SOMA Abstracts in 2009 Student Poster Competition

The following abstracts were presented during in the 2009 Student Poster Competition conducted at the American Osteopathic Association's 53rd annual Research Conference. Submitted through the Student Osteopathic Medical Association (SOMA), these abstracts constitute nearly 50% of the entries in the 2009 Student Poster Competition. The other student abstracts were submitted directly to the AOA Council on Research.

A judged event sponsored by both the AOA and SOMA, the Student Poster Competition took place November 1, 2009, during the Research Conference's annual poster session at the AOA's 114th Annual Osteopathic Medical Conference and Exposition in New Orleans, Louisiana.

The AOA Council on Research bestowed three first-place and six second-place awards, of which three of the second-place awards went to SOMA abstracts. Those SOMA abstracts are designated in the following pages with the symbol ♦.

All first-place and the remaining second-place student abstracts are among the poster session entries that were submitted directly to the Council on Research and that were published in the August 2009 issue of JAOA—The Journal of the American Osteopathic Association *(http://www.jaoa.org/cgi/content/full/109/8/425). A complete list of winning abstracts from the 2009 Student Poster Competition is available at http://www.do-online.org/index.cfm?au=D&PageId=res_contestwinners.*

The 2009 SOMA abstracts were organized into six series:

To enhance the readability of the SOMA abstracts, they have been edited for grammar and basic JAOA *style. The content of these abstracts has not been modified. The AOA Council on Research, SOMA, and* The Journal *do not assume responsibility for the abstracts' content.*

For information on submitting abstracts to future Student Poster Competitions, visit DO-Online at https://www.do-online.org/index.cfm?PageID=res_posterpresent.

AOA Research Fellowship

♦F1

Osteopathic Manipulative Treatments in the Emergency Department: Osteopathic Emergency Physicians' Perspectives

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Little is known concerning the frequency of use, type of osteopathic manipulative treatments (OMT) delivered, and attitudes to OMT by osteopathic physicians in the emergency department (ED). This study investigated the reported use of OMT by osteopathic emergency physicians, frequency of use for various techniques and on which patient populations they are used. The opinions of 431 osteopathic emergency physicians were obtained using a web-based survey tool. Fifty-nine percent reported that they never use OMT in the ED, whereas fewer reported monthly (30%), weekly (7%) and daily (4%) use. The four techniques reported to be most commonly used in the ED were soft tissue treatments (75%), muscle energy (74%), high-velocity, low-amplitude (49%) and counterstrain (45%). The most common patient populations for these techniques are back pain, headache and chronic musculoskeletal complaints. Seventy percent of respondents opined that OMT was underused in the ED with 83% indicating that time constraints was a reason for not using OMT. Osteopathic emergency physicians may be encouraged to use more OMT by further development of their OMT skills with an emphasis on time efficiency, familiarizing patients with OMT usage, and creation of formal guidelines for the use of OMT in the ED.

Osteopathic Manipulative Medicine/Osteopathic Principles and Practice

S18

Osteopathic Survey of Somatic Dysfunction and Zink Compensatory Patterns in Sololá, Guatemala

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Background: There is a paucity of research done in developing countries on J. Gordon Zink, DO's model of compensatory patterns of fascial restriction and ease. The Compensatory Pattern is assessed in a supine patient by noting ease of rotation at the following four junctions: craniocervical, cervicothoracic, thoracolumbar, and lumbosacral. The Common Compensatory Pattern has been Left/Right/Left/Right (LRLR). Is the "common" compensatory pattern truly common to all humans or is it an artifact of the lifestyle in industrialized countries? In Sololá, Guatemala, the pre-existing cultural category for a group of healers known as *sobadores* (bone-setters) made Solalá an ideal place for this study, where osteopathic manipulative medicine (OMM) was culturally well received by the community.

Objective: To survey Zink Compensatory Patterns among the Maya men and women in Sololá, Guatemala.

Materials and Methods: Patients were screened for musculoskeletal and visceral problems and referred to the OMM clinic by the physician at a local primary care clinic. Forty patients (30 female, 10 male) participated in the study. Verbal and/or written consent was obtained from all participants. Translation was provided as needed. Four TUCOM students, who had completed their first year of osteopathic medical education and had received additional training in Zink screening by faculty, performed an osteopathic structural exam using standard OMM tables. Results were noted on a Zink Fascial Screening Exam form developed by John C. Glover, DO, FAAO.

Results: Zink fascial screening data demonstrated a wide range of fascial strain patterns. Of our 40 patients, 26% had an LRLR compensatory pattern (the "common" pattern seen in prior research) and 6% had a RLRL compensatory pattern. Furthermore, 69% of all patients showed right rotational fascial preference at the cervicothoracic junction, 46% of whom had a left rotated compensatory pattern at their occipitoatlantal and thoracolumbar junctions.

Conclusion: The Common Compensatory Pattern of LRLR was the most consistently noted pattern among the Maya in Sololá, Guatemala. The wide variation noted in Zink patterns may actually represent the population studied, or be an artifact related to small n and intra-examiner reliability. Documentation of examiner reliability during the study, as well as prior to, should be included in future research. This study has been approved in its entirety by the TUCOM IRB Committee.

S23

The Efficacy of OMT by Osteopathic Medical Students on Musculoskeletal Pain and Somatic Findings

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Hypothesis: There will be a significant decrease in patient-reported pain scores and severity of somatic findings after osteopathic manipulative treatment (OMT) provided by osteopathic medical students.

♦Indicates poster that won the 2009 Student Poster Competition.

Materials and Methods: We performed a retrospective chart review of patients seen between February and April 2009 at the on-campus pre-doctoral teaching fellows' (PDTF) free clinic at Western University of Health Sciences in Pomona, California. As part of the standardized medical record form used at that clinic, patients were asked at the beginning and end of each treatment for their current global pain rated on the standard 0-10 pain scale. The PDTF fellow assessed tissue texture, asymmetry, range of motion, and tenderness (TART) for each region before and after treatment and graded the severity of the somatic dysfunction using the 0-3 scale as defined by the AOA standardized and validated Osteopathic SOAP note forms. Patients were included in this study if they had a pre-treatment pain score greater than or equal to 4/10 and had evidence of somatic dysfunction, with severity level >2 in at least one region. A decrease in pain post OMT was considered to be significant if it was 40% or greater from baseline level. Improvement of TART in each region was considered significant if there was a decrease of 2 or more severity scale levels. This retrospective chart review was exempt from IRB review.

Results: Forty-three charts met the inclusion criteria. Eighty-six percent (37/43) of the patients had a significant decrease in pain. The average percent decrease in pain was 67%±27.4%. The proportion of patients with significant decrease in TART severity level in any one region was 36/43 (84%). Per region the proportion of patients with significant decrease in TART were: cervical: 16/33 (48.5%); thoracic: 19/35 (54.3%); ribs: 14/24 (58.3%); lumbar: 7/18 (38.9%); pelvis: 10/25 (40%); sacrum: 6/22 (27.3%); UE: 5/12 (41.7%); LE: 1/9 (11.1%); cranium: 2/6 (33.3%). Global TART severity scores saw a 58.9% decrease from pre-OMT mean of 11.6±3.3 (range 5-19) to post-OMT mean of 4.8±2.5 (range 0-10). Of those with >40% decrease in pain, 32/37 (86.5%) had significant decrease in TART in at least one region.

Conclusion: Most patients treated with OMT by PDTF had both decreased pain as well as concomitant decrease in severity of somatic findings. This retrospective chart review demonstrates that OMT as performed by osteopathic medical students significantly reduces both pain and severity of somatic dysfunction.

S29

The Relationship Between Osteopathic Family Physicians' Use of Manipulative Therapy and Their Perceived Empathy for Patients

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Hypothesis: This study investigated the hypothesis that more frequent use of osteopathic manipulative treatment (OMT) by osteopathic family physicians would be associated with a higher level of physician empathy as perceived by patients.

Materials and Methods: First, 505 active members of the American College of Osteopathic Family Physicians in Ohio were surveyed concerning the use of OMT in their practices. Second, 59 physicians interested in the empathy portion of the study received 50 empathy rating forms for their patients to complete. Examples of the 10 items on the form included rating the extent to which the physician "is interested in you as a whole person" and "shows care and compassion." The empathy rating scores could range from 10 ("poor" on all 10 items) to 50 ("excellent" on all 10 items). This study was approved by the Ohio University institutional review board.

Results: The survey response rate was 36%, and osteopathic family physicians in Ohio performed OMT on a median of 10% of their patients. As of August 21, 2009, completed forms from 10 of the 59 physicians were received. These 10 physicians received high empathy scores after averaging the ratings from 25 to 50 patients per physician: average empathy scores ranged from 45.7 to 48.9 among the physicians, with an overall average score of 47.3, quite close to the maximum score of 50.

Conclusions: There was no association between greater use of OMT and perceived empathy scores in this study. The narrow range of physician empathy scores reduced the likelihood of finding an association between perceived empathy and any other variable. The high average empathy scores suggest that the osteopathic family physicians who completed the second part of the study have excellent relationships with their patients and are perceived as being attentive to and compassionate for each unique patient.

S32

Changes in sEMG, Clench Force, and Pain Following Osteopathic Counterstrain Treatment Directed to the Forearm and Upper Trapezius

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Hypothesis: We sought to provide evidence for the assertion that counterstrain releases muscle tension at the site of somatic dysfunction.

Materials and Methods: Fourty-six volunteers ranging in age from 23 to 62, were recruited from ATSU to participate in this 5-week study. We recorded muscle strength and fatigue using BIOPAC electromyography to analyze motor recruitment after treatment during once-weekly sessions. Subjects were tested for tender points (indicator of somatic dysfunction) present in the flexor muscles of the forearm and ranked the perceived pain level using a visual analog scale. An algometer was used to determine the level of force required to elicit the same level of palpated pain. The operator rated for the degree of tissue texture

changes felt at the tender point. Subjects squeezed an isometric hand dynamometer to measure the force of muscle contraction of the hand/wrist flexors in their dominant hand. Muscle recruitment was monitored by using EMG probes. Week 1 provided baseline data. In the following weeks, a Jones Counterstrain technique was applied to the forearm (weeks 2 and 3) and then the trapezius (weeks 4 and 5). Following treatment, each tender point found was rated by the subject and an algometer reading was taken. Muscle recruitment, tissue texture change, and force of muscle contraction were measured again.

Results: Counterstrain to the forearm corresponded with greater change in palpated tissue texture changes, pre- to post-therapy, than did administering therapy to the trapezius (P=.02). The impact of therapy on subjects' perception of pain resulted in an analogous change (P=.06) across locus of therapy. A correlation exists between the amount of pressure required to elicit pain and the amount of electrical activity in the forearm flexors (P=.001), but electrical activity in the forearm muscle did not change as a function of treatment. There was also a correlation between the amount of pressure required to elicit pain in the subject at the forearm tender point and the ability to recruit muscle in the forearm flexor as measured by clench force (P<.001).

Conclusion: The study shows that Counterstrain elicits tissue texture changes in muscle and the subjects' perception of pain pre- and posttreatment. It also illustrates changes in muscle function possibly associated with tender points. It fails to show changes in muscle electrical activity as a result of treatment.

S39

Using Osteopathic Manipulations to Decrease the Incidence of Acute Mountain Sickness

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Hypothesis: Osteopathic manipulative treatment (OMT) will facilitate high altitude acclimatization and decrease the incidence and intensity of Acute Mountain Sickness (AMS). Furthermore, results should yield important information relevant to understanding the pathophysiology & treatment of AMS.

Methods: Subjects were healthy volunteers from near sea level without recent exposure to high altitude. All were randomized into control and treated groups and were exposed to high altitude for 3 days and nights. OMT was performed at specific times. Assessments were performed on all several times daily. Scoring of AMS symptoms via the Lake Louise scoring system and measurement of hemoglobin O2 saturation (SaO2) was also performed. Retrospective analyses of sea level fitness testing will also be used to assess whether AMS symptoms, acclimatization and/or response to OMT correlate with overall cardiopulmonary fitness status of subject. The null hypothesis was rejected for P<.05 and the relation between different variables was explored using linear regression.

Results: 16 subjects (8 control and 8 treated) participated. Subjects had a mean age of 31 (23-54). Seven have a history of having had AMS in the past; meanwhile 13 experienced AMS during the study. 1 in each group never experienced AMS, and 1 control was excluded due to AMS severity. All sick subjects progressively improved over time (control=4; treated=7). However, those who became sick after Day 1 were only in the controls (n=2). The maximum AMS score with corresponding SaO2 for the control and treated groups were 5.5 at 85.3% and 5.3 at 83.9% respectively. The mean difference in SaO2 from Day 1-4 was 0.030 (P=.209; 95% CI, 0.024-0.084) for the controls and a statistically significant 0.060 for the treated (P<.001; 95% CI, 0.040-0.080). Correlating between SaO2 and corresponding AMS score between the groups for any subject with AMS symptoms, we observed a Pearson correlation coefficient of 0.202 for the controls and -0.2042 for the treated group with 2-tailed significance coefficients of 0.1392 and 0.0751 respectively.

Conclusion: The increased correlation between decreased AMS score and increased SaO2 only in those treated suggests evidence for OMT for prevention or amelioration of AMS. This preliminary study provides information and observations that are helpful in guiding future investigations. Further studies are needed with larger sample sizes. With this and more funding, it is also planned to incorporate a third group utilizing "sham OMT."

Clinical Studies

S7

The Effect of Tobacco Smoking on Length of Stay in Children With Bronchiolitis at Maricopa County Hospital

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Hypothesis: The presence of a smoker in the home will prolong length of stay among the bronchiolitis patients, but not among the gastritis patients. That is, patients who both: (1) have bronchiolitis and (2) have smokers in the home will have a longer length of stay than patients without both characteristics.

Methods: We examined the impact of smoking status on length of stay (LOS), adjusting for gender, race, birth weight, diagnosis (gastritis versus bronchiolitis) and number of siblings. Because LOS had a very non-normal distribution, which could not be corrected by any of the standard transformations, LOS was dichotomized into LOS less than or equal to 2 days (62% of the

cases) and LOS greater than 2 days (38% of the cases). This was used as the outcome variable in two logistic regressions.

Results: From June 2008 to August 2008, 2904 charts were examined. The dates of admission for eligible patients ranged from November 1, 2000, to January 1, 2007. Patients with bronchiolitis with a smoker in the home were no more likely to have an LOS longer than 2 days than were patients without both conditions (*P*=.990). The hypothesis was not confirmed. After adjusting for the effects of gender, race, birth weight, and number of siblings, the results were very similar to the unadjusted results. Patients with bronchiolitis with a smoker in the home were no more likely to have an LOS longer than 2 days than were patients without both conditions (*P*=.573). The hypothesis was not confirmed.

Conclusion: The presence of a smoker in the home did not prolong length of stay among the bronchiolitis patients, even after adjustment for potential confounding variables.

S8

Salivary Levels of Alpha-1-Antitrypsin: A Potential Noninvasive Biomarker of Pre-Diabetes

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Background: Proteomic studies have suggested that a-1-antitrypsin (A1AT) may be present at increased levels in the saliva of patients with insulin resistance and elevated fasting blood glucose. Therefore, its use as a non-invasive biomarker of pre-diabetes has been proposed. In order to design studies aimed at validating this potential biomarker, it is first necessary to develop reliable assay methods and determine the impact of factors such as salivary flow rate on A1AT concentration.

Hypothesis: It was hypothesized that A1AT levels in the saliva of healthy adults would be independent of age, flow rate, time since eating and total protein concentration.

Methods: After IRB approval, saliva samples that had been collected in a previous study were tested for A1AT levels using a commercially available immunoassay kit with modifications for detection in saliva. Sixteen samples from healthy adults along with associated data including age, sex, flow rate, total protein concentration and time since eating or drinking were analyzed. Pearson product-moment coefficients of correlation were determined for each parameter using SPSS software. Differences in A1AT levels between male and female subjects were investigated through unpaired t testing.

Results: A1AT concentration ranged from 0.647 to 41.7 mg/mL. The mean and median values were 6.46 mg/mL and 3.94 mg/mL, respectively. A1AT levels were higher in females than males but the difference was not statistically significant (*P*=.37). There was a significant correlation between A1AT levels and total protein concentration (r=0.611, *P*=.035). No correlation was found between A1AT concentration and flow rate, age, or time since eating or drinking.

Conclusions: Although based on a small sample size, the results of this study indicate that A1AT is present at a wide range of concentrations in human saliva. The information obtained regarding the relationship of A1AT levels to protein concentration, flow rate and time since eating or drinking will be helpful in the design of future studies to validate the use of A1AT as a non-invasive biomarker of pre-diabetes.

S34

Cardiovascular Assessment of Medical Students' Health; Preliminary Results Year One

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Background: Investigations of medical students' health are usually based upon assessments of psychological parameters such as anxiety and depression, related to academic and personal life stressors, utilizing confidential surveys and questionnaires, rather than direct measurements of physiological parameters. Specific information concerning cardiovascular problems manifesting in medical students by using measures of health such as electrocardiograms (ECGs) and blood pressure is limited.

Hypothesis: If medical school is a source of personal, career, and lifestyle stressors, then it is important to identify and assess those students whose vulnerability to these stressors has provoked abnormal ECGs, hypertension, or both.

Objective: To document abnormal ECGs and blood pressure readings that arise in a cohort of subjects during their first two years of medical school.

Materials and Methods: A bi-phasic single-arm study using a random selection algorithm to identify students for screening for hypertension and abnormal ECGs. Data was analyzed by calculating upper and lower 95% binomial confidence intervals for each parameter based on the proportion of students found to have abnormal ECG and blood pressure measurements. Potential subjects with pre-existing abnormal ECGs and/or hypertension were excluded from the study. Initial ECG readings and blood pressure measurements were obtained for incoming students of the Philadelphia College of Osteopathic Medicine class of 2012, following IRB approval for this study, followed by biweekly assessments of these parameters during the first two years of medical school. ECGs were interpreted by the participating cardiologist and blood pressures evaluated per the criteria of the *Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure*. Subjects with abnormal results were referred to a physician.

Preliminary Results-Year One: Total number of subjects enrolled=40. Two subjects withdrew for personal reasons unrelated to the study. There were 31 subjects who were documented with at least one incident of abnormally elevated blood pressure, and 26 subjects documented with at least one abnormal ECG.

Preliminary Conclusions-Year One: Preliminary data indicates that medical school stressors may have important implication for the health of these subjects. Early detection should enable timely intervention and management of these health issues in future osteopathic physicians.

S37

Classifying Enuresis, Voiding Dysfunction, and Treatment Outcomes in Children with Attention Deficit-Hyperactivity Disorder and Autism Spectrum Disorder: A Pilot Study

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Background: Enuresis and other voiding dysfunctions (VD) are debilitating to both the affected child and their families. Attention Deficit-Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD) are debilitating to the affected child and their families as well. Children who have both enuresis and VD along with ADHD or ASD are at even higher risk for social, psychological, and scholastic impairment. Studies have shown efficacy in treating children with ADHD, enuresis, and VD; however, no studies exist demonstrating treatment outcomes in children with ASD, enuresis, and VD.

Hypothesis: We believe that children with ADHD or ASD (autism and Asperger syndrome) who have enuresis and VD will respond to treatment with desmopressin (DDAVP) and antispasmodics similarly to children with enuresis and VD not diagnosed with ADHD or ASD. Accordingly, these children's urologic symptoms may not simply be a byproduct of their behavioral disorders and may be attributable to an underlying, organic cause.

Material and Methods: We performed a retrospective chart review of all patients with a presenting complaint of enuresis between January 1999 and November 2005. Complaints of enuresis, intermittent urinary incontinence during daytime (IUID), frequency, urgency, improvement or cure with DDAVP, and antispasmodic treatment were considered.

Results: Four hundred and eighteen children were identified. 77% (320/418) were children not diagnosed with either ADHD or ASD, 20% (82/418) had a diagnosis of ADHD, 2.4% (10/418) had a diagnosis of autism, and 0.7% (3/418) had a diagnosis of Asperger syndrome. In children without ADHD/ASD, 99% (318/320) had enuresis, 37% (118/320) had IUID, 40% (128/320) experienced frequency, 60% (192/320) experienced urgency, 79% (252/320) improved with DDAVP or antispasmodic treatment, and 63% (201/320) experienced complete resolution of symptoms. For children with ADHD, 99% (81/82) had enuresis, 49% (40/82) also had IUID, 45% (37/82) experienced frequency, 66% (54/82) experienced urgency, 92% (76/82) improved with DDAVP or antispasmodic treatment, and 63% (52/82) experienced complete resolution of symptoms. In children with autism, 90% (9/10) had enuresis, 90% (9/10) had IUID, 30% (3/10) experienced frequency, 90% (9/10) experienced urgency, 100% (10/10) improved with DDAVP or antispasmodic treatment, and 20% (2/10) experienced complete resolution of symptoms. In children diagnosed with Asperger syndrome, 100% (3/3) had enuresis, 67% (2/3) had IUID, 67% (2/3) experienced frequency, 67% (2/3) experienced urgency, 100% (3/3) improved with DDAVP or antispasmodic treatment, and 67% (2/3) experienced complete resolution of symptoms.

Conclusion: We successfully replicated results from previous studies showing the efficacy of DDAVP and antispasmodics in treating enuresis and VD in children with ADHD. Although no conclusion can be derived from the small sample size of patients with autism and Asperger syndrome, we feel that it shows a favorable trend toward efficacy of DDAVP and antispasmodic therapy in these children with enuresis and VD that warrants further, large scale investigation.

S41

Histomorphometric Comparison of Arterial Conduits Used During Coronary Artery Bypass Grafting of the Left Main Coronary and Posterior Interventricular Arteries

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Hypothesis: There exists a single optimally matching vessel to bypass the left main coronary artery and a single optimally matching vessel to bypass the posterior interventricular artery, the fourth and fifth most commonly encountered blockage sites of the coronary arteries, respectively.

Materials and Methods: From six cadavers, samples were taken of the bypass vessels and the left main and posterior interventricular arteries. Slides were stained with H and E and Von Geison stains. Measurements of arterial wall thickness (tunicas intima and media), lumen diameter, percent elastic and percent muscular were calculated.

Results: When examing the left main coronary artery (LM), all bypass vessels showed a statistical difference for luminal diameter ($P<.01$) and wall thickness ($P<.05$). The left internal thoracic, right gastroepiploic and right inferior epigastric arteries showed a statistical difference for percent elastic; no arteries showed a difference for percent muscle when compared to the LM. When

examining the posterior interventricular artery (PI), only one case showed a statistical difference in any parameter, with the diameter between the right radial artery (RA) ($P<.05$), with the RA being 0.8 mm larger on average. Thus, we are 95% confident to say that the diameters of the RA and PI are not equal. However, we cannot reject the null hypothesis (the mean difference is zero) for all the other values, because all have a *P* value greater than .05. *Not rejecting* the null hypothesis is not the same thing as *accepting* the null hypothesis, however. When using the student t test, one looks to show a difference. In our case, we are attempting to find similarities rather than differences. In order to attempt to find an equivocal artery, we used equivalence testing.

Conclusion: Best overall match for bypassing the left main coronary artery:

1. Right internal thoracic artery
2. Right radial artery
3. Left internal thoracic artery
4. Right gastroepiploic artery
5. Right inferior epigastric artery

Best overall match for bypassing the posterior interventricular artery:

1. Right inferior epigastric artery
2. Left internal thoracic artery
3. Right gastroepiploic artery
4. Right internal thoracic artery
5. Right radial artery

These rankings, based on anatomic features of the candidate graft vessels, should be considered along with other details specific to the patient when planning a coronary artery bypass.

Basic Sciences

S3

Obesity and Sex Specificity in the Metabolic Syndrome-A Novel of Non-mammalian Model

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Hypothesis: In a hyperphagic high weight line of chickens by inducing obesity, a sexually differentiating change in α_1 adrenergic femoral arterial response will be induced.

Materials and Methods: Animals: Twenty-four HW chickens, 12 male and 12 female, were reared from hatch to 196 days. Birds were randomly assigned to ad libitum or feed restricted treatments with 6 chickens per sex-treatment group.

Determination of Vasoconstriction: One mm chicken femoral arterial rings were mounted in a tissue bath and superfused with Krebs-Henseleit buffer (KH) at 37°C. Vessels were constricted with the addition of 80 mM KCl and washed out until maximum contraction was constant for each isolated vessel. Tissue baths were then washed out with KH until all vessels returned to and stabilized. Samples were then again constricted by the serial addition of the specific α_1 adrenergic (AR) agonist, phenylephrine at increasing concentrations from 10^{-10} through 10^{-4} M. Tissue baths were washed out with KH until all vessels stabilized. The specific antagonist of endothelial NO synthase, 10^{-5} M, g-nitro-L-Arginine-Methyl Ester (L-NAME) was added. Sixty minutes following L-NAME addition, vessels were again constricted with the serial addition of Phe.

Statistical Analysis for Vascular Experiments: Patterns in weight change, feed intake, and metabolite concentration across ages were evaluated. Body and abdominal fat weights were obtained and these data analyzed by ANOVA and *t* test.

Results: Males were heavier and had less abdominal fat than females. The percent fat to final weight was greater for females than males. Vascular data showed that in females ad libitum feeding decreased the amount of α_1 agonist required for half maximal response (EC_{50}) compared to males. With fed restriction, no statistical difference between sexes can be demonstrated. Furthermore, by eliminating NO through eNOS inhibition, differences between sexes associated with fed regimens are also eliminated. However, the ad libitum feeding, with the induction of high abdominal fat in this line of hyperphagic female chickens makes the female more sensitive to α_1AR stimulation.

Conclusions: 1. Sex-related changes in α_1 adrenergic responses elicited by manipulating body mass. 2. NO does not alter α_1 adrenergic efficacy in obesity.

S4

Ubiquitination of p75 Receptor Attenuates the Activation of Apoptotic Pathway

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Alzheimer disease (AD) is a neurodegenerative disorder characterized by global cognitive decline including a progressive loss of memory, orientation and reasoning. The neurotrophin receptor p75 is a potential mediator of AD pathogenesis. The p75 neurotrophin receptor regulates both neuronal survival as well as apoptosis. The p75 receptor interacts with TRAF6, and enhances NF-κB activation. TRAF6 possesses a RING finger domain and can function as an ubiquitin ligase. This prompted us to examine whether p75 is ubiquitinated in human control and AD brain samples. For this study, we used 6 AD and 6 control samples from post-mortem adult human brains. Our results demonstrated that ubiquitination of the p75 receptor is impaired

in samples of AD brain compared to those from non-demented controls. In control brain homogenate, p75 interacts with TRAF6 and p62, whereas in AD samples the interaction was abolished. Additionally, we show that the JNK pathway is activated in brains from AD patients but not in controls. In summary, our data suggests that in AD brain samples, p75 was not ubiquitinated due to the loss of interaction of TRAF6/p62, which could leads to activation of the apoptotic pathway.

Acknowledgment: This work was supported by the Midwestern University Office of Research and Sponsored Programs.

S5

Effect of pH, Citrate, and EDTA on Pancreatic Lipase Activity In Vitro

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Background and Aims: Irritant contact dermatitis represents a common skin condition in infants in the form of diaper dermatitis, and adult patients with fecal incontinence. Digestive enzymes present in feces, such as pancreatic lipase, are known etiological agents of skin irritation. We hypothesized that inhibiting lipase activity may reduce skin irritation due to contact with feces or stoma output. The purpose of this study was to investigate the effects of pH, citrate buffer, and EDTA on pancreatic lipase activity.

Materials and Methods: Pancreatic lipase activity was measured using a modification of a commercially available technique that utilizes the 5,5′-dithiobis(2-nitrobenzoic acid)-Dimercaptopropanol tributyrate (DTNB-BALB) method. Lipase activity was measured in phosphate buffered saline (PBS, 50 mM) and sodium citrate buffer (50 mM) in a pH range from 4 to 9, and in various concentrations of citrate buffer or sodium EDTA at a constant pH of 7.4.

Results: Lipase activity decreased as a function of pH from 375 ±7 U/L at high pH (~9) to 4±1 U/L at low pH (~5) ($P<.001$). Citrate buffer had a concentration-dependent inhibitory effect on lipase activity, falling from 1433±28 U/L at 0 mM citrate to 365±6 U/L at 50 mM ($P<0.05$). EDTA, a more potent chelator of calcium, was used to explore the possible role of calcium ion on the inhibition observed with citrate buffer. The addition of 20 mM EDTA virtually eliminated the lipase activity (3±8 U/L) ($P<.001$).

Conclusion: These results indicate that pancreatic lipase activity decreases as pH decreases. The addition of citrate buffer inhibited lipase activity at constant pH. EDTA quenched the lipase activity, presumably through the reduction in calcium ion availability. Although EDTA also chelates other divalent cations; previous studies have shown that calcium is the principle cation regulating pancreatic lipase activity. These results suggest that citrate buffer may be effective in reducing skin irritation caused by exposure to pancreatic lipase.

S6

Quantification of CFTR Protein Expression: A Comparison of Genistein-Treated and Control Male and Female Mice

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Background: The major route for cAMP-mediated chloride secretion across the intestinal epithelial apical membrane is presumed to be via the cystic fibrosis transmembrane conductance regulatory chloride channel (CFTR). The CFTR chloride channel is widely known to be activated in vitro by the naturally occurring phytoestrogen, genistein. We have previously shown in C57BL/6J mice, that daily injections of genistein (600 mg/kg body weight/day, 600G) significantly increase chloride secretion in jejunum freshly isolated from males (after 2 weeks duration) and females (after 1 week duration), compared to their genistein-free counterparts (DMSO, 0G). This study aimed to determine whether genistein-induced increases in intestinal chloride secretion were attributed to changes in CFTR protein expression.

Materials and Methods: Total CFTR protein expression was quantified, utilizing standard western blot techniques on freshly isolated jejunum removed from male and female mice exposed to either 600G or 0G. Total CFTR protein expression was normalized to total actin protein expression.

Results: CFTR expression was unchanged in female mice injected for 1 week with 600G (1.07±0.25, n=3) normalized to 0G (1.0±0, n=3), and similarly unchanged after 2 weeks injected with 600G (1.10±0.0.19, n=3) compared to 0G (1.0±0, n=3). CFTR expression was equally unaffected in male mice injected for 1 week with 600G normalized to 0G (1.24±0.18 (n=3) and 1.0±0 (n=3) respectively) and similarly unchanged after 2 weeks injected with 600G (0.99±0.12, n=3) normalized to 0G (1.0±0, n=3).

Conclusion: These data suggest that the genistein-mediated increases in intestinal chloride secretion are mediated without changing total intestinal CFTR protein expression.

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STUDENT CONTRIBUTIONS

S9

Shigella Dysenteriae and the New Small RNA Molecule, SraB

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Background: Small RNA molecules regulate many important bacterial processes. Apart from RyhB, little is known about the role of sRNA molecules in *Shigella dysenteriae*, a gram-negative bacterium that is responsible for the bloody diarrhea typical of shigellosis in humans. Thus, the ultimate goal of this project is to identify and characterize novel regulatory sRNA molecules controlling the expression of genes required for the survival and virulence of the human pathogen *S. dysenteriae*. Nothing is known about the function and the regulon of the newly discovered sRNA called SraB.

Methods: Reverse transcriptase polymerase chain reaction was used to confirm that SraB, a predicted small RNA molecule, is produced by *S. dysenteriae*. A promoter reporter study allowed us to determine the activity of SraB under different environmental temperatures, at different iron concentration, and in the presence of deoxycholic acid (DOC). An overexpression plasmid was used to induce the expression of *sraB* in *S. dysenteriae* and investigate its role, if any, in virulence.

Results: Preliminary results demonstrated that the newly discovered SraB small RNA molecule is constitutively expressed in *S. dysenteriae* and that expression is not dramatically altered by changes in environmental temperature or by the presence of DOC. Expression of *sraB* is slightly influenced by the iron concentration in the growth media. More work is being done to investigate how SraB affects virulence of *S. dysenteriae*.

Conclusion: These are only the first data to identify and begin to characterize SraB. Before definitive conclusions can be drawn, all these experiments will be repeated with statistical analysis. Five 15 percent of diarrheal cases worldwide are caused by *Shigella* and epidemics occur in underdeveloped nations. An understanding of what affects the virulence will allow us to better treat patients.

S10

Histology of the Left Stellate Ganglion in the Presence of Interventricular Septal Fibrosis

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Hypothesis: Evidence supports that the autonomic system plays a role in certain cardiovascular disease states. Ventricular arrhythmias have been associated with the level of sympathetic activation. We attempted to determine if the presence of fibrosis, a marker for previous ischemic events, correlates with an increase in the number of left stellate ganglion nerve cell bodies which is indicative of hypersympathetic stimulation to the myocardial tissue.

Materials and Methods: Left stellate ganglia and the corresponding interventricular septum of the heart were removed, sectioned and stained with hematoxylin and eosin and Masson's trichrome stain. The samples were described using a grading scale to quantify the percentage of fibrosis. Ganglion nerve cell bodies were then individually counted in three separate high-powered fields. A student's t test was used to statistically evaluate the data.

Results: Stellate ganglions were sampled from 32 cadavers. Fibrosis was present within 72% (23/32) of the interventricular septums. Nine interventricular septums were found to be free of fibrosis. For those interventricular septums that were positive for the presence of fibrosis, the mean left stellate ganglion nerve cell bodies was 39.7 (range, 26-51). For those interventricular septums that were negative for the presence of fibrosis, the mean left stellate ganglion nerve cell bodies was 34.3 (range, 27-46). The difference between the mean nerve cell bodies for interventricular septums with fibrosis and without fibrosis was found to be statistically significant (P=.046).

Conclusions: Histological changes in terms of the number of left stellate ganglion nerve cell bodies seem to be dependent upon the presence of fibrosis within the interventricular septum. Considering fibrosis of the interventricular septum is a marker for previous ischemic events, an increase in the number of nerve cell bodies of the left stellate ganglion in the presence of fibrosis suggests an association does exist between hypersympathetic stimulation to the myocardial tissue and myocardial infarction.

S12

Activation of the Cardiac Potassium Channel Herg May Be a Determinant of the Extracellular Potassium Dependency of Block of Herg by Terfenadine and Bepridil.

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Background: One form of Long QT syndrome, referred to as acquired Long QT syndrome, has been shown to primarily result from a reduction in the cardiac potassium channel HERG (human ether-a-go-go related gene) by a large number of pharmaceutical compounds. In some instances Long QT syndrome will degenerate into the potentially lethal arrhythmia torsade de pointes, characterized by a rapid heart rate and severely compromised cardiac output. Many patients requiring medication

present with abnormal serum electrolyte levels due to a variety of conditions including gastrointestinal dysfunction, renal and endocrine disorders, diuretic use, alcoholism, and aging. Extracellular electrolytes, in particular extracellular potassium, have significant influence on HERG channel behavior and have been shown to alter drug block of HERG. However, the mechanisms by which drug block is altered in different extracellular solutions are not well understood.

Materials and Methods: We used two-electrode voltage clamping of Xenopus oocytes to measure blockade of both wild-type HERG (WT) and a HERG mutant (D540K). The cRNA of either WT HERG or the D540K mutant was injected into enzymatically defolliculated oocytes and currents recorded 1 to 5 days after injection.

Results: Block of WT HERG by terfenadine and bepridil was unchanged with increasing extracellular potassium. Block of HERG by 1 uM terfenadine was 78%±1% in 0 mM K+ and 80%±1% in 20 mM K+. Block by 1 uM bepridil was 71%+1% in 0 mM K+ and 65%±3% in 20 mM K+. The mutant D540K displays an unusual gating property in that it opens upon hyperpolarization as well as depolarization. This is in contrast to WT HERG channels which only open with depolarization and then close with hyperpolarization. Block of D540K by 1 uM terfenadine and 1 uM bepridil decreased with increasing extracellular potassium. Block of D540K by 1 uM terfenadine was 90%±1% in 0 mM K+ and 83%±0% in 20 mM K+. Block of D540K by 1 uM bepridil was 58%±3% in 0 mM K+ and 31%±4% in 20 mM K+. In addition block of D540K by 1 uM bepridil in 20 mM Cs (a less permeant ion than K+) was 44%±4%.

Conclusions: Recent experiments indicate that terfenadine and bepridil can be trapped inside the channel after the channel closes and that the D540K mutant channel is unable to trap these drugs. In addition, we have reported that block of WT HERG by quinidine and cisapride, two drugs that are not trapped inside the channel after the channel closes, show a strong correlation with the permeant ion. Together these results suggest that the permeant ion is not able to destabilize a trapped drug (terfenadine and bepridil) but is able to destabilize a drug that is not trapped (quinidine and cisapride) and indicate a possible role for the activation gate in determining the extracellular potassium dependency of block of HERG.

S13

Expression of Transcriptional Co-activator With PDZ-Binding Motif (TAZ) in the Ovary

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Background: Peroxisome proliferator-activated receptor gamma (PPARγ) is a transcription factor regulating genes involved in metabolism and steroidogenesis. In ovaries, expression of PPARγ is found primarily in granulosa cells in developing follicles, as well as in theca cells and corpora lutea (CL). Thiazolidinediones (TZDs), insulin sensitizing drugs used to treat type II diabetes, bind to and activate PPARγ. TZDs can restore ovarian cycles in some women with polycystic ovary syndrome (PCOS), the leading cause of infertility in women of reproductive age. The efficacy of TZDs treating PCOS may be due to decreased peripheral insulin resistance and/or direct action via PPARγ at the ovarian level. Little is known regarding the natural regulation of PPARγ in the ovaries. Such knowledge would allow for a better understanding of various pathologies associated with the ovary, including PCOS. Recent research has shown that a novel transcription factor, TAZ, is involved in regulating PPARγ in fibroblasts.

Hypothesis: We hypothesize that TAZ may regulate PPARγ in the ovary. The present experiments were conducted to determine the expression pattern of TAZ and the quantity of mRNA for TAZ in the ovary during the reproductive cycle.

Methods and Materials: An anti-TAZ antibody was used to immunolocalize TAZ in ovarian tissue collected from female rats throughout the reproductive cycle. To measure levels of mRNA for TAZ, immature rats were treated with pregnant mares' serum gonadotropin (PMSG) to stimulate follicular development and 48 hours later treated with human chorionic gonadotropin (hCG) to mimic the luteinizing hormone surge causing ovulation and development of CL. Ovaries were collected 0 and 48 hours post PMSG, and 5 and 24 hours post hCG. RNA was isolated and processed for real-time PCR.

Results: Immunolocalization of TAZ in ovarian tissue samples demonstrated strong expression within luteal cells. There was a positive trend between the expression of mRNA for TAZ and the growth of follicles. TAZ mRNA also tended to increase with formation of CL.

Conclusion: These data illustrate that TAZ is expressed in the ovary and is primarily associated with CL. Therefore, TAZ may affect luteal expression of PPARγ. Future studies will determine if TAZ may regulate the role of PPARγ in steroidogenesis, which could impact progesterone production. Such an effect may impact fertility because luteal progesterone is essential for fertilization and implantation.

S14

Older Adults Exhibit More Intracortical Inhibition and Less Intracortical Facilitation Than Young Adults

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Hypothesis: We hypothesized that older adults (OA) would exhibit more intracortical inhibition than young adults (YA).

Materials and Methods: Using brain stimulation, we measured intracortical facilitation (ICF), short- and long-interval intracortical inhibition (SICI and LICI), and silent period duration inYA and OA (21.4±0.8 yrs and 70.9±1.8 yrs). These variables were assessed from the flexor carpi radialis of the non-dominant arm at rest and during contraction (15% of maximum).

Results: Under resting conditions, OA exhibited increased SICI and LICI (SICI: 29.0±9.2 vs. 46.2±4.8% of unconditioned pulse; LICI: 6.5±1.7 vs. 15.8±3.3% of unconditioned pulse; *P*=.04), and less ICF (74.6±8.7 vs. 104.9±6.9% of unconditioned pulse; *P*=.02). OA also exhibited a longer silent period during contraction (112.5±6.5 vs. 84.0±3.9 msec; *P*<.01).

Conclusion: These findings suggest increased GABA-mediated inhibition with age.

S15

Beta-Catenin Regulates Fine-Scale Pathfinding Behaviors of Optic Axons In Vivo

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Background: β-catenin is a cytoplasmic adaptor protein that signals in the Cadherin and Wnt pathways, two major axon guidance regulatory pathways. The roles of β-catenin in axon pathfinding in general, and in pathfinding of optic axons in the developing brain in vivo are not known.

Hypothesis: We hypothesized that β-catenin regulates several fine-scale pathfinding behaviors of optic axons in vivo, including fasciculation, directionality and growth cone protrusions.

Methods: We expressed active and inactive mutants of β-catenin and of its target gene -a-catenin- in small groups of optic axons in *Xenopus laevis* tadpoles. We assayed the effects of these mutants on defasciculation, directionality and growth cone protrusions of optic axons in the optic tract of whole mount brains.

Results: The β-catenin mutants NTERM (loss of function) and β-cat107 (gain of function) oppositely regulated the defasciculation and fine-grade directional growth of optic axons in the late optic tract in situ. NTERM and β-cat107 also selectively inhibited filopodial and lamellipodia protrusions in optic axonal growth cones. Expression of another β-catenin mutant CTERM that promotes transcriptional activation in the Wnt pathway did not alter these pathfinding behaviors of optic axons. Expression of an β-catenin mutant that inhibits interaction with β-catenin phenocopied the effects of the loss of function mutant NTERM.

Conclusions: These results suggest that β-catenin regulates several fine-scale pathfinding behaviors of optic axons in vivo, including defasciculation, directional growth and a balance between different type of growth cone protrusions. β-catenin likely regulates these pathfinding behaviors through structural pathways that are independent of gene transcription.

S16

The Effect of Treatment of Rat Granulosal Cells With High Concentrations of Homocysteine on Cystathione β Synthase (CβS) and Peroxisome Proliferator Activated Receptor-Gamma (PPARg) Gene Expression

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Context: Elevated levels of homocysteine (Hcy) are associated with reproductive sub/infertility. One potential mechanism by which elevated Hcy may interfere with fertility is by interfering with the expression and activity of PPARγ. PPARγ is a transcription factor expressed in the ovary primarily by granulosal cells (GC) of developing follicles. It is involved in regulating ovulation and steroidogenesis. Effects of Hcy on PPARγ in the ovary and the ability of the ovary to catabolize excess Hcy are not known.

Hypothesis: We hypothesized that exposure of rat GC to high levels of Hcy would (1) alter expression of PPARγ and (2) upregulate expression of CβS, an enzyme involved in regulating Hcy levels by conversion of Hcy to cysteine.

Methods: Follicular development was stimulated in immature rats by treatment with pregnant mares' serum gonadotropin (PMSG). The GC were harvested 48h later and cultured in defined media with (treated) or without (control) Hcy (18 μg/μL). Media were collected and replaced every 48h. After 6 days cells were collected and RNA isolated using Trizol. CβS and PPARγ were quantified using real-time PCR. Serum was collected from a second group of immature rats to determine circulating concentrations of Hcy during follicular growth (0 and 48 hours post-PMSG) and early luteal development (24 hours post-human chorionic gonadotropin [hCG]). Media from untreated cells and sera were assayed for Hcy using Axis-Shield. Data were analyzed by ANOVA followed with Tukey's test or *t* test using SPSS. ACUC #2009-3 WVSOM.

Results: GC are capable of producing Hcy and secretion was steady over time in culture. Treatment of GC with Hcy did not affect expression of mRNA for CβS, however, it tended to increase levels of mRNA for PPARγ. There was no difference in Hcy in sera collected during follicular development, but Hcy was elevated in sera 24 hours post-hCG compared to 48 hours post-PMSG.

Conclusions: The ability of GC to produce Hcy, and lack of effect of elevated Hcy on CβS mRNA might contribute to elevated follicular fluid concentrations of this amino acid which is associated with poor outcome in women undergoing IVF. From these data it appears that elevated Hcy might also impact ovarian function by modulating expression of PPARγ. Future studies will determine how Hcy impacts activity of CβS and PPARγ-medi-

ated gene expression, and investigate whether increased concentrations of circulating Hcy 24 hours post-hCG is due to ovarian or peripheral production.

S19

Investigation of Lower Extremity Aggregate Fascial 3D Tissue Geometry Using Visual Mapping Techniques and the Visible Human Male Image Set

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Hypothesis: The functional fascial architecture of the lower extremity (LE) involves the mechanical stressing and relaxation of interlocking anatomical components embedded within its configuration. These complex tissue interactions may be modeled using the dynamic tension-compression principles of tensegrity. This composite configuration may be thought of as a three-dimensional (3D) latticework of tension-producing radial, longitudinal and circumferential ligaments interspersed with compressive elements resulting in a dynamic, self-correcting 3D strutwork. There does not yet exist a precise visual mapping of this strutwork in such a form as to allow experimental exploration and biomechanical simulation. Our hypotheses is that the complex LE fascial assembly may be precisely represented as a set of digital data components that may be used as a visualization tool for anatomical simulations leading to biomechanical analysis.

Methods: We generated 3D surface models from sequential 2D vectorized contours of LE gross cross-sections, including the fascial layers, using cadaveric *Visible Human Male* images. This was accomplished using open source and commercial software tools. Finally, the spline-based sequential contours thus defined for each class of anatomical structure were lofted to generate renderable 3D polymesh objects.

Results: Our methods resulted in the synthesis of a comprehensive set of computable anatomical surface models representing LE skin, bone, muscle and fascia (both superficial and investing). This object library was comprised of 83 models. When these were rendered and visualized selectively in 3D space, they reconstructed desired LE volumetric anatomy of interest for this dataset with precision.

Conclusion: The results strongly suggest that complex anatomy may be modeled and rendered to illuminate the complex configurations described in the Background section using semi-automated, easily implemented digital techniques. The novel result of this work is the precise modeling and visualization of LE fascial architecture relative to its enclosed anatomy. Since all fascial components present were extracted as a unified model, this aggregate structure represents an anatomical unknown and will be investigated further to reveal what we believe may be a hierarchical assembly of connective tissue components extending from integument to periosteum. Our ultimate aim is to generate biomechanical simulations leading to optimized OMM techniques.

S20

Biomechanical Changes in Sacroiliac Joint Ankylosis - A Finite Element Study

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Background: Although sacroiliac (SI) joint ankylosis is common past the third decade, joint stiffening is often asymmetric. Previous studies have reported that as many as 77% of individuals over the age of 30 have asymmetries observable by CT. Alterations in motion, biomechanics, and stress distributions following asymmetric sacroiliac joint ankylosis are difficult to quantify in vivo, making the finite element method an ideal approach for studying these cases.

Hypothesis: Asymmetric SI joint ankylosis will lead to altered motion and stress distributions at both the ipsilateral and contralateral SI joints.

Materials and Methods: Finite element models were constructed in AMIRA 3.1.1 using frozen CT images from the male visible human project. Surface meshes were imported into MSC.Marc. All surface models were meshed with linear tetrahedral elements to create solid meshes. All ligaments (anterior sacroiliac, interosseous, inner posterior, long posterior, sacrotuberous, and sacrospinous) were modeled as 3D non-linear elastic truss elements. A cortical shell was simulated on both the sacrum and the iliac bones by adding a 1 and 3mm thick shell respectively. The SI joint cartilage was modeled as a 3D contact body between bony surfaces of the sacrum and ilium. In the ankylosed case, the left SI joint was fused by conferring bony material properties, rather than cartilaginous properties, to the 3D contact body in the joint space. 400N compressive loads at the sacral base were coupled with 40N*m flexion and extension moments while the acetabular cups were fixed. Angular motion at the sacral aspect of the SI joint and Von Mises equivalent of stress were measured for each case.

Results: When the 400N load was coupled with a 40N*m flexion moment, the ankylosed SI joint experienced a 9.6% increase in stress and a 74% decrease in the degree of flexion. The contralateral joint saw a 33.7% stress decrease and a 6.5% decrease in the flexion angle. For the 400N load coupled with a 40N*m extension moment, the stress at the ankylosed joint increased by 173%, while the joint angulation decreased by 4.3%. The contralateral joint experienced a 4.4% decrease in stress and a 0.3% decrease in angulation.

Conclusion: Following unilateral SI joint ankylosis, the fused joint experiences increased stresses and a decrease in motion in both flexion and extension. Both stresses and motion are decreased to a much smaller degree at the contralateral SI joint.

S21

Differential Osteopontin and NKT Cell Responses to RSV Infection in Neonates and Adults Determines the Subsequent Differential Adaptive Immune Responses and Susceptibility to Asthma

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Hypothesis: Pediatric RSV infection, as opposed to that in adults, is associated with a differential osteopontin and NKT cell response that skews the differentiation of dendritic cells to develop a subsequent Th2 adaptive immune response and hence, susceptibility to asthma in later life.

Materials and Methods: We infected neonatal and adult BALB/C mice with intranasal RSV. The lungs were collected for flowcytometric analysis to identify NKT cell ($CD19^{-}CD4^{+}CD1d^{+}$), plasmacytoid dendritic cells (pDC; $CD11b^{lo/-}$ $CD11c^{lo/-}$ $CD45Rb^{hi}$), and myeloid dendritic cells (mDC; $CD11b^{lo/-}CD11c^{hi}$ $CD45Rb^{lo/-}$). In selected experiments, bronchoalveolar lavage fluid (BALF) was collected to determine the amount of osteopontin produced by ELISA. The Th1 (IFN-?) and Th2 (IL-10) cytokines profile in BALF was detected by ELISA.

Results: Production of osteopontin in bronchoalveolar lavage fluid was markedly reduced in RSV-infected neonates compared to adults as revealed by ELISA. Flowcytometry analysis determined that there was an associated enhanced NKT cell ($CD19^{-}CD4^{+}CD1d^{+}$) response in the RSV-infected neonatal lungs compared to the infected adult lungs. In addition, polarization of neonatal lung DCs was skewed towards pDC ($CD11b^{lo/-}$ $CD11c^{lo/-}CD45Rb^{hi}$) over Mdc ($CD11b^{lo/-}CD11c^{hi}$ $CD45Rb^{lo/-}$). Finally, production of Th1 (IFN-?) and Th2 (IL-10) cytokines were markedly reduced and enhanced, respectively, in the neonatal lungs as opposed to the adult lungs in response to RSV-infection.

Conclusion: Differential osteopontin and NKT cell responses to RSV infection in neonates and adults modulate the subsequent pulmonary immune responses differently (Th1 in adults and Th2 in neonates) in these two age groups. Therefore, clinical interventions including exogenous osteopontin administration may induce an enhanced Th1 response to RSV infection in the neonates and may prevent development of bronchial asthma in later life.

♦S22

Genome-Wide Transcriptional Profile of Aging in Dermal Fibroblasts

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Hypothesis: Aging skin is an elusive yet highly investigated topic with widespread dermatologic implications including wound healing and cancer. Although there are many studies examining aging skin, the underlying molecular mechanisms illustrated by gene expression have not been demonstrated. An increasing aging population demands an understanding of aging skin at the molecular level, which is examined here with a transcriptional profile of dermal fibroblasts.

Materials and Method: Genome-wide steady-state mRNA levels in serum-deprived, quiescent human diploid fibroblasts from healthy donors aged 22 to 92 were measured. Fibroblast cultures, established from skin samples, were obtained from the GRC/NIA cell repository at the Coriell Institute. The donors were members of the Baltimore Longitudinal Study of Aging and were medically examined and diagnosed as "healthy." Cell lines were cultured and after 24 hours serum starvation, cell lysates were prepared and total RNA was isolated using an established protocol. Gene expression data were obtained using the genome-wide human Codelink bioarray containing 55,000 single-stranded oligonucleotide probes (GE/Applied Microarrays). Differential expressions of characterized genes between the young and old groups were determined with a cutoff $\pm$ 2.5 fold.

Results: Cells from old donors, compared to a group of young controls, contained increased transcript levels of genes encoding cytokines, chemokines, complement cascade, MHC I type molecular complexes, anti-apoptosis, and glycolysis. Conversely, there was decreased expression of genes encoding ribosomal, electron transport chain, and mitochondrial proteins. Based on gene ontologies, gene sets expressed in cells derived from old donors have been implicated in inflammation, stress, ubiquitination, glycolysis, and calcium binding. Prevailing groups of genes repressed in cells from old donors encode proteins in the electron transport chain, ribosomal and cytoskeletal proteins.

Conclusion: The observed transcriptional changes, affecting many metabolic processes, may be a consequence of mitochondrial respiratory dysfunction with increasing human age. Our results are consistent with the view that low-grade inflammation, a hallmark of many age-associated diseases, is a cell-autonomous phenomenon part of a cellular survival process in aging fibroblasts possibly in response to compromised mitochondrial function.

♦Indicates poster that won the 2009 Student Poster Competition.

S24

Dissecting Cellular Pathways of Oncogenic Ras Isoforms

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Background: Cancer is the second leading cause of death in the United States. It was estimated that 1,437,180 people were diagnosed with and 565,650 people died of cancer in 2008. In 2005, cancer care cost taxpayers $209.9 billion and the costs are rising every year. A better understanding of the cancer biology is needed to help the development of effective cancer therapeutic intervention. Cancer cells are known to harbor multiple genetic changes, including activations of oncogenes and inactivations of tumor suppressor genes. For instance, ras gene encodes a GTP-bound protein that, upon activation, mediates various important cellular responses through cascades of protein kinase signaling pathways. A single nucleotide mutation in codon 12, 13, or 61 of the ras gene results in constitutive activation of the Ras proteins, which exhibit oncogenic potential. Importantly, activated mutations in the ras gene are found in 30% of all human cancers. Four ras genes have been identified in mammalian cells: H-ras, K-ras (4A, 4B), N-ras. All Ras proteins share highly homologous sequences in their N-termini, but each has a unique C-terminus. Studies have suggested that activated mutations in each ras gene are associated with specific types of cancer. It is, however, unclear whether activation of these Ras isoforms triggers different biological activities in human cells. The goal of this research proposal is aimed at dissecting the cell signaling pathways mediated by oncogenic Ras isoforms.

Hypotheses: Activation of each Ras isoform is linked to a specific signaling pathway, leading to differential cellular responses. We also hypothesize that specific codon mutations in the ras gene may trigger differential cellular outcomes.

Materials and Methods: Human normal fibroblasts as well as tumor cell lines will be retrovirally transduced with H-RasV12, N-RasV12, K-RasV12, K-RasL61 or an empty vector control. Cells were selected and monitored for cell growth and changes in the expression of various signaling proteins.

Results: Expression of each of the Ras isoform trigger differential cellular outcomes likely through distinct signaling pathways

Conclusion: Specific oncogenic Ras proteins are associated with distinct types of cancers. Understanding the differential signaling pathways mediated by specific oncogenic Ras isoforms may impact future development of pharmacological agents that will be more specific in targeting and treating the underlying cause of cancer.

S25

Bee Venom Inhibits Cell Growth of SKBR3, MCF-7, and MDA-MB-231 Breast Cancer Cells

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Background: Bee venom (BV) is a popular homeopathic treatment for rheumatoid arthritis due to its anti-inflammatory properties. Recent research investigated the effects of BV as a possible cancer treatment. Current data has suggested that BV works through a Bcl-2 dependent mechanism. Interestingly, breast cancer cells overexpress the protein Bcl-2, an anti-apoptotic protein, making it an ideal model to investigate. Furthermore, the National Cancer Institute estimates that in 2009 more than 194,200 patients in the United States will be newly diagnosed with Breast Cancer, and for more than 40,500 patients the disease will be fatal.

Hypothesis: It is our hypothesis that SKBR3, MDA-MB-231, and MCF-7 breast cancer cells will exhibit varying degrees of apoptosis when treated in a time and dose dependent manner with BV. The BV will selectively induce apoptosis in these breast cancer cells based primarily on the expression of Bcl-2. The long-term goal of this project is to understand the role BV plays in cancer cell apoptosis.

Materials and Methods: Western blots were used to verify the expression of Bcl-2 in each cell line. SKBR3, MDA-MB-231, and MCF-7 breast cancer cells were cultured in normal media containing serum and treated in a dose and time dependent manner with BV. Treated cells were then assayed using the MTT Assay and the LDH assay. All TD50s were calculated using SigmaPlot. Use of DAPI staining to investigate nuclear structure confirmed the induction of cell death in these cells.

Results: Our data indicates that BV induced cell death in each of the three breast cancer cell lines evaluated, independent of Bcl-2 expression. Western blot revealed that Bcl-2 was present in high amounts in MCF-7 and SKBR3 cells, but not present in MDA-MB-231 cells. Apoptosis still occurred irrespective of the cell line evaluated. The TD50s were calculated for the following cells: SKBR3 — 10.54 ug/mL, MDA-MB-231 — 15.54 ug/mL, and MCF-7 — 10.72 ug/mL. The LDH assay and DAPI staining further confirmed these findings.

Conclusion: Recent data, although limited, suggests that BV induces cancer cell death through a Bcl-2 dependent mechanism. However, our results indicate that BV also induces apoptosis within MDA-MB-231 cells, a Bcl-2 negative cell line. This suggests that cell death is occurring through a Bcl-2 independent pathway. We must now determine through which mechanism the BV is working in MDA-MB-231 cells.

STUDENT CONTRIBUTIONS

S28

Ischemia and Reperfusion Injury of Mouse HL-1 Cells Treated With Fas Antagonist

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Background: It is shown that Fas ligand is expressed during ischemia/reperfusion which results in apoptosis of cardiac cells. The Akt/glycogen synthase kinase-3b (GSK3b) pathway also plays an important role in the cell death/survival pathway after a variety of cell death stimuli. Furthermore, the sustained elevation of ribosomal S6 protein kinase, which can be activated by p38, is shown to precede apoptosis of cardiac cells. The hypothesis of the study is that Kp7-6, a Fas antagonist, will alter ischemia/reperfusion-induced caspase 3 activity, Akt/GSK3b activity, and phosphorylation of p38 and ribosomal protein S6 in mouse HL-1 cells and, therefore, potentially reduce injuries during induced ischemia and reperfusion.

Materials and Methods: Mouse HL-1 cardiac myocytes were used in this study of ischemia/reperfusion injury. The cells were placed in an ischemia buffer in an anoxic chamber for 2 hours to achieve ischemia. The cells were then reperfused for 6 hours with normoxic Krebs-Henseleit buffer containing either 1 mg/mL Kp7-6 or vehicle control and caspase 3 activity was measured. Alternatively, cells were reperfused for up to 2 hours and the phosphorylation, and hence activation state, of Akt, GSK3b, p38 and S6 was measured using Western blotting.

Results: Following ischemia/reperfusion, elevated caspase 3 activity was noted. However, caspase 3 activity was decreased in cells treated with Kp7-6. Ischemia/reperfusion caused a transient decrease in Akt and GSK3b phosphorylation, which peaked at 5-15 minutes and returned to baseline at 2 hours, with no significant difference when treated with the Fas antagonist. In contrast, the phosphorylation state of p38 and ribosomal protein S6 was increased at 2 hours of reperfusion, and was reduced in cells receiving Kp7-6.

Conclusion: Inhibition of Fas during the reperfusion phase of ischemia/reperfusion, decreases caspase 3 activity and activation of p38 and ribosomal protein S6, serving as a protective factor against apoptosis of cardiac cells. However, the cross protection of Fas inhibition against the phosphorylation of Akt/GSK3-β was not demonstrated. The results indicate that Fas inhibition may play a significant role in the cell death/survival pathway during reperfusion therapy after a myocardial infarction.

Acknowledgment: This research was supported by a WVSOM intramural grant.

S30

Paclitaxel Increases Ischemia/Reperfusion Injury in Cardiomyocytes

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Background: Recovery of cardiomyocytes following periods of ischemia depends upon adequate reperfusion. However, this causes further damage known as reperfusion injury. Two consequences of ischemia/reperfusion (IR) are apoptosis and necrosis, which can increase infarct size and contribute to long-term morbidity and mortality post-myocardial infarction (MI). In some individuals who suffer an MI, reperfusion therapy consists of the placement of drug-eluting stents. One such drug, paclitaxel, inhibits cell proliferation by stabilizing microtubules, thus causing G2-M cell cycle arrest and preventing smooth muscle proliferation, excessive neointima formation and restenosis. However, paclitaxel is also used as a cancer chemotherapeutic agent due to its ability to induce apoptosis. Therefore, is it possible that paclitaxel could exacerbate cell death in cardiac myocytes?

Hypothesis: Paclitaxel enhances IR-induced apoptosis and necrosis in cardiac myocytes potentially causing further damage post-MI.

Materials and Methods: HL-1 cardiomyocytes received two hours of ischemia, followed by two to six hours of reperfusion. During reperfusion, cells were treated with 10 or 1000 ng/mL of paclitaxel or vehicle control. At the completion of reperfusion, apoptosis and necrosis was determined by measuring caspase 3 activity and LDH release, respectively.

Results: Two hours of ischemia alone did not activate caspase 3. In contrast, ischemia followed by 6 hours of reperfusion induced apoptosis as measured by an increase in caspase 3 activity (.084±.009 vs .187±.028 pmol/min/mg protein, $P<.05$). While low dose paclitaxel had no effect on this IR-induced caspase 3 activation, high dose paclitaxel potentiated the effect (.187±.028 vs .304±.06 pmol/min/mg protein, $P<.05$). In contrast, paclitaxel alone did not induce caspase 3 activity in HL-1 cardiomyocytes. Similarly, preliminary data suggest that paclitaxel augments IR-induced LDH release, an indicator of necrosis.

Conclusion: In HL-1 cells, paclitaxel potentiated IR-induced necrosis and apoptosis. Reperfusion therapy is a critical factor in treating an MI. However, a certain amount of cell death inevitably occurs by both apoptosis and necrosis. These results indicate that paclitaxel, which would be released from drug-eluting stents during reperfusion, has the potential for augmenting reperfusion injury of cardiomyocytes.

Acknowledgment: This research was funded by a WVSOM intramural grant.

S31

TRPM8 Expression is Higher in Smaller Neurons of Human Trigeminal Ganglion

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Background: TRPM8 is a non-selective cation channel involved in the integration and transmission of sensory information in pain and neurogenic inflammation. It is a temperature-sensitive channel activated by both innocuous and noxious cold temperatures, aiding in our ability to perceive and avoid potentially harmful thermal conditions. TRPM8 has been implicated in many prevalent pain disorders.

Hypothesis: Smaller neurons, those likely responsible for the transduction of pain and temperature, will express more TRPM8 (as measured by immunoreactivity).

Materials and Methods: Four human trigeminal ganglia (TG) were sectioned (12 μm) onto slides, incubated (20 min) in 0.6% H2O2 followed by 1 hour incubation in normal goat serum (1:25). Samples were then incubated for 24 hours with anti-TRPM8 antibody (1:100), normal goat serum (1:25), and Triton-X (1:100). Following rinsing, a biotinylated secondary anti-rabbit antibody with normal goat serum (1:25) was applied to the TG samples. Vectastain Elite ABC Reagent was applied and immunoreactivity revealed with DAB. All solutions were diluted in PBS. The TG sample slides were rinsed, dehydrated, cleared, cover-slipped, and viewed under the Zeiss Axioscope 2 microscope. Collected images were analyzed with Image J Software. Mean grey values, membrane staining, and signal to noise ratios (SNRs) were determined and statistical analyses with t tests were performed.

Results: Mean grey values among TG cells decreased as cell size increased. Results showed 84% of small-diameter neurons with TRPM8 produced immunopositive membranes, whereas only 57% of medium-diameter neurons and 32% of large-diameter neurons were immunopositive. Neurons with immunopositive membranes had higher SNRs. All of the data were statistically significant.

Conclusion: We determined there is a correlation between TRPM8 expression and neuron size in the human TG. Smaller neurons (more likely to be nociceptive) expressed more TRPM8, versus the larger neurons involved in the perception of pressure and touch. Our research supports the osteopathic principle that structure and function are interdependent. Specific physiological functions are linked to neuron size, thus changes in these components can lead to alterations in the perception of pain. Changing the neuron membrane anatomy with TRPM8 will change its function. Our results suggest the development of novel non-narcotic analgesics can be targeted to TRPM8 channels.

S33

Monocrotaline-Induced Pulmonary Hypertension in the Rat Downregulates Oxytocin Receptors in Right Ventricular Hypertrophy

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Hypothesis: Pulmonary hypertension (PH) ultimately causes failure of right ventricle (RV) with a lethal outcome. PH can be induced in the rat with monocrotaline (MCT), a pyrrolizidine alkaloid from the plant *Crotolaria spectabilis* that damages the pulmonary artery endothelium leading to thickening of the pulmonary arteries and increased vascular resistance. This subsequently results in RV hypertrophy, inflammation, nitric oxide (NO)-associated coronary endothelial dysfunction and increment of natriuretic peptides (NP) in the circulation. We verified the hypothesis that pathogenesis of PH involves the oxytocin receptor (OTR) because of its functional association with inflammatory cytokines and release of atrial natriuretic peptide (ANP) and NO.

Methods: Male Sprague-Dawley rats weighing 220-250 g received a single subcutaneous injection of 60 mg/kg of MCT. Six to 7 weeks following the injection, rats were sacrificed and gene and protein expression were detected by real-time PCR and western-blot analysis, respectively, in the RV and LV (left ventricle).

Results: MCT-treated rats displayed significant increases in lung weight and RV weight. RV hypertrophy was evident as the ratio of the RV to LV plus septum weight was significantly higher in MCT-treated rats compared to control rats. MCT treatment increased transcripts of ANP (3.7-fold in the LV and 8-fold in RV) and brain NP (2.7-fold in the LV and 10-fold in RV). Transcripts for three NP receptors significantly increased (0.3-2 fold) only in the RV. iNOS (inductible NO synthase) protein expression also increased selectively in the RV. In contrast, the endothelial NOS and neural NOS transcripts heightened (0.5-2 fold) in the LV. Both OTR mRNA and protein were decreased by 50% in the RV, whereas an up-regulation of cytokines IL-1β and IL-6 was observed. Nab1 mRNA, a marker of pathological hypertrophy, increased two-fold in the RV.

Conclusion: Increased gene expression of NP in the RV of the MCT-treated rat correlates with upregulation of NP receptor transcripts indicating local NP action in the RV during PH. OTR expression is attenuated in the RV possibly by inflammatory cytokines because OTR promoter region contains multiple putative interleukin-response elements. Lowering OTR in RV during pulmonary hypertension can influence cardiac function since OT regulates heart rate and cardiac contractility.

STUDENT CONTRIBUTIONS

S35

Design, Synthesis, and Characterization of 6-beta Naltrexol Analogs, and Their Selectivity for In Vitro Opioid Receptor Subtypes

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Hypothesis: The global hypothesis is that substituents at C6, coupled with conformation of the C ring, influence molecule binding to specific functionally relevant mu opioid receptor (MOR) conformations.

Materials and Methods: Standard synthetic methodologies were employed to generate compounds. Gravity flash chromatography with silica gel was used in compound purification. The structures of final compounds were characterized using 1D and 2D experiments on a Jeol 300 MHz NMR spectrometer. High Resolution Mass Spectrometry was completed by contract with the University of Illinois. Biological assays were executed by Dr. Rothman's lab using previously established procedures.

Results and Discussion: Because the mu opioid receptor (MOR) is known to be involved in therapeutically relevant pathways that lead to pain and addiction, we are currently studying the specific structural characteristics that promote antagonism at the MOR. The opiates 6β-naltrexol and 6β-naltrexamide function as neutral antagonists in in vitro and in vivo systems previously exposed to the mu agonist morphine, and are under investigation as improved treatments for narcotic dependence. In this research, we synthesized four new naltrexone derivatives that do not contain a protic group at C_6 (mesylates 20 (6a) and 14 (6b), 6b-tosylate 21, and 6b-carbamate 22) using a reduction, protection, O-derivatization and deprotection sequence. These four compounds and their intermediates were characterized by ^{1}H and ^{13}C NMR, IR, and HRMS prior to evaluation in two types of in vitro biochemical assays: (1) opioid receptor affinity assays and (2) functional binding assays in opiate-dependent cells. Affinity assays indicate that 14 binds with nanomolar affinity to the MOR, although it is the least subtype selective compound in the 6b-series. Functional binding characterization of 14 proved that the mesylate functioned as a partial inverse agonist (IA) rather than a neutral antagonist (NA). We are currently awaiting data on the three 6b-derivatives synthesized to further investigate how C_6 stereochemistry impacts their subtype specificity and functional behavior.

Conclusion: Overall, our collective results suggest that the absence of a hydrogen-bond donor on the C_6 oxygen enhances rather than impedes the in vitro affinity of naltrexol derivatives for the MOR. Furthermore, we deduce that C_6 substituent identity can be altered from naltrexol's –OH or naltrexamide's amide to hone the desired functional properties of an antagonist.

S36

Examination of Microorganisms in Traditional Cultured Milk Kefir

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Background: Over the course of history, different and isolated communities worldwide developed methods for preserving dairy products through microbial fermentation. The original cultures and methods used for dairy fermentation, including kefir, have continued to be handed down through family members or friends. While those traditions have remained relatively unknown to the mainstream US consumer, recent studies have shown evidence for notable therapeutic benefits of probiotic fermented milks. In practical terms however, kefir fermentation is usually carried out under the unsterile and varying conditions of a household kitchen. These conditions represent significant potential for introduction and growth of pathological organisms and may pose a health risk. An understanding of the microbial composition of the milk kefir is required to understand the potential health benefits and risks associated with its preparation and consumption.

Hypothesis: Our goal was to study the microbial composition of a traditional, self-propagating milk kefir culture (kefir "grains"). We hypothesize that kefir grains comprise a polymicrobial colony existing in a highly organized matrix.

Methods: The kefir grains used in this study were obtained from a household where they were used to culture milk that was consumed on a daily basis. Every 2 days the grains were propagated by straining and placing in fresh, pasteurized milk, and left at room temperature. Examination of the grains involved: (1) morphological study through light, scanning electron, and transmission electron microscopy; and (2) identification of component microorganisms by differential plating and staining methods.

Results: Morphological examination of the kefir grains revealed a network of bacteria and yeasts held together by a thick matrix. Bacterial and yeast cells appeared to be distributed differently within the grain: the grain wall was composed of tightly-packed bacilli, while the inner and outer surfaces were primarily yeasts. This distribution of microorganisms likely reflects the symbiosis and function of each of the organisms within the grain structure. Identification of isolated colonies revealed the presence of at least five different organisms that were characterized in this study.

Conclusion: Our results demonstrate the complexity of kefir grains and suggest that further investigation may significantly contribute to our knowledge of the benefits of kefir and our ability to assure its safety.

S38

Quantitative Analysis of the Human Facial Nucleus in Autism

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Hypothesis: Autistic children exhibit deficits in facial expression. To account for this, we hypothesize a derangement in the organization of the facial nucleus. To explore this possibility, we are investigating the cytoarchitecture of the facial nucleus in postmortem brain tissue from the Autism Tissue Program. We conducted the first quantitative analysis using a control brainstem, of an 8-year-old, and two autistic brainstems, of an 8- and 2-year-old, to determine how measured morphological differences play a role in specific autistic symptomatology.

Materials and Methods: Brainstem tissue was obtained from the Autism Tissue Program. This presentation is based on control tissue from an 8-year-old female and tissue from two individuals diagnosed with autism (8- and 2-year-old males). Brainstem tissue was sectioned at a thickness of 50 microns and stored in 70% EtOH. Every 4th free-floating section was mounted onto glass slides, air-dried and stained for Nïssl substance using Giemsa.

Results: Tissue sections were examined using an Olympus BX45 microscope and photographed with an Olympus DP12 digital camera. A collection of 10 human brainstems was used as a reference for locating the facial nucleus in autistic tissue. For morphometric analyses, only neurons with visible nucleoli and contours away from the surface of the tissue section were selected; only cell bodies that were completely within the tissue section were included. Cells bodies were traced with the aid of a drawing tube; tracings were digitized and grayscale tracings were analyzed using ImageJ software (at http://rsb.info.nih.gov/ij/). An index of circularity was calculated for each soma using the following:

$$\text{Circularity} = [4\pi * \text{Area} / \text{Perimeter}^2]$$

The 8-year-old control sample had 9388 neurons in the facial nucleus, averaging neuronal area of 487.53 sq. microns and circularity of 0.595. The 8-year-old Autistic sample's neuron count was 16384, area of 608.21 sq. microns, and circularity of 0.527. The 2-year-old Autistic sample had a neuron count of 536, area of 602.13, and circularity of 0.663.

Conclusion: Results show increased neuron count in the 8-year-old Autistic brain compared to controls. The parameters measured to assess neuron morphology indicate differences in neuronal structure. The 2-year-old Autistic brain had a reduced neuron count and increased circularity differing from the 8-year-old Autistic brain; this data set is inconclusive, more age-matched samples need to be studied to develop conclusions.

Medical Education

S2

Evaluation of Medical Student Stress and Well-Being

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Hypothesis: Preparation for a career in medicine is a long and arduous journey. Medical students are reported to have a higher perceived level of stress than their peers and were expected to have increased manifestations of depression and anxiety. This study examined the perceptions, manifestations and management approaches to stress in a cohort of second year Preclinical medical students.

Materials and Methods: A survey was developed and presented to a class of medical students at the completion of their second year course work. The survey was designed to assess perceived stress Before and After matriculation to medical school. Categorical questions were used to examine behaviors indicative of stress and measures used by students to relieve their stress. Other factors assessed included: relationship and support structures, age, gender, marital status and children. Data were analyzed using SPSS 17.0. Descriptive measures were reported as frequencies and factorial analysis was performed using a chi-square procedure with a significance threshold of α=.05. Student participation was voluntary and anonymous; this project was approved by the University IRB Committee.

Results: The response rate was 96% (205/214). A significant increase in stress was noted during the preclinical years. A variety of stress indicators were noted to increase in students experiencing stress, most notably: concentration difficulties sleep problems, anxiety/depression, weight gain, contemplating withdrawal from school and prescribed use of antidepressants and anxiolytics. Interestingly, 26% of the students reported no stress related problems. Students in the lowest academic quartile appeared to experience the most stress. Long distance relationships tended to deteriorate during the preclinical years and related to the stress. Gender had no apparent influence on perception of stress. Eating and exercise were the two most common stress relief measures reported. Mitigating factors included marriage, children, close friends at school and family support.

Conclusion: The preclinical years of medical education are a stress-inducing endeavor that can take a significant toll of a student's mental and physical performance. Medical school students, faculty, and administrators need to be aware of the indi-

cators of stress and the risk of these leading to anxiety disorders and frank depression. Measures such as family support and a network of friends can serve to moderate the impact of the stress.

S11

Anterior Cruciate Ligament Function in Osteopathic Medical Education

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Hypothesis: The anterior cruciate ligament (ACL) of the knee and the function of its' anteromedial (AM) and posterolateral (PL) bundles, have become a focus of orthopedic research. The purpose of this project is to asses the degree to which the AM and PL bundles are discussed within basic science medical curricula.

Materials and Methods: A survey was mailed to all osteopathic medical schools within the United States accredited through the Commission on Osteopathic College Accreditation (COCA) addressing different aspects of anatomical ACL education.

Results: Nine (42%) institutions indicated that the AM and PL bundles of the ACL are discussed within their basic science curricula, while 12 (57.14%) do not discuss them. Four (44%) discuss that the bundles are parallel in extension and crossed in flexion. Nine (44%) instructed that the AM bundle is a major anterior-posterior restrictor, 12 (56%) schools discuss the kinematic contribution of the PL as a major rotational stabilizer of the ACL. Seven (33%) schools identified the AM and PL bundles via direct visualization during anatomical dissection of the ACL.

Conclusion: Due to the probability that 3rd and 4th year medical students will encounter ACL injuries during their clinical rotations; it is paramount that students fully understand the function of the ACL to ensure effective use of osteopathic diagnostic techniques. This in-turn requires an understanding of AM and PL bundles as the two distinct functional components of the ACL. Our findings suggest the need for enhanced presentation of the AM and PL bundles within basic science medical education, thereby providing a more comprehensive anatomical education in osteopathic medical institutions.

S26

The Virginia College of Osteopathic Medicine Emergency Response Model for Honduras Disaster Relief and Future Mission Trips

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♦ Indicates poster that won the 2009 Student Poster Competition.

Hypothesis: The implementation of a disaster response model paired with a written medical student documentation of their immersion experience will allow for both: future streamlined disaster response in addition to a greater educational experience for the medical student.

Materials and Methods: A Medical Mission Team of 50 VCOM faculty doctors, students and staff traveled to Tegucigalpa, Honduras November 21-28, 2008, a few weeks after severe flooding in the country. Hundreds of thousands of Hondurans were forced from their homes and exposed to dangerous living conditions, diseases and lack of health care and related interventions. The VCOM Mission Team provided emergency medical care and distributed food, clothing, and supplies to the poorest and most affected regions. By the time VCOM students and doctors arrived, the situation was stable and the emphasis of the trip was primary care.

Results: The purpose of the research was to (1) determine the effectiveness of an emergency response model intervention and (2) assess a protocol for student reflections on lessons learned. The primary strategy was testing an organizational model to provide food, clothing and medications and to pilot test a protocol for student documentation of their experiences. Program planning and implementation elements were clustered in four areas: (1) Application and Registration Process; (2) Orientation Sessions and Materials; (3) Accommodations and Logistics; (4) Education and Mission Trip Experiences. Based on analyses of experiences gained, the four organizational clusters proved successful in simplifying complex logistical and management elements. This original research has led to further development of diagrams, forms and flow charts to create a complete portfolio and framework for mission trips including disaster response. These materials now reside on the VCOM intranet. The protocol for student reflections included (1) Introduction, (2) Discussion of Key Issues/Questions, (3) Diagnosis, (4) Treatment Decision, (5) Conclusion and (6) Reflections along with pictures obtained with patient release forms.

Conclusion: The reflective framework was successful to help students analyze their learning experiences. Findings indicated that preceptor review and signature on student reflections added clarity and factual presentation as well as contributing to the student learning experience. Further refinement is in process for producing medical mission journals published by students

♦S27

A Study to Investigate the Efficacy of a Novel Interactive Web-Based Virtual Clinical Scenario System (Virtual People Factory) in Medical Education

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Background: In recent years, there have been numerous innovations and developments with web-based virtual patient (VP) simulations. Despite improvements to these learning tools, VP simulations are still limited by a labor intensive and highly technical development process, lack of a common platform, and an inability to mimic human-to-human conversational fluidity. A team of developers at the University of Florida have built a software platform (Virtual People Factory, VPF) that implements a uniquely designed conversational technology (Human-centered Distributed Conversational Modeling, HDCM) that addresses the fore mentioned issues. Using this software, we have created a web-based interactive clinical scenario that enlists users for rapid and robust script development. Our goal is to demonstrate that an encounter with the VPF software will be found to have an educational value and increase medical student preparedness for actual standardized patient encounters.

Methods: Forty-six second year medical students at Georgia Campus–Philadelphia College of Osteopathic Medicine (GA-PCOM) used VPF to interact with a VP complaining of a suspicious headache, general malaise, fever, and nuchal rigidity. Following a brief online tutorial of the platform, students were directed to the VPF via a web link where students accessed the clinical vignette. Students were allowed to obtain an H&P, request lab results, and other diagnostic tests. The testing was a two-fold process. First, script errors were identified by the users and quickly corrected by a script editor. Upon completion users received performance feedback and completed a survey regarding the educational value of the application.

Results: Of the students in the test group, 63.27% found educational value in the VPF (rating >3), 71.43% found the system to be easy to use (rating >3), 26.53% found the virtual patient to simulate a real-life interaction (rating >3), 79.59% found the program to be beneficial in their preparation for the live standardized patient encounter (rating = yes), 91.84 % would like to have the virtual patient technology available for future training purposes (rating = yes).

Conclusions: Implementation of a user-friendly virtual patient encounter was shown to be educationally valuable and to complement student-patient interaction. VPF provides an alternative for students to learn history-taking and physical examination skills. Immediate and objective performance feedback from the system provides students the opportunity to improve their performance in conducting a patient interview and familiarize themselves with the H&P process. The interaction allows the user to identify any problems with recognizing classic signs and symptoms of specific diagnoses. Future testing of the VPF can be targeted at monitoring the academic performance of students that use the VPF tool prior to a live SP encounter.

Acknowledgments: The VPF software and HDCM technology was created in conjunction with the Department of Computer Science at the University of Florida and the Medical College of Georgia. (Publication on the technology is referenced here: Rossen B, Lind DS, Lok B. Human-centered distributed conversational modeling: efficient modeling of robust virtual human conversations. Presented at: 9th International Conference on Intelligent Virtual Agents 2009; September 14-16, 2009; Amsterdam, The Netherlands.) Funding for this project was provided by the PCOM D'Alonzo Memorial Award, Dr Paul Evans, and Dr Gary Watson. Special thanks to Dr Gary Watson and Dr David Lind for their academic and clinical guidance. We would also like to acknowledge Dr Michael Sampson and the Department of Clinical Assessment at GA-PCOM for their coordination in student testing.

Health Policy

S40

Analysis of the Use and Maintenance of Latrines and Water Purification Systems in South-East Haiti

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Hypothesis: The purpose of this study is to examine the use and maintenance of latrines and water purification systems in south-east Haiti. Due to a lack of health education and resources in the community, it is suspected that few homes will demonstrate proper use and maintenance of these systems.

Materials and Methods: As part of a privately-funded public health program in southeast Haiti, 400 water purification systems were distributed to homes throughout the Seguin Plateau in May 2007. These systems were sold at minimal cost to families in the community. Each home that received a water purification system was mapped using GPS technology. In September 2007, homes with purification systems began to receive latrines. As of August 2009, about 70 latrines have been built. In August 2007-2009, surveillance data was collected on the use and maintenance of latrines and water purification systems. This data focused on the knowledge of families on how to use the systems, whether they chose to use the systems, whether they had the proper resources for using the systems, and whether their systems were functioning properly. Data was collected door-to-door by two Haitian water-technicians and one American worker.

Results: The data collected from August 2007-2009 demonstrated that while initial use and maintenance of latrines and water purification systems was encouraging, these numbers drastically declined through 2009. Declines in use and maintenance were most marked between August 2008 and 2009. Overall, data from August 2009 demonstrated that proper use and maintenance of latrines and water purification systems was rare. Most latrines are improperly used and dirty. Similarly, almost a third

of water purification systems were not being used at all. Those in use often contained improperly purified water.

Conclusion: Although data reveals that proper use and maintenance of latrines and water purification systems is sporadic, the demand for these improvements continues to be high. In order to increase proper use and maintenance of latrines and water purification systems, a nurse educator was hired to visit each of the homes in the study and provide a series of lessons. In addition, two water technicians have been hired to visit each of the homes enrolled in the study to ensure that the water purification systems are functioning properly and that homes are well-stocked with Clorox and/or aquatabs.

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You Look Great, How do You Feel? A Hairstylist Depression Screening and Referral Pilot Study

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Hypothesis: We set out to discover whether hairstylists could be effectively trained to deliver depression screening and referral to their clients.

Materials and Methods: We trained 27 student hairstylists as a supplement to their training program in a two-hour lecture and question-answer style training about depression. The training consisted of what depression is, how to recognize it, how to communicate with clients about depression, and who in the community to refer the client to. The stylists were given a pre-training survey gauging their self-reported understanding, comfort and confidence about depression, and a post-training survey gauging the same aspects. Furthermore, the stylists were surveyed about the frequency of certain conversational topics in their client-stylist encounters.

Results: We found positive correlations between the ability to recognize depression and likelihood to talk with their clients about depression (Pearson=0.62), likelihood to talk about depression and refer (Pearson=0.71), and likelihood of referral and referral power (Pearson=0.54). We also found stylist-client conversations were frequently centered around the following topics: health (46%), stress (31%), marital issues (31%), sleep (38%), and work (54%), all of which lend to the use of a quick depression screen.

Conclusions: The results show that hairstylists may be an untapped source of lay health advisors. They can be trained to effectively and efficiently screen their clients for depression and properly refer them for management. Unique to their profession, much of their time is spent conversing with clients and frequently conversation is related to topics, which lend themselves to easy utilization of depression screening. Also, their profession allows frequent encounters and a distinctive relationship with their clients that they feel would improve the effectiveness of their referral, represented by the aforementioned referral power measure. In all, this pilot study warrants a much larger study to look further into the role of hairstylists as lay health advisors.

Let the Osteopath follow the course of the blood from the heart to its destination and return, and remove all obstructions, open all doors; for on it we depend for all the joys of perfect form and functioning, which is health.

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