7 days. Naltrexone was administered to minimize opioid effects. Blood samples for pharmacokinetics were obtained through 48 hours postdose. Institutional review board approval and subject informed consent were obtained.

Results: Of the 20 subjects enrolled, pharmacokinetic data were analyzed from 17. Oxymorphone peak plasma concentration (Cmax) increased 14% and 80% with 20% ethanol and 40% ethanol, respectively, compared with water. Oxymorphone area under the curve (AUC) was relatively unaffected by ethanol, decreasing 5% and increasing 15% with 20% ethanol and 40% ethanol, respectively. Median time to Cmax occurred earlier (2 hours) with 40% ethanol compared with 20% ethanol (5 hours) or water (5 hours); elimination half-life was unaffected.

Conclusion: Interactions between ethanol and Oxy-CRF 40 mg resulted in ethanol dose-related increases in oxymorphone Cmax with AUC relatively unaffected. Oxy-CRF did not exhibit dose-dumping with ethanol under fasted conditions.

Funding: This study was funded by Endo Pharmaceuticals, Chadds Ford, Pennsylvania.

C15

Bioequivalence of Crush-Resistant Oxymorphone Extended Release 40 mg and Oxymorphone Extended Release 40 mg Under Fasted Conditions

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Hypothesis: The pharmacokinetics of 40 mg single dose tablets of oxymorphone extended release (Oxy-ER) and a new oxymorphone extended release tablet formulation designed to be crush-resistant (Oxy-CRF) are expected to be bioequivalent under fasted conditions.

Methods: In an open-label, randomized, 2-sequence, 4-period, replicated-dosing study, healthy adults received 2 single doses each of Oxy-CRF 40 mg and Oxy-ER 40 mg under fasted conditions with each treatment separated by 7 days. Naltrexone was administered to minimize opioid effects. Blood samples for pharmacokinetics were obtained through 48 hours post-dose. Institutional review board approval and subject written informed consent were obtained.

Results: Of 34 enrolled subjects, pharmacokinetic data were analyzed from 30 who fully completed and 1 who partially completed the study. Similar mean (SD) oxymorphone peak plasma concentrations (Cmax), 2.4 (0.9) and 2.4 (1.2) ng/mL, and areas under the curve (AUC), 32.6 (10.9) and 33.0 (11.6) ng•h/mL, were observed for Oxy-CRF and Oxy-ER, respectively. The 90% confidence intervals for Cmax (98%-112%) and AUC (95%-104%) met bioequivalence criteria (ie, within 80%-125%).

Conclusion: Oxy-CRF 40 mg is bioequivalent to Oxy-ER 40 mg under fasted conditions.

Funding: This study was funded by Endo Pharmaceuticals, Chadds Ford, Pennsylvania.

♦C16

Increase in Paraoxonase I Activity After Hemodialysis Is Not Caused by Changes in High-Density Lipoprotein Subclasses

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Background: It has been previously documented that the activity of paraoxonase 1 (PON1) decreases in renal failure patients. However, PON1, activity has been shown by our laboratory to increase with hemodialysis (HD). The mechanism for this PON1 rescue phenomenon is presently unknown. Could it be due to changes in the high-density lipoprotein (HDL) subclasses? The research attempted to answer this question.

Hypothesis: We tested the hypothesis that PON1 activity increases after hemodialysis because of changes in HDL subclasses.

Methods: High-density lipoprotein samples from a random sampling of HD patients from the Department of Internal Medicine and Laboratory Medicine at Showa University, Yokohama, Japan, were first tested for PON1 enzyme activity (n=35). PON1 activity was measured kinetically by arylesterase assay on both pre- and posthemodialysis samples. Specimens were then subjected to HDL subclass analysis using a Lipoprint LDL and HDL Subfractionation System.

Results: This research confirms previous work demonstrating that PON1 activity increases after HD. In this case, a 45.1% increase in post-HD PON1 activity (55.9 ± 22.5 vs 81.1 ± 27.4 U/L, *P*=.000000027) was observed. Subsequent Lipoprint analysis yielded the following results: large-HDL profile (48.1 ± 13.3 % vs 44.4 ± 19, *P*=.36), intermediate- (41.9 ± 6.8 % vs 41.9 ± 9.9, *P*=.37), and small-HDL profile increased by 19.4% (11.4 ± 6.9 vs 13.6 ± 11, *P*=.37). These results suggest that the HDL subfractions remain relatively unchanged through the course of HD therapy, although a trend for a shift towards smaller particles after dialysis became apparent.

Conclusion: Our work provides new insights into the rescue of HDL/PON1 activity produced by hemodialysis. The findings suggest that this increase in activity is unrelated to changes in the HDL subclass distribution. Instead this could be the consequence of the removal of metabolic waste products that would otherwise inhibit PON1 activity.

♦C17

Lymphomatoid Papulosis and Pityriasis Lichenoides et Varioliformis Acuta Overlap: An Enigma

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Controversy exists as to whether lymphomatoid papulosis (LyP) is a version of pityriasis lichenoides et varioliformis acuta (PLEVA) or a variant of the malignant lymphomas. Both LyP and PLEVA present similarly clinically and are benign papular cutaneous eruptions that heal spontaneously. Recent studies show that these 2 disorders can be delineated immunohistochemically and are indeed 2 distinct disorders, although Rogers suggests that the similar clonal T-cell receptor gene rearrangements may argue that there is an interrelationship between these 2 entities. LyP is part of a spectrum of CD30 (Ki-1)-positive cutaneous lymphoproliferative diseases that histologically suggests malignancy. This disease tends to be chronic and benign, although patients with LyP have a 5% to 20% risk of associated malignant lymphoma at some point in their life. PLEVA, on the other hand, has characteristically been reported to have no risk of malignancy and rarely or never expresses the CD30-antigen. However, there have been recent reports of the development of large plaque parapsoriasis in patients with pityriasis lichenoides et varioliformis acuta that have led to reconsideration of its malignancy rate. A case of a 42-year-old woman found to have histologic findings of both LyP and PLEVA is reported.

C18

Impact of Oral Glucose Tolerance Test on Hepatic Glycogen and de Novo Lipogenesis

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Background: After an oral glucose tolerance test (OGTT), most of the glucose is found in the blood circulation but some can be taken up by the liver to be either stored as glycogen or converted to lipids.

Objectives: The first aim of this study is to assess the contribution of the overall glucose flux stored as hepatic glycogen after a 75 g glucose load (OGTT). The second aim of the study is to determine whether some of the OGTT glucose is converted to lipids through hepatic de novo lipogenesis (DNL). We hypothesized that a small fraction of the OGTT load remains in the liver as hepatic glycogen and a smaller part is converted to fat to be exported by the liver as very-low-den-

♦ 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.

sity lipoprotein triglycerides (VLDL-TG).

Methods: A healthy adult male volunteer was admitted to San Francisco General Hospital. The subject underwent tracer studies to measure hepatic uridine diphosphate (UDP)-glucose flux and hepatic DNL. D-glactose-1d was infused to label the hepatic UDP-glucose pool, which in turn was sampled by acetaminophen, used as a "pharmacologic probe." Acetaminophen was conjugated with UDP-glucose in the liver to form acetaminophen glucuronide (GlcUA), which was subsequently excreted in the urine. Urine and blood samples were collected regularly for 5 hours following the OGTT. Labeled urinary GlcUA was isolated by HPLC and derivatized for GC/MS analysis. U-13C-glucose was added to the OGTT load to estimate glucose production. The tracer dilution method was used to calculate the flux of UDP-glucose (the amount of glucose stored as glycogen over time) and suppression of glucose production. TG-rich lipoproteins were isolated from plasma samples by ultracentrifugation and processed for GC/MS analysis to measure DNL.

Results: The hepatic glycogen flux was highest during the initial 30 minutes following the OGTT load (4-5 g of glucose stored as glycogen) and was lowest between 90 and 120 minutes (2 g of glucose). Glucose production suppression peaked between 90 and 120 minutes after the OGTT load. Some of the glucose from the OGTT was converted to lipids by DNL representing 6.54 % of the TG exported as hepatic VLDL-TG.

Conclusion: As hypothesized, a small portion of the OGTT load remains in the liver as hepatic glycogen. About 15% of the glucose load is stored as hepatic glycogen. After an OGTT, a smaller portion of glucose is converted to lipids. Mathematical modeling will allow us to further evaluate the significance of these metabolic pathways in terms of total hepatic glucose disposal after an OGTT.

C19

Diclofenac Sodium 1% Gel in Patients With Hand Osteoarthritis: Effectiveness in Patients With First Carpometacarpal Joint Involvement

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Hypothesis: The safety and efficacy of diclofenac sodium 1% gel (DSG) for treatment of osteoarthritis (OA) of the hand is similar for the first carpometacarpal (CMC-1) joint and the intraphalangeal (IP) joints.

Methods: Patients (aged ≥40 y) with radiographically verified hand OA applied DSG or vehicle gel, 2 g to each hand, 4 times daily for 8 weeks. Outcomes assessed at 6 weeks included OA pain intensity (100-mm Visual Analog Scale [VAS]) and Australian/Canadian Osteoarthritis Hand Index (AUSCAN) subscales for pain, stiffness, and function. Efficacy was compared in patients with OA in CMC-1 only, OA in CMC-1 plus ≥1 IP joint (CMC-1 plus), and only IP involvement (no CMC-1). No statistical analysis was performed.

Results: Of 384 patients, 241 had CMC-1 plus, 110 had no CMC-1, and 34 had CMC-1 only. Percent reduction of OA pain intensity from baseline with DSG was superior to vehicle in all subgroups for VAS pain (CMC-plus [DSG 49.2%; vehicle 37.1%], CMC-1 only [DSG 41.6%; vehicle 34.0%], no CMC-1 [DSG 40.3%; vehicle 34.6%]) and AUSCAN pain (CMC-plus [DSG 43.3%; vehicle 30.5%], CMC-1 only (DSG 30.1%; vehicle 27.7%), no CMC-1 [DSG 34.1%; vehicle 29.5%]). AUSCAN stiffness and function improved from baseline more with DSG than with vehicle in patients with CMC-plus (stiffness, DSG 41.8%; vehicle 27.3%; function, DSG 41.3%; vehicle 27.7%) or no CMC-1 (stiffness, DSG 34.5%; vehicle 22.5%; function, DSG 35.3%; vehicle 24.0%). For CMC-1 only, improvements from baseline in AUSCAN stiffness (DSG 23.8%; vehicle 21.4%) and function (DSG 25.3%; vehicle 25.4%) were similar between DSG and vehicle.

Conclusion: DSG was superior to vehicle in reducing pain in all finger joints but was superior to vehicle in improving function and stiffness only in patients with OA of the IP joints. Because neither DSG nor vehicle have disease-modifying mechanisms, pain relief alone is likely responsible for the observed improvement of function and stiffness. Function and stiffness may be less amenable to improvement by pain relief in CMC-1 than in IP joints.

Funding: This study was funded by Endo Pharmaceuticals, Chadds Ford, Pennsylvania.

C20

Safety of Oxymorphone Extended Release for Chronic Low Back Pain in Patients With Diabetes, Hypertension, or Cardiovascular Disease

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Hypothesis: Use of oxymorphone extended release for chronic low back pain has a safety profile in patients with hypertension, diabetes, or cardiovascular disease that is similar to its safety profile in patients without these comorbidities.

Methods: This was a post hoc subanalysis of 2 multicenter, enriched enrollment, randomized-withdrawal trials conducted at multiple US multidisciplinary pain centers. Opioid-naive and opioid-experienced adult patients with chronic low back pain (cLBP), including subpopulations with comorbid hypertension, diabetes, or cardiovascular disease, were included. Open-label oxymorphone ER was initiated at 10 mg/d (5 mg every 12 hours) in opioid-naive and opioid-experienced

patients at a dose equianalgesic to their previous opioid plus 5 mg oxymorphone immediate release rescue medication every 4 to 6 hours, as needed. Oxymorphone ER was titrated to a dose that was tolerated and effectively reduced pain to 40 mm or less on a 100-mm Visual Analog Scale (VAS). Safety was assessed from reports of adverse events (AEs); efficacy was assessed by pain intensity on the VAS.

Results: During up to 4 weeks of open-label titration of oxymorphone ER, 348 of 575 patients achieved a tolerated, effective dose (median, 40 mg); incidence of 1 or more AEs was similar in patients with and without hypertension (69% vs 69%), diabetes (74% vs 69%), and cardiovascular disease (74% vs 68%). During 12 weeks of double-blind treatment, incidence of ⩾1 AE was less similar but not significantly different in patients with and without hypertension (58% vs 49%, *P*=.27), diabetes (56% vs 52%, *P*=.82), and cardiovascular disease (73% vs 50%, *P*=.07). Treatment-related AEs included nausea, constipation, somnolence, headache, and diarrhea. Increases in VAS pain intensity from baseline to final visit were observed for placebo patients with and without hypertension (27.9 vs 28.4), diabetes (26.6 vs 28.4), and cardiovascular disease (30.3 vs 28.0). With oxymorphone ER, a smaller increase in VAS was observed for patients with and without hypertension (9.8 vs 9.6), diabetes (13.3 vs 9.2), and cardiovascular disease (13.0 vs 9.2).

Conclusion: The safety profile of oxymorphone ER for cLBP in patients with comorbid hypertension, diabetes, or cardiovascular disease was similar to that in patients without these comorbidities during up to 4 weeks of titration followed by 12 weeks of treatment.

Funding: Supported by Endo Pharmaceuticals Inc, Chadds Ford, Pennsylvania.

Basic Sciences

♦B1

Cerium Oxide Nanoparticles Protect Against MPTP-Induced Dopaminergic Neurodegeneration in a Mouse Model for Pakinson Disease

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Cerium oxide nanoparticles (CeONP) are regenerative free radical scavengers that protect against oxidative stress. CeONP extend cell and organism longevity, protect against oxidative stress, and are effective in tissue culture models of neurodegenerative disorders. Given that Parkinson disease is strongly associated with oxidative stress, we hypothesized that CeONP may be a promising disease-modifying therapy for treatment of Parkinson disease. For these studies, 2-month-old male mice were treated with CeONP via 3 tail vein injections for a total dose of 0.05, 0.5, 5.0, and 50 µg/g. Five days after the CeONP injections, mice received 4 IP injections of 1-methyl-4-phenyl-1,2,3,6-tetrahyropyridine (MPTP, 20 mg/kg) every 2 hours for 1 day, to induce Parkinson disease. All mice were killed 7 days after the last MPTP injection. Striata were removed from half the mice, for measurement of dopamine and DOPAC via HPLC. In the second half, mice were transcardially perfused and fixed. Brains were removed, stereotaxically sectioned into 50 µm slices, and stained for tyrosine hydroxylase (TH) with Nissl counterstain. Five sections through the substantianigra (SN) were counted for TH+, Nissl+ staining. CeONP at the 0.5 and 5 µg/g dose increased striatal dopamine content by 29% and 45% respectively. MPTP induced an 89% decline in striatal dopamine, which was inhibited by CeONP over the dose range of 0.05 to 5 µg/g, with 0.5 µg/g being the most effective. At this dose, striatal dopamine in MPTP-treated animals matched that of controls not receiving MPTP. Similar results were seen in DOPAC. In the substantia nigra (SN), CeONP increased the numbers of TH+ neurons at the 0.05 and 0.5 µg/g doses, as compared to untreated controls. MPTP induced a 52% decline in TH+ neurons in the SN. CeONP-treated animals maintained numbers of TH+ neurons similar to animals not exposed to MPTP. Importantly, the doses of CeONP at which neuroprotection was observed were low, resulting an approximate doubling in brain cerium concentration. We currently lack effective treatment and prevention for Parkinson disease and other neurodegenerative disorders associated with oxidative stress. Here, we demonstrate for the first time that very low doses of CeONP protect against Parkinson disease in a mammalian model. These results strongly support a role for CeONP as a nanopharmaceutical that may halt or slow the progression of Parkinson disease, and possibly other neurodegenerative disorders.

♦B2

Investigation on the Effects of Acute Maternal Ethanol Exposure on c-fos Gene Expression in the Hippocampus of Murine Offspring

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Background: Fetal alcohol syndrome (FAS) is a condition that describes an abnormal pattern of developmental changes due to maternal ingestion of alcohol during gestation. This syndrome leads to various neurological abnormalities. The hippocampus is one of the primary brain regions affected by prenatal exposure to alcohol. The expression of c-fos has been studied and shown to be suppressed in the hippocampus

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with maternal alcohol exposure, mediating the neurological abnormalities observed with FAS.

Hypothesis: Our hypothesis states that acute prenatal ethanol exposures have measurable affects on fetal hippocampus development and c-fos gene expression.

Objectives: To investigate the ethanol effects on the histological changes of the hippocampus in maternally exposed offspring via in situ hybridization to analyze the expression of c-fos having a characteristically defined role in the hippocampus and qRT-PCR to quantify the change of hippocampal c-fos gene expression.

Methods: In the control treatment group, pregnant females were not injected with ethanol during gestation. In the acute treatment group, pregnant females were injected with 0.175 mL of 25% ethanol/g body weight at 10 days, 6 hours, and 10 hours of gestation (E10.25 and E10.42). Embryos and offspring adult brains were then isolated from each treatment group at 18 days of gestation (E18.0) and 8 weeks after birth, respectively, for analysis via in situ hybridization and qRT-PCR.

Results: The acute treatment group revealed a significant ($P<.05$) decrease in c-fos expression post maternal ethanol exposure in 18-day fetal brains, whereas a significant ($P<.05$) increase in c-fos expression post maternal ethanol exposure was observed in 8-week adult brains.

Conclusion: The change of c-fos expression from normal basal levels may play a considerable role in the neurological defects in humans with FAS. Thus, continued research will hopefully provide an increased understanding of teratogenic effects of ethanol in the development of the fetal brain and its correlation with the symptoms exhibited by humans with FAS.

♦B3

The Morphology and Distribution of Somatostatinergic Neuronal Elements in the Human Hypothalamus

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Somatostatin is a 14-28 amino acid peptide that is located in the gastrointestinal system and in multiple sites of the human brain. The inhibitory effect of somatostatin on growth hormone secretion of the pituitary gland is a well-known phenomenon; however, the precise distribution and morphology of the somatostatinergic elements in the hypothalamus has not been entirely elucidated. In the present study, we used single-label immunohistochemistry to reveal the somatostatinergic elements in the human diencephalon. The majority of the observed somatostatin-immunoreactive (IR) perikarya were located in the infundibulum/median eminence (arcuate nucleus) and in the periventricular area of the preoptic and infundibular regions. Numerous somatostatin-IR neurons were found in the suprachiasmatic and ventromedial nuclei and in the nucleus of the diagonal band of Broca. Cell bodies were also present around the mammillary nuclei and in the supramammillary nucleus. Few somatostatin-IR perikarya surrounded the fornix in the tuberal region. The paraventricular nuclei also contained several perikarya, while only negligible amount of somatostatinergic elements were detected in the supraoptic nucleus. A number of cell bodies were seen in the lateral hypothalamus, particularly at the infundibular and posterior hypothalamic regions. Few perikarya along with fiber varicosities were seen in the lamina terminalis. Somatostatin-IR axonal varicosities were abundant in the infundibulum and periventricular area of the preoptic and infundibular regions, while the medial hypothalamic regions contained only few somatostatin-IR fibers. The widespread distribution of the somatostatinergic elements in the human hypothalamus suggests that somatostatin, apart from its endocrine function to regulate growth hormone secretion, may play a pivotal role in the regulation of other hypothalamic functions as well as a neurotransmitter/neuromodulator. Further studies are required to reveal the associations of the somatostatinergic neuronal elements with other hypothalamic neurotransmitter systems in human.

♦B4

Intimate Associations Between the Endogenous Opiate Systemes and the Growth Hormone-Releasing Hormone (GHRH) System in the Human Hypothalamus

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Intrauterine growth restriction (IUGR) represents a frequent attribute of pregnancies of opioid-dependent mothers. Although it is a general consensus that opioids suppress growth, the mechanism of this phenomenon is largely unknown. Since endogenous opiates, such as endorphin or enkephalin, use the same receptor as morphin, these peptides may be one of the key regulators of growth in human by impacting growth hormone (GH) secretion, either directly, or indirectly, via growth hormone-releasing hormone (GHRH) release. We have previously described the morphology and distribution of the enkephalin, endorphin, and GHRH systems in the human hypothalamus. In the present study, we examined the possibility of juxtapositions between the enkephalinergic/endorphinergic axonal varicosities and GHRH-immunoreactive (IR) perikarya. These putative associations may represent the mechanism of the endogenous opioid-influenced GH release. Because of the long post mortem period and consequent lack of optimal preservation of the

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cell membranes in the brain, electron microscopy could not be utilized to detect the presence of synapses between the enkephalin/endorphin and GHRH neurons. Therefore, we used light microscopic double label immunocytochemistry combined with high magnification microscopy with oil immersion to identify putative juxtapositions between these systems. Our findings revealed that the majority of the GHRH-IR perikarya formed intimate associations with enkephalinergic axonal varicosities in the infundibular nucleus/median eminence. In contrast to these findings, no significant endorphinergic-GHRH associations were detected. The density of the abutting enkephalinergic fibers on the surface of the GHRH perikarya suggest that these juxtapositions may be functional synapses and may represent the morphological substrate of the impact of enkephalin on growth. The lack of apparent innervation of the GHRH neurons by the endorphin system indicates significant differences between the regulatory roles of endogenous opiates on growth in humans.

♦B5

Substance P-Immunoreactive Axon Varicosities Appear to Innervate GHRH-IR Neurons in the Human Hypothalamus

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Substance P (SP) is an undecapeptide with multiple effects on the gastrointestinal, cardiovascular, and urinary systems as well as complex central nervous system functions such as learning, memory, and sexual homeostasis. Previous studies also revealed that SP exhibits regulatory effect on growth possibly via influencing hypothalamic GHRH release in humans.[1] However, the morphological substrate of this phenomenon has not been elucidated yet. In our previous studies, we described intimate associations between the SP and LHRH neurons that may represent the morphological basis of the effect of SP on gonadal functions. In the present study, we examined the putative presence of such juxtapositions between the SP and GHRH systems. Because of the long postmortem period and consequent lack of optimal preservation of the cell membranes in the brain, electron microscopy could not be utilized to detect the presence of SP-GHRH synapses; therefore, light microscopic double-label immunocytochemistry combined with high magnification microscopy with oil immersion was used to identify putative juxtapositions between these systems. Our studies revealed dense SP fiber network abutting on the surface of the majority of GHRH neurons in the human hypothalamus. The density and the morphology of these intimate associations suggest that SP influences growth by regulating hypothalamic GHRH release by direct synaptic contacts.

Reference

1. Coiro R, Volpi L, Capretti G, et al. Intravenously infused substance P enhances basal and growth hormone (GH) releasing hormone-stimulated GH secretion in normal men. *Peptides.* 1992;13(4):843-846.

♦B7

Semi-automated Method for Measurement of *Escherichia coli* and *Staphylococcus aureus* Growth, Capsule, and Biofilm Formation

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Overview: Determination of bacterial growth rates, biofilm formation, and exopolysaccharide production are labor and time intensive. We tested whether the Bioscreen C (GrowthCurves, Inc) would have utility as a high-throughput tool in the measurement of fundamental phenotype expression.

Hypothesis: Equipment designed to measure bacterial growth rate can be adapted for multipurpose measurement of biofilm formation and exopolysaccharide production.

Methods: Muller-Hinton (MH), yeast nitrogen base with 1% peptone (YNBP) and artificial urine media with strains of *E coli* or *S aureus* (10^5 CFU/mL) were used to fill Honeycomb microwell plates (100 wells/plate with 2 identical plates tested concurrently; 200 µL/well, continuous shaking; n=4 – 8 per test condition). After growth, the plates were washed, dried (37°C), stained with either crystal violet (biofilm; 1% w/v) or Alcian Blue (capsule; 1% w/v) and absorbance measured (600 nm and 405 nm, respectively). Data were directly imported into InStat and Prism (GraphPad) for statistical analysis and graphing, respectively.

Results: In general, over a 48- to 72-hour period (depending on conditions used for drying of the plates), we obtained reproducible, statistically significant data on the affects of growth conditions on generation time, capsule production, and biofilm formation (maximally for 25 different conditions per 24-hour run cycle; n=4). *S aureus* strains (MH) with deletions in sarA and agr grew significantly slower than parent strain (1.6 fold slower) and produced significantly ($P<.05$) less biofilm (~2 fold) than the parent strain but produced similar amounts of capsule. *E coli* growth rate, biofilm, and capsule production in simulated nephropathic urine medium was similar for urine with insulin (20 µU) or amino acid alone as compared to control. Addition of insulin to urine medium with proline increased generation time, capsule, and biofilm production. Although growth in YNBP with amino acids was significantly faster than growth in urine medium, capsule and biofilm production was overall not affected by the addition of insulin.

Conclusion: Post-growth curve staining of microplates from Bioscreen C is a rapid, reproducible, and easily manipulated

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system to concurrently measure bacterial growth, biofilm formation, and capsule production. There is potential for further applications of this system by expanding the types of detector dye used.

♦B8

Quorum-Signaling in *Staphylococcus aureus*: A Role for Menaquinone (Vitamin K_2)

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Hypothesis: Menaquinone (vitamin K_2) functions as a quorum-signaling compound affecting phenotypic expression in *Staphylococcus aureus*.

Overview: Menaquinone (vitamin K_2) is released by *S aureus* during growth. Although it plays a role in electron transport, this does not explain the additional release of menaquinone into the environment. This extracellular menaquinone could function as a chemical communication signal (ie, quorum-signaling compound). To test this, menaquinone's affect on *S aureus* biofilm formation, capsule production, carotenoid production, and adherence to fibronectin was measured.

Methods: Methicillin-sensitive and -resistant *S aureus* (MSSA and MRSA, respectively) laboratory and clinical isolates (n=8) were tested. Biofilm formation, capsule production and adherence to fibronectin were determined by growing the strains (24 hour, 37ºC, static) in various concentrations of menaquinone (0.005-12 μg/mL). After growth, plates were washed, stained and examined spectrophotometrically. Cellular carotenoid levels in methanol/heat extracted samples grown in the presence and absence of vitamin K_2 were determined spectrophotometrically. All experiments were performed in triplicate, repeated at least once and analyzed using InStat.

Results: Menaquinone (0.01 μg/mL) significantly increased ($P<.05$) adherence to plastic in a bacterial population concentration dependent manner ($10^4 > 10^6$ CFU/mL starting concentration) while having no effect on capsule production. In addition, menaquinone (0.05-4 μg/mL) significantly increased ($P<.05$) adherence to fibronectin in 4 MSSA strains and 1 MRSA strain by 2 to 6 fold as compared to media controls, although adherence of *S aureus* mutants lacking agrsarA were not affected. Menaquinone also decreased both rate of growth by 6 to 18 minutes and significantly ($P<.05$) inhibited carotenoid production in a vitamin concentration and strain specific manner.

Conclusion: These findings show that menaquinone plays a role in *S aureus* physiology beyond electron transport and supports the hypothesis that menaquinone may function as a quorum compound.

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♦B9

Differential Binding of FITC-Insulin to *Escherichia coli*

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Hypothesis: Wild type *Escherichia coli* and parent strain will exhibit high levels of FITC-insulin polar fluorescence as compared to methyl-accepting chemotaxis proteins (MCP) null mutants.

Overview: Translocational movement in a chemical gradient by *E coli* correlates with expression of polarly-located MCPs that are known cell-signaling receptors in chemotaxis. Prior findings show that mammalian insulin modulates *E coli* chemotactic movement (chemorepellent). We measured the binding pattern of FITC-insulin to *E coli* that expressed all MCPs and strains lacking all MCPs (null mutants).

Methods: *E coli* strains used were: ATCC 25922 (wild type); RP437 (parent); UU1250 MCP null (Δaer,tar-tap,trs,trg); and UU2612 MCP null (Δtar-tap, tsr, aer, trg). All strains were grown in yeast nitrogen base with 1% peptone to mid-logarithmic growth phase. Cells were then pelleted (3000 rpm; 25 minutes; 5ºC), washed, placed on glass slides, methanol fixed and stained (30 minutes; 37ºC) with fluorescein isothiocyanate (FITC)-insulin (25 μg/mL PBS; Sigma-Aldrich). The cells were then counter-stained with DAPI (1:1000; 30 minutes; 37ºC). Slides were coded and examined by epifluorescence microscopy. Percent of fluorescent cells was determined from photographs of representative fields (n=3). All analyses were done in a blinded fashion.

Results: All bacterial cells regardless of *E coli* genotype exhibited specific FITC-insulin binding. However, the pattern of fluorescence (polar; punctuate, +1 to +3; or diffuse) varied. Wild type *E coli* primarily exhibited diffuse fluorescence (68% population), while the parent strain (RP437) exhibited primarily +2 to +3 punctuate fluorescence (67% of population). The FITC-insulin binding to MCP null mutants was similar to that observed for wild-type and parent strains (ie, UU2612 principally exhibited diffuse fluorescence [66%] and UU1250 exhibiting +3 punctuate fluorescence [44%]). The autofluorescence of unstained cells was similar to the glass background.

Conclusion: Binding of FITC-insulin to *E coli* appears to be unrelated to chemotactic behavior and polar localization of MCP. This indicates that the signaling pathway for insulin modulation of *E coli* chemotactic behavior is distinct from the previously-reported MCP signaling pathways.

♦B10

Molecular Neuroanatomy of Neocortical Layer I Heterotopia: A Novel in Silico Approach

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Background: A diverse number of neocortical malformations are observed in humans and often arise from defective neuronal migration during fetal and early postnatal periods. Whether caused by genetic mutation, injury, or environmental insult, neocortical malformations are a significant risk factor for intellectual delay, life-long cognitive impairment, and epilepsy. Therefore, greater understanding of the anatomic and physiologic changes that characterize neocortical malformations have broad and significant implications for affected individuals. Molecular layer heterotopias (MLH) are small malformations seen in the neocortex of individuals with a number of neurodevelopmental disorders including: cobblestone lissencephaly, dyslexia, Fukuyama muscular dystrophy, and epilepsy. Surprisingly, the cellular constituents and neuronal circuitry of MLH remain poorly understood despite their presence in these diverse conditions with a broad spectrum of clinical presentations. C57BL/6J mice also have MLH with identical cytoarchitecture to that seen in humans, providing a model for greater study of the cellular constituents of these malformations.

Methods: The Allen Brain Atlas was used to search for the cellular constituents of MLH based on cell-type specific gene expression in mice with MLH.

Results: We demonstrate that a diverse group of neuronal cell types are present in MLH including excitatory and inhibitory neurons. In addition, diverse groups of glial cell-types are present in MLH including oligodendrocytes and astrocytes.

Conclusion: MLH in mice serve as a useful model of heterotopia in humans. Our data suggest that a diverse number of cell-types are likely present in human MLH. These data provide clues to the mechanisms of function brain changes in humans with MLH.

♦B11

Glial Cell Line-Derived Neurotrophic Factor Gene Mutations in Parkinson Disease

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Hypothesis: Glial cell line-derived neurotrophic factor (GDNF) is a potent survival factor for nigrostriatal dopaminergic (DA) neurons, which are known to degenerate in Parkinson disease (PD). GDNF was identified independently in our lab as a PD candidate gene through a study of Copy Number Variation in PD. The neuroprotective properties of GDNF have been widely studied as a potential therapeutic tool to treat patients with PD, and altered GDNF expression levels are believed to be involved in the pathophysiology of PD. We hypothesize that the frequency of GDNF mutations will be increased in PD.

Design: We employed a retrospective case-control methodology comparing mutation frequencies in subjects with PD (cases) against normal population frequencies (controls).

Methods: DNA from 95 anonymous subjects with PD was obtained from the Coriell Cell Repositories (NDPT-089). Polymerase chain reaction (PCR) primers were designed using Primer 3 (http://frodo.wi.mit.edu/primer3) to amplify all GDNF coding regions. Following PCR amplification, samples were submitted for standard Sanger sequencing. Sequencing data were analyzed using CodonCode sequence assembly and alignment software. All novel mutations and known polymorphisms were catalogued in an Excel database. Polymorphism frequencies were compared to normal control frequencies in dbSNP (online SNP database at http://www.ncbi.nlm.nih.gov/projects/SNP).

Results: There are polymorphisms that exist in the exonic regions of GDNF that are known to be non-synonymous and may potentially affect GDNF function or expression. We will present the frequency of these polymorphisms and novel mutations in a representative PD cohort.

Conclusion: Further studies in larger cohorts are necessary and planned to better ascertain the relative contribution of these GDNF genetic variations to PD and understand the molecular basis of PD pathogenesis.

♦B12

Nanocarrier-Based Mitochondria-Targeted Delivery of Glutathione

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Encapsulation of reduced glutathione (rGSH) into mitochondrial-targeted liposomes is a strategy aimed at accumulating large amounts of rGSH at the mitochondrial membrane, leading to an increase of intramitochondrial rGSH. In the mitochondrium, rGSH acts to reduce reactive oxygen species (ROS) that are generated during metabolic processes like cellular respiration, as well as during pathologic processes such as chemotherapy, ischemia-reperfusion injury, type 2 diabetes mellitus, and glucose-6-phosphate dehydrogenase deficiency. An excess of ROS may lead to apoptotic cellular death. Therefore, targeting antioxidants like rGSH into

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the mitochondria may alleviate the damage that ROS can cause to the cell and may even prevent apoptosis of the damaged cell. This study addressed the encapsulation, measurement, and characterization of rGSH in 2 types of liposomes; dehydration-rehydration vesicles (DRV), and small unilamellar vesicles (SUV). It was found that rGSH leaks from the liposomal membrane of both DRVs and SUVs and is subsequently oxidized by environmental oxygen at a constant rate (9% loss per 24 hours) over time. Regardless of initial rGSH encapsulation concentration (200 mM, 100 mM, 50 mM) or liposome preparation method (DRV or SUV) rGSH leaked from liposomes at the same rate. Samples of rGSH encapsulated DRVs and SUVs were stored at 4°C in sealed, plastic test tubes. The average (standard deviation) encapsulation efficiency of rGSH was determined to be 67% (2%) for DRVs and 12% (2%) for SUVs. In addition to preparing and charactering liposomal rGSH formulations, an optimal protocol was established for mimicking oxidative stress for cells in vitro. Murine 4T1 cells were exposed to different concentrations of H_2O_2 for different period of times and a gradient of cellular stress was revealed. Two assays were used to determine the cell viability after exposing cells to H_2O_2. The Celltiter Glo assay measures intracellular ATP concentrations by immunofluorescence, the MTS assay measures intramitochondrial reductive enzymes that are used to produce ATP. An optimal concentration of H_2O_2 used to reproduce oxidative stress on cells was determined to be 10 uM for MTS assay and 12 uM for Celltiter Glo assay. Having developed protocols for preparing, measuring, and characterizing liposomal rGSH and for oxidatively stressing cells with H_2O_2, subsequent work will involve testing these liposome formulations for their potential to rescue cells exposed to H_2O_2 from apoptotic cell death.

♦B13

Autoantibodies Are Abundant in Human Sera and Are Useful for Disease Diagnostics

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Our previous studies have shown that brain-reactive antibodies are ubiquitous in the blood, independent of age or the presence or absence of disease. The underlying reason for the presence and abundance of brain-reactive autoantibodies in human sera, especially in younger and otherwise healthy individuals, is not known. We suggest that the presence of active disease causes the production and release of cellular products as a result of cell damage and death related to ongoing pathology, resulting in an immune response that leads to the production and appearance of a relatively large number of self-reactive autoantibodies in the blood. Because each disease involves certain tissue that is specific to that disease, the end result is the generation of a disease-specific autoantibody profile in the blood that is characteristic for each disease and the specific cell types involved, such as neurons in the case of Alzheimer disease (AD). Although some autoantibodies may be vestiges of past diseases and reflect a history of immunologic activity, it is possible that many autoantibodies are also present in the blood as a result of existing or ongoing diseases. It is this latter group that will benefit most from early detection and diagnosis of long-standing, progressive diseases like AD. The purpose of the present study is to get an estimate of the number of autoantibodies that are in the blood at any one time. To test this point, we used human protein microarrays containing 9486 proteins with a total of 23,000 spots probed with human serum from 90 individuals (50 subjects with AD, 40 controls) to identify the individual protein targets of these human brain-reactive autoantibodies. Results indicate that individual sera contain 500 to 1500 different autoantibodies that are detectable using the available protein microarrays. Because these arrays contain roughly one-third of the human proteome, the actual number of autoantibodies translates to 1500 to 4500 per individual serum sample. Although it appears that the presence of this astounding number of autoantibodies in sera may be a generally unappreciated feature of the blood, with a function that remains to be elucidated, we have found that the presence (or absence) and titer of specific autoantibodies in the blood can reflect the presence of certain diseases, and thus are potentially useful as diagnostic indicators of diseases, including AD.

♦B14

DNA Circles in the Hourglass of Life

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Hypothesis: We hypothesize that health and longevity in humans are based on cellular mechanisms involving extrachromosomal, circular, ribosomal DNA (rDNA). We propose that the progression of age, at least in part, is encoded by cytoplasmic accumulation of rDNA circles formed in nucleoli and escaping through nuclear pores. If proven correct, cytoplasmic rDNA accumulation could trigger innate immunity mechanisms designed to respond to viral infections. Infected cells would then undergo apoptosis which may represent a major driving force in both normal aging and age-related diseases.

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Design: Our experimental goals are based on the observation that lifespan and replicative potential of yeast (*S cerevisiae*) is directly proportional to the abundance of rDNA circles. In this context, it is known that the nicotinamide adenine dinucleotide (NAD+)-dependent protein deacetylase, Silent Information Regulator 1 (SIRT1), generally prevents formation of rDNA circles. Whereas previous approaches assessing phenotype and quantity of rDNA circles were technically laborious, our much simplified quantitative PCR (QPCR)-based strategy simultaneously assesses virtually any type of circular DNA in the cell nucleus or cytoplasm. Our experiments are therefore designed to identify nuclear and cytoplasmic rDNA species in established human cell lines and assess the effects of SIRT1 activity on their formation and translocation.

Methods: Three human cell lines: breast carcinoma (MCF-7), neuroblastoma (SH-SY5Y), and embryonic kidney (HEK-293) were grown under standard culturing conditions. SIRT1 was activated with 50 µM resveratrol for 48 hours. To purify DNA circles, nuclear and cytoplasmic fractions were successively subjected to exonucleaseIII, RNaseA, and ProteinaseK. Samples were then used for standard QPCR using the following gene-specific DNA primers: 5SrDNA, 45SrDNA, and Alu microsatellite circles (used as internal standard).

Results: With our newly developed methodology, we detected 5SrDNA and 45SrDNA circles in the nucleus and cytoplasm of human cells. Resveratrol-dependent SIRT1 activation caused a downward trend in the abundance of nuclear rDNA circles and a significant decrease of cytoplasmic rDNA circles.

Conclusion: We are the first to correlate cell health and longevity through SIRT1 activation, with subsequent reduced cytoplasmic rDNA. These data support our hypothesis and warrant further understanding of this intracellular relationship.

♦B15

The Effects of TNF-a on GM3-Synthase Levels and Its Subsequent Activation of the Insulin Receptor in C2C12 Cells

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Recent research has shown that TNF-α has an effect on the insulin signaling pathway by up-regulating the production of GM3-synthase. GM3-synthase, the protein responsible for the synthesis of GM3, is produced in higher levels in the presence of TNF-α. In this research study, C2C12 mouse myoblasts were grown in order to perform experiments on ganglioside GM3-synthase and its effects on insulin receptor phosphorylation. Cells were grown and differentiated into skeletal myotubes and then treated with TNF-α, insulin, and TNF-α antagonist individually and in combination. The treated cells were frozen and lysed, and protein concentrations were determined. Western blots were run with the cell lysates and protein expression was determined using antibodies to GM3-synthase, insulin receptor-β, phospho-IR, and β-tubulin (tubulin was chosen as a standard for total protein loading). Preliminary results have shown that TNF-α treatment plays a role in increasing GM3-synthase levels and decreasing p-IR levels. We have also seen the opposite effect when cells were treated with both TNF-α and the TNF-α antagonist. Using image analysis software, we have been able to see higher levels of GM3-synthase in cells treated with TNF-α as compared to those treated with the antagonist. There also appears to be a reversal effect in cells treated first with TNF-α and later with TNF-α antagonist, as these cells presented lower levels of GM3-synthase than those cells treated with TNF-α alone. These results suggest that a TNF-α antagonist could be used in order to decrease GM3-synthase levels and increase the level of insulin receptor phosphorylation. These data could provide information relevant to the treatment of patients with type 2 diabetes mellitus.

♦B16

Anatomical Distribution and Functional Significance of Dopachrome Tautomerase in the Adult Mammalian Brain

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Hypothesis: D-Dopachrome Tautomerase (DT) is a member of the tyrosinase family involved in the biosynthesis of melanin. Although it is well established that the gene product DT is widely expressed in cardiac melanocytes, no study has mapped the distribution of DT in the adult mammalian brain. Here we address this void and hypothesize that DT may be sensitive to changes in ambient temperature, particularly during states of general anesthesia.

Design: A well-characterized rabbit polyclonal antiserum was used for immunocytochemical localization of DT in mice and humans. To further verify localization of DT, Western blots were carried out in samples containing hippocampal, hypothalamic, striatal, and cerebellar material. To measure changes in DT expression in mice undergoing general anesthesia, quantitative polymerase chain reaction (QPCR) was performed in mice treated with the dissociate anesthetic ketamine (5 mg/kg).

Methods: Immunocytochemistry was performed using a DT polyclonal primary antibody. Coronal sections (50 µm) through the rostrocaudal extent of the mouse brain were processed and visualized under light microscopy. For the human brain, hippocampal and hypothalamic sections (50 µm) were processed for dual immunocytochemical localization of DT and

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catecholamine enzymes. For Western blotting, cytoplasmic and nuclear proteins were extracted using nuclear extraction kits. For QPCR measurements, one set of mice (n=5) was exposed to ketamine-induced hypothermia (35°C), while another set of anesthetized mice (n=5) was placed on heating pads, thus maintaining normal core-body temperature (37°C).

Results: DT was found to be present in discrete populations of hippocampal neurons (CA-3, means [SEM], 95.6 [3.47] cells per 40× magnification). Western blot analysis confirmed our immunocytochemical data showing DT localized to areas of the brain involved in cognition. QPCR analysis of the aforementioned brain area showed that anesthesia-induced hypothermia up-regulated DT activity in mouse hippocampus (2.5-fold decrease in hypothermic mice relative to non-hypothermic mice, $P<.05$).

Conclusion: Here we show the anatomic distribution of DT in the adult mammalian brain. Our data point to the conclusion that DT plays an important role in hippocampal transmission and in the regulation of homeostatic functions in the nervous system.

♦B17

Thiocyanate Prevents Myeloperoxidase-Mediated Loss of Paraoxonase Activity From Human High-Density Lipoprotein

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Background: During inflammation, myeloperoxidase (MPO) uses chloride (Cl-) and thiocyanate (SCN-), along with hydrogen peroxide, to generate pro-oxidant HOCl and HOSCN. HOCl targets high-density lipoprotein (HDL) causing loss of cardio-protective paraoxonase (PON1) enzyme activity. Consequently, HDL becomes dysfunctional and unable to protect low-density lipoprotein (LDL) from atherogenic transformation. However, although SCN- is MPO's preferred substrate, the potential effect of HOSCN on HDL has not been investigated.

Hypothesis: The aim of our study was to evaluate potential pro-oxidant effects of SCN- on HDL's PON1 activity.

Methods: Isolated HDL was incubated with MPO (30 nM), hydrogen peroxide (100 uM), and physiologic concentrations of Cl- (150 mM) and SCN- (100 uM) added either separately or in combination. Subsequently, HDL's PON1 activity and its ability to protect LDL from atherogenic transformation were measured. Formation of HOCl and HOSCN by our MPO system was confirmed by monitoring oxidation of 5-thio-2-nitrobenzoic acid.

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Results: During incubation with the MPO system containing Cl-, HDL's PON1 phosphotriesterase activity, a marker for cardiovascular disease risk, declined to 61% ± 4% of normal ($P<.05$ compared with untreated HDL) after 4 hours. A similar, though not statistically significant, decline to 79% ± 13 % of normal was observed when SCN- replaced Cl-. However, when HDL was incubated with the MPO system containing both Cl- and SCN-, mimicking the physiological state, PON1's phosphotriesterase activity did not decline. Instead, it remained stable at 102% ± 12% of normal for 4 hours; a subsequent decline in activity was only observed during prolonged incubation (8 hours). A similar protective effect was observed for both PON1's aryl esterase and lactonase enzyme activities, and HDL's ability to protect LDL from atherogenic transformation. Interestingly, despite different effects of MPO incubations containing Cl- and/or SCN- on PON1 activity, HDL protein oxidation, assessed as loss of reduced thiols, was essentially the same under all experimental conditions.

Conclusion: Thiocyanate may be an important anti-inflammatory molecule that lessens MPO's ability to render HDL dysfunctional.

Funding: Financial support was provided by Midwestern University. Student Doctor Kosmach was a 2010 Kenneth A. Suarez Summer Research Fellow.

♦B18

Lymphatic Pump Technique Facilitates the Clearance of Respiratory Infection With *Streptococcus pneumonia*

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Background: Osteopathic manipulative treatment (OMT) techniques are thought to remove restrictions in the lymphatic vessels and enhance the flow of lymph. One such technique, the lymphatic pump technique (LPT), is thought to aid in the removal of built-up metabolic wastes, toxins, exudates, and cellular debris that occur during infection or edema. Furthermore, LPT has been reported to enhance immune function. *Streptococcus pneumoniae* is known to be a common cause of otitis media, meningitis, and respiratory infections. It is additionally a major cause of pneumonia in the elderly and infants and has been reported to cause disease secondary to infection with the influenza virus.

Objective: To determine if LPT would enhance the clearance of *S pneumoniae* respiratory infection.

Methods: Rats were nasally infected with ~5×10^7 *S pneumoniae* CFUs. Rats were divided into control, sham, or LPT treatment groups. For 1, 3, or 7 consecutive days, rats received either (1) a daily sham treatment consisting of intravenous administration of 10 mg/kg propofol anesthesia followed by

4 minutes of light touch, (2) 4 minutes of LPT daily under anesthesia, or (3) no treatment or anesthesia (control). At days 2, 4, and 8 postinfection, lungs were collected and measured for *S pneumoniae* bacteria and the number of pulmonary leukocytes. In addition, blood and spleens were collected to measure the extrapulmonary immune response.

Results: LPT treated animals showed a statistically significant ($P<.05$) decrease in the total CFU/lung at day 2, 4, and 8 postinfection. The decrease was determined to be 20- to 30-fold during each of the 3 points when compared to control animals. At 2 and 4 days postinfection, there were no significant ($P>.05$) differences in total lung leukocyte numbers between the treatment groups, suggesting LPT did not increase the numbers of leukocytes trafficking into the lungs at this time point. At 8 days postinfection, lung leukocyte concentrations were highest in control rats, which were unable to clear pulmonary bacteria. Both LPT- and sham-treated rats had fewer bacteria in their lungs at day 8 postinfection, suggesting the infection was subsiding; therefore, it was not surprising these rats had fewer leukocytes in their lungs.

Conclusions: We have shown that LPT enhances the clearance of pneumococcal bacteria in the lungs after 1, 3, or 7 applications. Our initial findings support the clinical use of LPT to treat patients with pneumonia.

♦B19

Immune Cell Phenotype Expression Patterns in a Compression Model of Spinal Cord Injury

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After spinal cord injury (SCI), the blood-spinal cord barrier (BSCB) is damaged, allowing peripheral immune cells to enter the CNS. Over time, the integrity of the BSCB is restored, but immune cells continue to accumulate, likely in response to changes in the endothelium and cytokines released in the injury site. To gain access to the CNS parenchyma, immune cells interact with endothelial cells of the BSCB. We hypothesized that molecules expressed by vascular endothelial cells influence the functional profile (ie, phenotype), and thus the effects of the immune cells that enter the CNS after injury. Within the CNS, an M1 (classically activated) macrophage phenotype is thought to play a neurodegenerative role after SCI while the M2 (alternatively activated) phenotype is thought to play a reparative role, possibly limiting secondary injury in spinal cord lesions. Here we characterize the pattern of BSCB damage and restoration and the phenotype of macrophages and T cells over a 6-week period after compression injury of the thoracic spinal cord. The renin-angiotensin system (RAS) has recently been reported to promote inflammation in a variety of CNS neurodegenerative disorders. Furthermore, RAS can modulate the phenotype of macrophages and T cells. Thus, we sought to evaluate the role of RAS in SCI. We hypothesized that inhibition of RAS would have beneficial effects after injury by altering the phenotype of the vascular endothelium and thus the phenotype of the infiltrating immune cells. Female Sprague-Dawley rats were given a SCI by compressing the thoracic spinal cord with specially modified forceps. Locomotor recovery was evaluated at specific time points post-injury until killing. The animals were perfused transcardially after which the spinal cords were removed, sectioned, and mounted onto coated slides. Immunohistochemical analyses were performed to identify molecules on the vascular endothelium and the phenotype of infiltrating immune cells. In a second set of experiments we assessed the role of RAS in SCI by treating animals with captopril, an inhibitor of angiotensin-converting enzyme (ACE), and evaluating functional recovery after injury. We found no significant differences in the degree of spontaneous locomotor recovery in captopril-treated and vehicle-treated rats.

B20

Lymphatic Pump Treatment of Rats With Adjuvant-Induced Arthritis

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by synovial inflammation and lymphocyte infiltration. Lymphatic pump treatment (LPT) is clinically used to reduce edema and aid the immune response through increasing lymphatic circulation.

Purpose: This study examined the effect of LPT on arthritis using the rat adjuvant-induced arthritis (AIA) model of RA. We hypothesized that LPT of rats with established arthritis would result in reduced inflammation through increased lymphatic circulation resulting in T regulatory cell (Treg) function.

Methods: AIA was initiated in 12 female Lewis rats by subcutaneous injection of killed Mycobacterium butyricum above the base of the tail. Arthritic rats were randomly placed in 2 groups; the LPT group and the sham group which was held. LPT was performed 6 times over 11 days without anesthesia and utilized a diaphragmatic approach generating a pulsation in the fluids and tissues by rhythmically pressing below the rib cage 30 to 40 times over 30 seconds. Arthritis was assessed by measuring ankle circumferences and by articular index scoring. Blood smears and flow cytometry were performed to study leukocyte populations.

Results: The LPT rats trended to have less swollen ankles

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throughout the usual peak of arthritis, which was greatest on day 21 when the ankle circumferences were 3.1 mm smaller (~40% less) than the sham-treated animals. The LPT rats also trended to have lower articular index scores. During the peak of inflammation, the LPT animals had an articular index score of 1.75 compared to sham-treated animals score of 2.17. Overall, the articular index scores of the LPT animals seemed to plateau while the articular index scores of the sham-treated animals continued to rise. The LPT group had a higher percentage of CD3+ cells than the sham group (76.3% to 67.8%). This suggests that more lymphocytes were circulating in the blood with LPT. Also, the percentage of peripheral blood Treg cells (CD4+, CD25+) were increased with LPT compared to the sham group (0.7% to 0.51%).

Conclusion: These results suggest that the progression of arthritis can be blunted with LPT. Specifically, analysis of ankle circumference (a direct measure of edema) and articular index score (a subjective measure of inflammation) suggests that LPT resulted in reduced inflammation particularly during the peak inflammatory period of AIA. The LPT may be accomplishing this through increasing lymphocyte circulation.

♦B21

Cadmium Alters the Small RNA Expression Profile in a Human Lung Alveolar Epithelial Cell Line

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Background: Cadmium (Cd) is known to cause acute lung toxicity which is initially characterized by pulmonary edema and hemorrhaging with subsequent development of inflammation and fibrosis. Chronic exposure to Cd is associated with the development of chronic obstructive pulmonary disease and possibly an increased risk of lung cancer. While many studies have addressed the toxic effects of Cd, the specific molecular mechanisms of Cd toxicity have yet to be fully elucidated. Recently, small RNAs such as microRNAs and fragmented tRNAs have been identified as important regulators of cellular function.

Hypothesis: In this study, we investigated the hypothesis that Cd treatment alters the small RNA expression profile in the A549 human alveolar epithelial cell line.

Methods: Sub-confluent (70% to 80%) cells were exposed to CdCl2 (10 microM) for 24 hours in serum-free medium. Cytotoxicity was evaluated using trypan blue and hemacytometer cell counting, and WST-1 assays. Total RNA was extracted from control and Cd-treated cells and microRNA array analysis was performed. Differentially regulated microRNAs were validated through Northern blot analysis and real-time PCR assays.

Results: MicroRNA array analysis showed that acute exposure to a relatively non-cytotoxic concentration of Cd caused a statistically significant (*t* test, $P \leq .05$) alteration in the expression of 6 human microRNAs (miR-3127, miR-23b, miR-23a, miR-92b, miR-222, and miR-1915) and one fragmented tRNA (previously known as miR-1308). Northern blot analysis for miR-1308 confirmed the array results and indicated increased expression with Cd-treatment. Real time PCR analysis demonstrated a 7-fold and a 3.2-fold statistically significant (*t* test, $P \leq .05$) increase in the expression of mir-1308 and mir-3127, respectively.

Conclusion: These finding suggest that alterations in the small RNA expression profile may contribute to Cd-induced lung injury since both microRNAs and fragmented tRNAs are known to have biological function.

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B22

Nontraditional Induction of Osteogenic Gene Expression in C2C12 Cells

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Background: C2C12 cells are a bipotent cell line obtained from subcloned myoblast cells with a propensity to differentiate towards osteoblasts or myocytes. The ability of C2C12 cells to differentiate into muscle or bone lineages depending on the extracellular signaling and growth factors present, such as bone morphogenetic protein-2 (BMP-2) and low serum conditions, has been established.

Hypothesis: As BMP-2 is an expensive morphogen, we attempted to prompt osteoblastic gene expression in a C2C12 line using other means.

Methods: The C2C12 line was obtained from ATCC (Manassas, Virginia) and the C2C12-Osterix line was obtained from Dr Beniot de Crombrigghe (Houston, Texas). Cell lines were cultured using alpha MEM containing F12 mix and 10% FBS. For differentiation experiments, DP media, which contains β-glycerophosphate and 50 µg/mL ascorbic acid, was used. Collagen 1a1 stock solution and ethanol were used to create collagen matrices. Matrices were covered in alpha mem for roughly 1 hour before cells were seeded. Tetracycline was added to the media in various concentrations after cells were

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seeded. Kill curves were conducted in order to properly utilize the C2C12-osterix line. Bone differentiation was determined by positive alkaline phosphatase staining, mineralization, and presence of transcription factors.

Results: We first studied the exposure of cells to osteoblast differentiation promoting media, which showed evidence of osteoblastic conversion. Then, our laboratory examined the ability of a collagen matrix without BMP-2 treatment to induce osteogenic differentiation. While cells plated onto both plastic and collagen exhibited positive ALP staining, the cells grown on the collagen matrices showed more diffuse staining than cells grown on plastic substrates. Even without BMP-2, the collagen matrices were able to serve as an effective substrate for osteoblast differentiation. The third option we evaluated was a cell line stably carrying the osteoblast transcription factor osterix in a tet on/tet off system. In this C2C12-osterix line, the osterix expression vector is turned off with the addition of tetracycline (tet). We optimized the conditions necessary for using this system and are able to show robust differentiation of C2C12 cells towards the osteoblastic lineage.

Conclusion: Our results suggest that osteogenic gene expression can be instigated without the traditional means of adding BMP-2 or other proteins specific to bone formation.

♦B23

Insulin Signaling and Caveolae: Role of Saturated Fatty Acids in the Development of Insulin Resistance in 3T3-L1 Adipocytes

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Caveolae are membrane microdomains recently recognized as critical for proper compartmentation of insulin signaling; however, their role in the pathogenesis of insulin resistance is not fully understood. Caveolae are particularly abundant in adipocytes and are especially critical for insulin signal transduction in these cells. These microdomains are rich in caveolin-1, a scaffolding protein that has previously been shown to exhibit an insulin receptor-binding motif. Research in adipocytes demonstrated that the inhibition of insulin signaling and the elimination of insulin receptors from the caveolae were associated with an accumulation of glycosphingolipids such as ganglioside GM3. A growing body of evidence implicates that saturated fatty acids such as palmitate and ceramide, which are precursors of glycosphingolipids, play a role in the pathogenesis of insulin resistance and have been shown to effect downstream molecules in the insulin signaling pathway. The aim of this study is to determine the effect of palmitate and ceramide on the activation of insulin receptor and its localization to the caveolae microdomain, Glut-4 localization to the caveolae microdomain, as well as the effect on the functional structure of the microdomain and its associated caveolin-1 proteins. We provide evidence for the disruption of insulin signaling and displacement of its compartmentation from the caveolae after treatment of 3T3-L1 adipocytes with palmitate and ceramide. Our analysis demonstrates a downregulation of total insulin receptor and caveolin-1 with no changes in the overall concentration of Glut-4 and phospho-insulin receptor in cells treated with ceramide. Immunoflourescence analysis of palmitate treated 3T3-L1 adipocytes shows a decrease in the amount of caveolin-1 present in the membrane as well as a downregulation of Glut-4 and insulin receptor. These results suggest that adipocytes exposed to excess free fatty acid concentrations are likely to respond by up-regulating receptors involved in the metabolism and function of insulin. In addition, the displacement of these receptors from the caveolin micro-domain indicates a possible mechanism by which the surrounding tissue may develop insulin resistance.

B24

The Role of cAMP in Arginine Metabolism in Human Gingival Fibroblasts

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Hypothesis: Arginine levels may regulate the enzymes involved in arginine and ornithine metabolisms in human gingival fibroblast (HGF) in periodontitis. Thus, the expression of enzymes such as arginase I (ARGI), arginase II (ARGII), ornithine decarboxylase (ODC), and ornithine aminotransferase (OAT) appears to be essential in the regulation of the cellular immune response and the inflammatory process.

Design: Excessive NO is associated with tissue destruction and inflammation; NO synthesis through nitric oxide synthase (iNOS) is increased in periodontitis. Arginine is a common substrate of both iNOS and arginase enzymes. Upregulation of arginase is speculated to decrease arginine availability for iNOS and may down-regulate NO overproduction. Pro-inflammatory cytokines have been found to upregulate iNOS in HGF, but regulation of arginase enzymes, ODC, and OAT in this tissue type has yet to be determined. Presence of ARGI and ARGII in HGF may provide a source of ornithine for production of polyamines, and ultimately for collagen synthesis and cellular proliferation via ODC and OAT.

Methods: Human gingival fibroblast cultures were established by trypsin dispersion and maintained in Eagle's Minimal Essential Medium supplemented with 10% fetal bovine serum and antibiotic. Cultures in passage 3-6 were used for RealTime-PCR, Western blot analysis, and arginase assay. Cytokines and stimuli were added at the appropriate amount.

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Western blot analyses of proteins were performed from whole cell extracts. Each experiment was repeated at least 3 times. Data are expressed as mean (SD). Comparisons were made by Student's *t* test (2-tailed for paired samples).

Results: In presence of cAMP and dexamethasone, mRNA and protein levels of all 4 enzymes showed a significant increase compared to control. Interferon g decreased ARGI and ARGII ex-pression. Pro-inflammatory cytokines inhibit the enzymes expression and attenuated their enhanced expression via cAMP. In this study, we have determined the transcription and expression of the enzymes important in homeostasis arginine and ornithine in HGF in absence and presence of T helper II cytokines and glucocorticoids.

Conclusion: ARGI, ARGII, ODC, and OAT are expressed constitutively in HGF. These enzymes may modulate the inflammatory response in periodontitis, limiting NO production by iNOS and exerting anti-inflammatory effects.

B25

In Vitro Dose Response of Modeled Myofascial Release in 3-Dimensional Bioengineered Tendons: Effects on Human Fibroblast (HF) Hyperplasia, Hypertrophy, and Cytokine Secretion

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Background: We have reported the effects of modeled myofascial release (MFR) on 2-D HF constructs. MFR suppressed various inflammatory cytokines and attenuated the increased apoptosis/hyperplasia and actin rearrangement in cells previously strained cyclically. These data build upon evidence describing MFR clinical efficacies for treating various somatic dysfunctions. In this study, we aim to improve on our model by using 3-dimensional (3-D) bioengineered tendons (BETs) to investigate the effects of various MFR dosing times.

Hypothesis: We hypothesize that different durations of MFR generate unique profiles in human fibroblast (HF) hyperplasia, hypertrophy, and cytokine secretions.

Methods: Bioengineered tendons (3-D HF/collagen gel constructs) were attached at 2 anchor points while remaining freely suspended from an elastomeric well bottom. Vacuum pressure was applied to increase the distance between the 2 anchor points resulting in strained BET. Bioengineered tendons were stretch 6% beyond L0 and held for 0.5, 1, 2, 3, 4, and 5 minutes. Conditioned media were then collected 48 hours poststrain and analyzed for cytokines via protein microarrays (n=2 per group). In addition, HF were extracted from the BET by collagenase and intracellular protein and DNA content were measured (n=3-4/group).

Results:

	Mean (SEM) Duration of MFR, min					
Measure	A 0.5	B 1	C 2	D 3	E 4	F 5
DNA, pg/mL	27.3 (0.3)	26.3 (0.1)	26.6 (0.2)	26.4 (0.4)	26.2 (0.3)	27.1 (1.4)
Protein, μg/mL	187.6 (16.6)	251 (28.9)	186.8 (14.3)	166.5 (28.8)	183.6 (18.1)	193.7 (15.7)
GRO	8.9 (9.1)F	11.6 (15.2)	0.9 (16.4)	19.3 (0.05)F	36.3 (18.9)	42.0 (3.8)A,D
IL-3	-10.8 (3.8)F	-3.5 (8.9)F	-7.5 (24.04)	-1.04 (0.1)F	28.48 (19.5)	31.5 (4.7)A,B,D
IL-8	-1.9 (2.1)F	3.8 (7.8)F	-11.1 (32.8)F	22.0 (9.4)F	27.1 (16.7)	74.9 (8.2)A-D
TARC	1.6 (1.8)	0.3 (0.6)F	-0.7 (0.8)F	4.4 (2.2)	6.7 (2.2)	8.6 (1.7)B,C
GCSF	-4.7 (2.1)F	-4.3 (6.9)F	-0.8 (0.5)F	-5.4 (1.9)F	6.5 (4.0)	14.48 (1.9)A-D
Angiogenin	13.5 (5.1)F	16.85 (1.1)F	12.6 (32.0)	18.8 (2.9)F	31.3 (8.9)	57.1 (6.8)A,B,D

Letters signify $P<.05$ vs the value in the corresponding row and column.

Abbreviations: GCSF, granulocyte colony-stimulating factor, GRO, growth regulated oncogene; TARC, thymus and activation regulated chemokines; SEM, standard error of the mean.

Conclusion: Although time-dosed MFR did not affect BET DNA or intracellular protein content, BET cytokine secretion is time dose-dependent. Longer MFR duration leads to significant step-wise increases in angiogenic growth factor secretions. These studies provide in vitro evidence for time-dependent MFR dose responses which may be useful for mechanistic understating and future refinements of osteopathic manipulative treatment regimens.

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Medical Education

ME1

Improving Resident Research Projects by Providing a Focused Research Infrastructure and Training on Evidence-Based Practice

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Context: The *Basic Standards for Residency Training in Osteopathic Family Practice and Manipulative Medicine* states that residency programs must provide instruction in critical evaluation of medical literature, including assessing study validity. Fur-

thermore, it requires the participation of each resident in an active research/scholarly activity. The Standards define research to include a number of activities, including papers on healthcare topics, presentations at meetings, or original research.

Hypothesis: This retrospective review evaluated whether a structured research requisite can result in advancing primary care research within a family medicine department.

Methods: Initiated by the departmental vice-chair, the Department of Family Medicine at the University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine has, since 2005, as part of the residency graduation fulfillment, required all residents to develop and complete an institutional review board–approved original research study. Prior to this initiative, residents fulfilled their scholarly research requirement by writing a review paper or developing a case presentation. In support of this new research requirement and to advance the research agenda in primary care, the department built an infrastructure providing a number of support services, including a faculty-led journal club; instruction in research design; a faculty-directed IRB submission process; a faculty statistical analyst; and focused training in information mastery, which includes instruction in evidence-based medicine and medical informatics.

Results: Since the inception of this research requirement, 34 studies have been completed. Included in these projects have been 43 residents and 18 faculty members. The poster presentations describing these studies have won national awards and a number of them have been published in peer-reviewed journals.

Conclusion: Research in primary care can be advanced by initiating an original research requirement, providing faculty development, and building a supportive infrastructure.

Health Policy

HP1

A Comparison of Citizens' Perceptions of Their Countries' Health Insurance Systems: Canada, France, the United Kingdom, and the United States of America

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Hypotheses: As a result of current policy debates, health insurance systems receive much attention. This research compared citizens' perceptions of Canada's National Health Insurance model, France's Bismark model, the United Kingdom's (UK) Beveridge model, and the United States' (US) mixed-model of healthcare. It hypothesized significant differences exist between citizens' perceptions of their countries' health insurance systems regarding excellent quality of care from one's primary care doctor (H1), patient-centered primary care (H2), cost-related access barriers to care (H3), serious problems paying medical bills (H4), and concerns about long time lags for diagnoses (H5).

Methods: Secondary data analysis of the 2010 Commonwealth Fund International Health Policy Survey, a cross-sectional survey of citizens from 11 countries, focused on Canada, France, the UK, and the US (n=8616). Kruskal-Wallis tests and Goodman and Kruskal tau tests tested for significant differences among citizens' perceptions of their countries' health insurance systems for the 5 hypotheses.

Results: Significant differences exist between perceptions of excellent quality of care from one's primary care doctor (H1, P=.001), patient-centered primary care (H2, P=.05), cost-related access barriers to care (H3, P=.001), serious problems paying medical bills (H4, P=.001), and concerns about long time lags for diagnoses (H5, P=.001). The results suggest that, although the United States leads in patient-centered primary care, it ranks lowest in citizens' ability to afford medical care. A third of US respondents reported not filling prescriptions/skipping doses and/or skipping a medical test, treatment, or follow-up for financial reasons. Canadians were the most likely to have concerns about long time lags for diagnoses. The UK had the highest percentage of citizens reporting excellent quality of primary care; the lowest percentage skipping diagnoses, treatments, and follow-ups for financial reasons; the lowest incidence of citizens reporting serious problems paying medical bills; and the lowest incidence of citizens concerned about long time lags for diagnoses. France ranked in the middle for each variable tested.

Conclusion: These results suggest no system consistently satiates citizens' interests. Different health insurance systems accomplish different ends. Policy makers and administrators can apply these results to policy development and reform decisions within their particular contexts.