2012 Student Abstracts and Poster Competitions

As stated in the editorial by Kelsey S. Neufeld, OMS IV, on pages 8 to 10, this issue of *The Journal of the American Osteopathic Association* (*JAOA*) contains abstracts submitted through the Student Osteopathic Medical Association and entered into the 2012 Student Poster Competition, an annual judged event held during the Poster Session at the AOA Research Conference.

For more information about this competition and to see a list of the first- and second-place winners, see Student Doctor Neufeld's editorial beginning on page 8.

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

AOA Research Fellowship

S4

Mutations in VHL Gene Result in Loss of Protein With Minimal Effect on Protein Function

Kristin Huntoon, PhD, OMS IV[1]; Chunzhang Yang, PhD[2]; Zhengping Zhuang, MD, PhD[2]; Russell Lonser, MD[2]

[1]New York Institute of Technology College of Osteopathic Medicine, Old Westbury; [2]SNB, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

Background: von Hippel-Lindau disease (VHL) is a heritable multisystem cancer syndrome caused by a germline mutation of the *VHL* gene. von Hippel-Lindau disease is associated with benign and malignant tumors of the viscera and central nervous system. Missense mutations of the *VHL* gene account for the majority of the germline mutations in VHL, but their precise mechanism of tumorigenesis is not known.

Methods: To determine the mechanism in which missense mutations underlie the pathogenesis of VHL, molecular characterization, functional analysis, and cell culture of VHL-associated tumors (renal cell carcinoma and hemangioblastoma) was performed.

Results: Western Blot analysis demonstrated quantitative reduction of mutant protein in VHL-associated tumors (renal cell carcinoma [86% to 93%] and hemangioblastoma [73% to 85%]), but messenger RNA expression remained at normal physiologic levels. Western blot analysis revealed that mutant VHL protein products retain the fundamental function as an E3 ligase to degrade hypoxia inducible factor (HIF) in stable transfections of 786-0 cells, a VHL-deficient cell line. However, mutant proteins were highly unstable and with significantly reduced protein half-life as determined by timed cyclohex-

imide treatment (from 3.3 to 1.5 hours) of transfected 786-0 cells. Immunoprecipitation revealed that the loss of missense VHL proteins correlated with an imbalance of HSP70/HSP90 binding that results in rapid posttranslational destruction of these proteins resulting in clearance of mutant VHL that still has the capability to destabilize HIF1. Lastly, Western blot analysis confirmed that treatment with suberoylanilide hydroxamic acid (SAHA) and other proteasomal inhibitors elongated the half-life of missense VHL protein (from 1.5 hours to 2.5 to 10 hours).

Conclusion: Missense VHL protein has the ability to function as wild-type VHL protein under circumstances that increase the longevity of the protein. Additionally, SAHA and other proteasomal inhibitors may function in the future therapy of patients with VHL that harbor missense mutants in decreasing their tumor burden in response to HIF1 upregulation.

SOMA Research Fellowship

S29

Reproductive Health Seminar Program: Evaluating Its Impact on Educators and the Youth They Serve

Emily E. Young, MA, MPH, OMS IV; Katherine Beditz, MPH; Andrea Schaeffer, MPH; Lauren Bello, MPH; Lindsay LaCorte, DO, MPH; Janet Hamstra, EdD, MS

Nova Southeastern University College of Osteopathic Medicine, Fort Lauderdale, Florida

Objective: The purpose of this study was to evaluate the effect of the Reproductive Health Seminar Program (RHSP) on the knowledge and attitudes of health professional student educators and the youth they serve in South Florida.

Hypothesis: With an aim of providing quality, comprehensive, age-appropriate reproductive health education to youth in an open and safe environment, we hypothesized that involvement in the RHSP increases the knowledge and skills regarding reproductive health of the health professional student educators and the youth they serve.

Methods: The RHSP was designed and launched in January 2011 by graduate students of Nova Southeastern University College of Osteopathic Medicine. This program has trained students enrolled in medical, physician assistant, nursing, and public health programs to become reproductive health educators who commit to a year of leading 1.5-hour seminars to at-risk youth in local community shelters and alternative schools. Each educator is trained on 1 of 6 possible seminars offered through the program. Educators were assessed via survey prior to program initiation in October 2011 and again in May 2012. These pre- and postprogram assessments were analyzed together for changes in educator knowledge and attitudes during their program involvement. Participating youth completed pre- and postseminar surveys at each seminar encounter. These seminar surveys are analyzed for changes in youth knowledge and skills, reflecting educator teaching effectiveness. All data were collected from the educator assessments and youth surveys were stored in Excel and imported into SPSS software for data analysis.

Results: Approximately 750 youth have participated in the RHSP. Data from educator assessments are currently being analyzed. Data from youth surveys indicate a statistically significant average increase of 29% in youth knowledge and skills across all seminars. The following increases in knowledge and skills were observed between the youth before and after assessments, per seminar:

Seminar 1: Anatomy and Puberty—37%
Seminar 2: Abstinence and Condoms—31%
Seminar 3: Understanding Pregnancy—32%
Seminar 4: Birth Control Options—51%
Seminar 5: Sexually Transmitted Infections—21%
Seminar 6: Healthy Relationships—0%

Conclusion: The Reproductive Health Seminar Program is a student-led, seminar-based, objective-driven educational initiative that positively impacts

the knowledge and attitudes of administering health professional students and the youth they serve. The program is uniquely structured as it incorporates a purposeful variety of health professional students, individualized training to educators in only 1 seminar, and flexible scheduling between the student educators and youth-serving sites. This inexpensive program has been highly valued by all involved and fulfills a high need within the local community. It serves as a reproducible model for similar health education programs and community partnerships across the United States.

Osteopathic Manipulative Medicine/Osteopathic Principles and Practice

S5

Survey of Patient Knowledge of Osteopathic Physicians and Osteopathic Manipulative Treatment

Peter D. Snell, OMS II[1]; Obaydah A. AbdurRaqeeb, DO, MS[2]; Tiffany L. Smith, BA[2]; Matthew M. Huckabee, BE[1]; Amy Keenum, DO, PharmD[2]

[1]Lincoln Memorial University-DeBusk College of Osteopathic Medicine, Harrogate, Tennessee; [2]University of Tennessee, Knoxville

Objective: This study sought to assess outpatient knowledge of osteopathic physicians (ie, DOs) and osteopathic manipulative treatment (OMT) by comparing them to other common allied health doctors and medical therapies, respectively, at a family medicine clinic where DOs both practice and administer OMT.

Hypothesis: Our hypothesis was that respondents would be (1) less familiar with DOs when compared with other common allied health practitioners and (2) less familiar with OMT when compared with other common medical therapies.

Methods: In this cross-sectional study, 124 patients were approached to complete a written survey while waiting to be seen by their physician (DO or allopathic physician [ie, MD]) at a family medicine clinic where DOs practice and administer OMT. Using a Likert scale, respondents were asked to state how familiar they were with what an MD, chiropractor (ie, DC), dentist (ie, DDS), optometrist (ie, OD), podiatrist (ie, DPM), physical therapist (ie, DPT), and DO does. Similarly, respondents were asked to state how familiar they were with homeopathic remedies (HM), physical therapy (PT), chiropractic therapy (CT), and OMT. Also, respondents were asked to rate how similar a DO is to an MD, DC, DDS, OD, DPM, and DPT.

Results: Twelve patients refused to participate, leaving 112 patients who were enrolled in the study. A 5-point Likert scale, from not at all familiar (1) to extremely familiar (5) was used. The mean score for familiarity with DOs (2.76) was lower than that of every other type of practitioner (MD, 4.21; DC, 3.79; DDS, 4.23; OD, 3.85; DPM, 3.62; DPT, 4.23). Further, those who were at all familiar with DOs were significantly more familiar with every other type of practitioner than those who were not at all familiar with DOs ($P<.01$). On the other hand, respondents felt that DOs were more similar to MDs as opposed to any other type of practitioner (MD, 3.21; DC, 2.79; DDS, 2.21; OD, 1.94; DPM, 2.08; DPT, 2.48). Also, the mean score for familiarity with OMT (1.70) was lower than every other type of therapy (HM, 2.60; PT, 4.46; CT, 3.63).

Conclusion: Our survey revealed that respondents were overall (1) less familiar with what a DO is than an MD, DC, DDS, OD, DPM, and DPT and (2) less familiar with OMT than HM, PT, and CT. Participants stated, when compared with an MD, DC, DDS, OD, DPM, and DPT, a DO is most similar to an MD. This lack of familiarity coupled with the high percentage of respondents who chose "unsure" demonstrates an osteopathic knowledge deficit. Better education must be implemented to increase patient knowledge of DOs and OMT.

S9

Stiles Screening Examination Can Be a Useful Didactic Teaching Paradigm for First-Year Students as the Results Correlate to Viscerosomatic Dysfunction

Jeffrey Brodovsky, OMS II[1]; Eiman Mahmoud, MD[2]; Janet Burns, DO[3]; Thomas Liggett, PhD[3]

[1]Touro University College of Osteopathic Medicine (TUCOM), Vallejo, California; [2]Basic Science, TUCOM, Vallejo, California; [3]Osteopathic Manipulative Medicine, TUCOM, Vallejo, California

Background: Patients experiencing viscerosomatic dysfunction often present with visceral complaints and less often with musculoskeletal complaints. However, on physical examination, somatic dysfunction can often be found rapidly through a screening protocol with a patient standing, sitting, or lying supine.

Objective: The authors determined whether somatic dysfunctions in patients presenting to the urgent care clinic of the Meir Hospital in Israel showed any correlation between nonmusculoskeletal chief complaints (NMCCs) and somatic dysfunction by a first-year student in this proof-of-point study.

Methods: A total of 50 patients were examined by a first-year osteopathic medical student during a clinical elective abroad. Prior to embarking, the student received 6 hours of training in a modified Stiles screening examination followed by a reliability assessment by Janet Burns, DO. Results were recorded on the Outpatient Osteopathic SOAP Note, and the area of greatest restriction (severity) was determined on the basis of ICD-9 regions, of which some were subdivided to be more specific to the screening paradigm. The severity of somatic dysfunction for different areas was rated on a scale of 0 to 3, where 3 was the most significant somatic dysfunction and 0 was no dysfunction.

Results: The data showed that 2 notable ICD-9 subregions were found to correlate with NMCCs. In patients with chest pain, there was a greater average score on the Stiles screening examination in the thoracic T1-T4 areas than for any other NMCC (1.48 chest pain vs 1.19 average of all NMCCs). In patients with generalized abdominal pain, the T5-T9 area had a greater average as well (1.39 abdominal pain vs 1.08 average of all NMCCs).

Conclusion: Through this study, we have shown that known viscerosomatic reflexes, as related to chief complaint, can be rapidly identified by the Stiles screening examination. This proof-of-point study indicates that a protocol-specific training regimen for osteopathic medical students can be didactically useful, as a first-year student with limited training can easily learn the modified Stiles screening examination. Furthermore, the Stiles screening examination provides a basis of which region of a patient to treat first. We speculate that this screening examination can be used by physicians practicing in urgent care settings where it is critical to determine a diagnosis rapidly. Additional research is warranted to increase the number of participants and student-operators.

S17

Use of OMT in Recreational Runners as Preventive Medicine

Jonathan Daly, OMS II[1]; Colin Zhu, DO[2]; Jandy Hanna, PhD[1]

[1]West Virginia School of Osteopathic Medicine, Lewisburg; [2]Centrastate Hospital, Freehold, New Jersey

Background: Recreational runners, compared with trained runners, are at greater risk of injury of the lower limb. Many of the mechanisms of these injuries are not well understood, but some may be associated with overpronation or excessive fascial tract. Both of these somatic dysfunctions may be improved with osteopathic manipulative treatment (OMT). Osteopathic manipulative treatment has been used to treat athletes in conjunction with normal injury protocol and was found to decrease lower limb injuries.

Hypothesis: We postulated that the use of OMT may affect biomechanical parameters during running, which may improve somatic function and prevent injury.

Methods: We examined the biomechanics of running in 10 participants using a 3-dimensional motion analysis system and a force plate. Each participant completed a pre-OMT and post-OMT running session. Markers were placed on bony landmarks to facilitate hip, knee, ankle, and talus angle calculation. Normality of each angle variable was examined and then variance between pre- and post-OMT measures were examined using Bartlett test and Welch analysis of variance (ANOVA) at foot touchdown and foot lift off. Peak and impulse force means were compared between pre- and post-OMT runs by ANOVA.

Results: For the majority of participants, hip, knee, and ankle angle at touchdown exhibited a significant reduction in variance after OMT ($P \leq .05$). Limb angle variance at lift off was inconsistent in terms of significant differences in variance. In terms of forces, the majority of participants exhibited a significant difference in peak and impulse propulsive forces (for each participant: $P \leq .05$, df=9).

Conclusion: These results suggest that OMT has a statistically significant effect on reduction of variance during running, although the mechanisms are not completely understood. Reduction in variance (although not in mean values) suggests that OMT may function like prestretch, and the change in forces suggests the muscles were reset to a different point along their physiologic length-tension curve. In keeping with these suppositions, the majority of treatments were muscle energy and counterstrain techniques. Further research should examine these mechanisms to determine whether recreational runners could benefit from these techniques.

Acknowledgments: This study was approved by the West Virginia School of Osteopathic Medicine Institutional Review Board (JH12012007) and supported by an intramural grant.

Clinical Studies

S25

Time Required for Habituation to Simulated-Barefoot Running, as Measured by Variance, Kinematics, and Running Pattern

Madeline Teisberg, MS, OMS III; Katrina Ostermann, DO; William Fredericks, DO; Maria Soukup, BS; Katherine Lee, DO; Jandy Hanna, PhD

West Virginia School of Osteopathic Medicine, Lewisburg

Background: Habituation to a given shod condition may affect running biomechanics and injury rate. Simulated-barefoot running (SBR) is a recent topic of interest, as it may reduce risk of injury. Runners naïve to SBR report more Achilles tendon injuries and discomfort than runners habituated to SBR.

Objective: To ascertain the minimum transition period for runners changing their shod condition (ie, style and type of footwear or lack thereof) by determining a mean time to habituate to SBR.

Methods: The study measured footfall, step length, and kinematic parameters by video recording each participant at different speeds over 9 weeks during 3 stages. Stage I, pre-habituation, included 3 visits in week 1. Participants ran for 2 minutes at 3.0 m sec-1 (6.7 mph) in different shod conditions over 3 visits. Participants were given Vibram 5-finger shoes (V) at the final stage I visit. Stage II, habituation, included a total of 3 visits at weeks 3, 5, and 7 to measure footfall pattern in the V condition over time. Stage III, after habituation, repeated the stage I protocol. The criteria for determining habituation were: (1) the participant must run in the V more often than in his or her personal shoes, (2) the covariation in knee and ankle angles at touchdown between V visits must be less than 5%, and (3) the contact time and stride length during V running must be less than the initial shod visit measurements, so the V stride frequency measures greater than initial shod stride frequency.

Results: Out of 5 participants, 4 met the habituation criteria by week 9. For these 4 participants, the mean habituation time was 42.67 days. Three of those 4 participants were habituated by the latter 2 criteria earlier than the first criterion (eg, ran in V more often than in personal shoes). The participant who did not habituate exhibited large variation in joint angles during V running and longer contact times during V running than during personal shoe running.

Conclusion: These data show that the naïve runner habituates to SBR within 7 weeks on average. The participant who did not habituate ran less often (twice per week) and less far (5 miles per week) than the other participants (average 4 times per week, 12 miles per week). If there are advantages to V running, minimal runners may not initially benefit as they may not habituate as swiftly as consistent runners.

Acknowledgments: This study was approved by the institutional review board (JH03122012) and supported by an intramural grant.

S33

Selective Activation of SIRT1 Reduces Ischemia-Reperfusion Injury in Cardiac Myocytes

Jeremiah X. Karrs, OMS II; Ryan H. Khal, BA; Bethany Hampton, BS; Bonny Dickinson, PhD; Brian Griffith, PhD; Judith Maloney, PhD

West Virginia School of Osteopathic Medicine, Lewisburg

Background: Reperfusion therapy is central to the management of myocardial infarction (MI). However, it depletes intracellular NAD^+ levels, resulting in localized oxidative damage and cardiomyocyte cell death. NAD^+ plays a critical role in regulating cellular redox and also controls the activity of the NAD^+ dependent class III histone deacetylase SIRT1. Studies show that resveratrol, a natural phenol found in red wine, is a SIRT1 activator and is implicated in cardioprotection after ischemia-reperfusion (IR). Whether the cardioprotective effect of resveratrol is linked to its activation of SIRT1 remains to be examined.

Hypothesis: Increasing SIRT1 activity will decrease IR-induced apoptosis in HL-1 cardiac myocytes.

Methods: To examine the effect of IR on NAD^+ depletion in murine HL-1 cardiac myocytes, cells were subjected to 2 hours of simulated ischemia followed by 0.5 4 hours of simulated reperfusion, and the NAD^+/NADH ratio was measured by using a colorimetric assay. To determine the effect of SIRT1 activation on IR-induced cell death, during reperfusion, HL-1 cells were treated during 16 hours of simulated reperfusion with 10 mM resveratrol (a nonspecific SIRT1 activator) or 50 μM DCHC (a highly selective SIRT1 activator), and cell death was assessed with annexin-V/propidium iodide staining by flow cytometry.

Results: NAD^+/NADH ratios were significantly decreased as early as 0.5 hours of simulated reperfusion and remained depressed for up to 4 hours. Consistent with the role of NAD in maintaining cell viability, HL-1 apoptosis was observed by 16 hours of simulated reperfusion. The specific SIRT1 activator DCHC reduced IR-induced annexin V staining, 10 mM resveratrol had no effect on IR-induced apoptosis. In contrast, there was no effect of resveratrol or DCHC on annexin V staining in the absence of ischemia.

Conclusion: While nonspecific activation of SIRT1 with resveratrol had no effect on HL-1 apoptosis during IR, the selective SIRT1 activator DCHC significantly reduced HL-1 apoptosis. These data suggest that the use of highly selective activators of SIRT1 may be required to provide cardioprotection during IR.

S35

Influence of Gaze on Static Postural Control in Individuals With Huntington Disease

Kelly H. Beers, OMS III[1]; Lisa M. Muratori, PT, EdD[2]; Lauren T. Cooke, BS[1]; Ely Rabin, PhD[1]

[1]New York Institute of Technology College of Osteopathic Medicine, Old Westbury; [2]Stony Brook University, New York

Background: It has been noted that patients with Huntington disease (HD) have poor postural control and often sway during quiet stance, and it is possible that increased postural sway in HD is related to poor ocular control. Posture is controlled with visual feedback, among other modalities. To interpret retinal feedback, corresponding feedback about eye position is required to distinguish eye movement from external movement. One of the first presenting symptoms of HD is abnormal ocular motor control, which includes impairments in voluntary eye movements (saccades) and the vestibulo-ocular reflex (VOR), which stabilizes gaze during head movement. Patients with HD have impaired fixation and saccade control (including suppression).

Objective: Here, we study how impaired ocular control affects balance in people with HD, specifically whether ocular control of individuals with HD contributes to postural instability.

Methods: In the experimental task, participants stood as still as possible for 30 seconds with eyes open and fixating on a visual target and then stood for 30 seconds with eyes closed. We measured participants' head and eye positions. First, we compared postural sway with visual fixation to sway with eyes closed. Second, we determined if postural control errors were related to eye movement errors. We measured head and eye position and analyzed postural shifts following errors in visual fixation to determine if posture is responding to visual feedback. For example, if fixation is followed by a postural shift, then it is possible that visual feedback is interpreted as a body movement.

Results: Data were analyzed and ocular movements were correlated with postural movements using Matlab software. We found that participants with HD sway more during fixation than in dark conditions. Patients with HD showed postural sway at a lower frequency than saccadic movements during grating conditions.

Conclusion: Despite less feedback with eyes closed, participants with HD sway less than with their eyes open. The increase in frequency of postural sway in participants with HD corresponds to the primary frequency of their eye movements.

Basic Sciences

S1

Pseudognaphlium obtusifolium Slows Melanoma Cell Growth

Mark Braswell, OMS II[1]; Dale J. Rosado, PhD[2]; Elizabeth Brandon, PhD[3]

[1]Kansas City University of Medicine and Biosciences' College of Osteopathic Medicine, Kansas; [2]Chemistry and Biochemistry, Mississippi College, Clinton; [3]Biology, Mississippi College, Clinton

Background: Native plants have been used for medicinal purposes in North America. One such plant, *Pseudognaphalium obtusifolium* (*Gnaphalium obtusifolium*, rabbit tobacco), has served as a medicinal plant for inflammatory ailments, but little research has been conducted on the bioactive compounds in the plant.

Objective: We are using melanoma cells, a cancer for which there are few treatment options, aside from surgery, to test whether *P obtusifolium* contains any compounds with anticancer properties. The identification of novel compounds that slow tumor growth could augment traditional chemotherapies and surgery. To our knowledge, no research has been conducted with *P obtusifolium* and melanoma.

Hypotheses: Our hypotheses were that *P obtusifolium* would slow melanoma cell growth and enhance melanoma cell death.

Methods: To determine the effect on cell proliferation, aqueous extracts from the leaves of *P obtusifolium* were added to the growth medium of B16F10 mouse melanoma cells. Serial dilutions of the extracts were made and added to cells at the beginning of each experiment. Cells were plated, synchronized, and treated for 24 and 48 hours. Cells were counted with an automated cell counter (Nexcelom). To test the effect of *P obtusifolium* on cell survival, serial dilutions of the aqueous extracts were prepared in medium with 300 mM sodium azide (NaN_3). Cell viability was measured with an MTT assay. Briefly, cells reduced 3-(4.5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolim bromide (MTT) to formazan, which is then solubilized. The absorbance of formazan was obtained with a microplate reader (PolarSTAR Optima, BMG Labtech).

Results: Our results revealed that cells treated with a 1:10 dilution of *P obtusifolium* extract had a slower growth rate than control cells ($P=.04$, control vs 1:10 dilution at 48 hours). In contrast, *P obtusifolium* promoted cell survival acutely during NaN_3 treatment ($P=.01$, positive control vs 1:10 dilution).

Conclusion: *P obtusifolium* decreased melanoma cell growth rate, but promoted melanoma cell survival. The mechanism by which survival is conferred is unknown but may relate to cell cycle arrest in the *P obtusifolium*–treated cells. These results indicate that compounds in the leaf extracts of *P obtusifolium* affect the cell cycle. Further purification of these extracts is needed to identify the active compound or compounds and to determine the mechanism by which the cell cycle is affected.

S3

Changes in Paroxonase-1 in Response to a Cerebrovascular Accident: A Pilot Study

Christopher R. Engdahl, MS[1]; Crystal Takada, MS[1]; Russell Caccavello, BS[1]; Satoshi Kimura, MD, PhD[2]; Alejandro Gugliucci, MD, PhD[1]

[1]Glycation, Oxidation and Disease Laboratory, Touro University College of Osteopathic Medicine, Vallejo, California; [2]Department of Internal Medicine and Laboratory Medicine, Showa University, Yokohama City, Kanagawa Prefecture, Japan

Background: It is well established that high-density lipoproteins (HDLs) play a vasculoprotective role in humans. A growing amount of evidence suggests that paraoxonase-1 (PON1), an enzyme component of HDL, is in part responsible for this function. Like most enzymes, PON1's activity depends on strict homeostatic parameters. Disruption of this steady-state, which occurs in many disease processes, has a deleterious effect on PON1 function. In particular, PON1 suppression is strongly correlated with worsening atherosclerosis. While this phenomenon has been studied in cardiovascular and renal disease, the changes in PON1 activity over the course of a cerebrovascular accident (CVA) remain largely unknown.

Hypothesis: We tested the hypothesis that HDL subclass distribution changes and PON1 activity decreases in the aftermath of a CVA and attempted to quantify this phenomenon.

Methods: Sera-containing HDL from a random sampling of patients with CVA (n=9) from the Department of Internal Medicine and Laboratory Medicine at Showa University in Yokohama, Japan, were assayed for PON1 activity. At CVA onset, and in the days and weeks in recovery, PON1 activity was measured kinetically by an arylesterase assay. The specimens were also subjected to HDL subclass analysis using the Lipoprint LDL and HDL Subfractionation System, which has been approved by the

US Food and Drug Administration. The results were analyzed with Lipoware software to determine the HDL subclass content of the samples. These data were then analyzed and plotted for trends.

Results: Lipoprint analysis demonstrated that HDL subclasses fluctuate dramatically during the early hours of a CVA. However, in the days and weeks following the event, a shift toward smaller HDL subclasses and a decrease in PON1 activity were consistently observed.

Conclusion: In the hours and days following a CVA, HDL subclass distribution and PON1 activity is dramatically altered. This decline in PON1 function is observed despite a variety of CVA presentations, diagnoses, and interventions. While representing the first study of this kind, our findings are consistent with our current understanding of PON1 suppression. These findings may have implications for predicting and preventing future vasculoocclusive events in the CVA patient. This pilot study serves as a proof of principle for a larger follow-up study to confirm the results and to assess their predictive value.

S6

Cyclin O, a Novel Cyclin Impacting the Cell Cycle and DNA Damage Response

Anastasia Cruz, OMS II[1];
Salvatore Caradonna, PhD[2]

[1]University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine, Stratford; [2]Molecular Biology, University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine, Stratford

Replication of DNA can induce replicative stress within a cell, which in turn activates a DNA damage response of coordinated pathways that limit expansion of a damaged cell. Regulatory proteins in the DNA damage response work in concert to limit damage by cytostatic mechanisms or inducing apoptosis. Cyclin O is an example of a regulatory protein, which we have shown leads to an accumulation of cells in S phase when this protein is overexpressed in cells. We have also shown that overexpression of cyclin O increases cell death. Other preliminary data in our laboratory indicate that cyclin O associates with a cyclin dependent kinase and induces phosphorylation of 2 key DNA damage response proteins, namely Rad9 and RPA2. This project is working to characterize the pathway in the DNA damage response that cyclin O is involved in. Analysis of how cyclin O influences cell cycle and its location within the cell may provide more insight to the function of cyclin O. Based on our preliminary data and cyclin O's relationship with Rad9 and RPA2, our hypothesis states that cyclin O plays a role in the DNA damage response.

We have created an inducible vector of cyclin O to be able to control the expression of the protein in different scenarios. Exposure to DNA damaging agents will allow comparison of genotoxic stress and normal cell conditions. Inducing the protein at different times in the cell cycle enables us to view how cyclin O works in different stages. Through flow cytometry, we can analyze the percentage of cells in each phase of the cell cycle to understand cyclin O's effect on cell cycle.

Immunofluorescence allows us to localize where exactly in the cell the protein is present when induced and its connection with other regulatory proteins of the DNA damage response. Our results thus far have shown cyclin O to be a negative regulator of cell replication. These findings may lead to new insights for rational drug design for cancer chemotherapy.

S7

Increased Atrial Fibrillation Inducibility in a Rat Myocardial Infarction/Heart Failure Model: Effects of B-type Natriuretic Peptide Treatment

Diana Teplitsky, OMS III; Yuefeng Chen, MD, PhD; Martin A. Gerdes, PhD; Youhua Zhang, MD, PhD

Biomedical Sciences, New York Institute of Technology College of Osteopathic Medicine, Old Westbury

Background: Atrial fibrillation (AF) and heart failure (HF) are 2 global cardiovascular epidemics that frequently coexist. Rat myocardial infarction (MI) is a commonly used small animal model to study HF pathophysiology and therapy. However, little is known about whether AF inducibility is increased in this MI/HF model.

Hypothesis: We hypothesized that there is in fact increased AF inducibility in this animal model, and that using an effective treatment such as B-type natriuretic peptide (BNP) can reduce AF risk associated with HF.

Methods: This study consisted of 3 groups of female Sprague Dawley rats: sham MI rats, MI rats, and MI rats treated with BNP. Myocardial infarction was produced in all rats by ligation of the left anterior descending coronary artery except in the sham MI rats. Osmotic mini-pumps were implanted in all rats during surgery for drug delivery. Rats in the BNP group received 4 weeks of BNP treatment (6 μg/kg per day) treatment, while placebo (saline) was administrated in both the sham MI and MI groups. After 4 weeks of treatment, left ventricular chamber dimension and function were determined using echocardiography. Cardiac electrophysiological tests using an intracardiac catheter approach were performed to determine right atrial effective refractory period and AF inducibility with burst pacing.

Results: Compared with the sham control animals, MI rats developed left ventricular chamber dilatation (left ventricular diastolic internal diameter 6.7±0.4 mm in sham vs 9.2±0.5 mm in MI, $P<.01$) and reduced left ventricular function (fractional shortening 42±5% in sham vs 22±4% in MI, $P<.01$). Moreover, MI also increased AF inducibility (AF duration increased from 0.2±0.2 seconds in sham to 1.3±1.6 seconds in MI rats, $P<.05$), although no significant changes in atrial effective refractory period was found. Treatment with BNP significantly reduced AF inducibility in the MI rats (1.31±.6 seconds in MI vs 0.2±0.2 seconds in BNP, $P<.05$), despite the fact that BNP treatment did not produce significant changes in left ventricular chamber dimension and function.

Conclusion: Our data demonstrated for the first time, to our knowledge, that an increased AF inducibility exists in MI/HF rats. Furthermore, long-term BNP treatment after MI can significantly decrease AF inducibility, which may not be associated with significant left ventricular functional improvement.

S8

Cystathionine-β-Synthase Expression and Ovarian Regulation of Homocysteine in Rats

Steven M. Alsobrook, OMS II; Michelle Vanoy-Warner, BS; Christina Sparks, MS; Carolyn Komar, PhD

West Virginia School of Osteopathic Medicine, Lewisburg

Background: High levels of circulating homocysteine (Hcy) are negatively correlated with female fertility. Women with PCOS, the number 1 cause of subfertility and infertility in premenopausal women, have elevated concentrations of Hcy. Poor oocyte quality is also associated with follicles that have high concentrations of Hcy in follicular fluid. Restricted expression of cystathionine-β-synthase (CβS) limits the number of tissues capable of metabolizing Hcy via transulferation. The current study investigated if the ovary is capable of regulating local concentrations of Hcy by determining whether ovarian tissue expresses CβS, and if so, whether its expression changes during the ovarian cycle.

Hypothesis: We hypothesized that CβS is expressed in the ovary and that its expression may be regulated by the changing endocrine environment created by the ovarian cycle, circulating concentrations of Hcy, or both.

Methods: Immature rats were injected with pregnant mares' serum gonadotropin (PMSG) to stimulate follicular development; 48 hours later they received human chorionic gonadotropin (hCG) to induce ovulation and formation of corpora lutea (CL). Ovaries were collected 0 hours and 48 hours after PMSG and 5 hours and 24 hours after hCG. One ovary from each animal (n=3-6/time point) was analyzed by means of immunohistochemistry (IHC) using an antibody for CβS; the second was processed for immunoblot analysis of CβS. To determine if circulating concentrations of Hcy can influence CβS expression, normally cycling rats were treated with 1.5 gm of Hcy per 500 mL water daily (n=4) or were untreated (n=3) for 16 days. Ovaries were collected and processed for IHC.

Results: Cystathionine-β-synthase expression was similar throughout the ovarian tissue, distributed primarily in the granulosa cells and theca. At 24 hours after hCG, expression was seen in CL as well. Levels of CβS were highest 5 hours after hCG compared with the other time points ($P<.05$). The expression of CβS in ovarian tissue from Hcy-treated and Hcy-untreated animals killed on the same day of the estrous cycle was similar. The pattern of expression in the cycling animals paralleled findings in the immature, gonadotropin-primed animals at comparable stages of the cycle.

Conclusion: Our data show that CβS is expressed in the ovaries and suggest that its expression is regulated by the estrous cycle. Exogenous administration of Hcy does not appear to alter the pattern of expression of CβS in cycling animals. These findings show that the ovary is capable of regulating the amount of Hcy locally, independent of the peripheral circulation.

S10

Investigation Into a Potential Role for Telomerase in the Herpes Simplex Virus 2 Life Cycle

Scott B. Roberts, OMS II[1]; Prajakta Pradhan, MS[2]; Marie L. Nguyen, PhD[2]

[1]Des Moines University College of Osteopathic Medicine, Iowa; [2]Immunology and Microbiology, Des Moines University College of Osteopathic Medicine, Iowa

Background: Herpes simplex virus (HSV) is a major cause of disease in humans. Primary infection is usually noted by the presence of small painful ulcerations around the mouth or genitals. Herpes simplex virus-2 is commonly associated with genital herpes, while HSV-1 is typically associated with oral lesions. Herpes simplex virus can also cause more serious complications such as encephalitis. Once HSV has been contracted, it remains in a latent phase in neural ganglia and cannot be eradicated. It has the ability to reactivate from this latent stage to cause recurrent outbreaks. Current therapies are aimed at reducing the severity and number of outbreaks. Acyclovir, a nucleoside analog that targets the viral DNA polymerase, is the treatment of choice. One possible alternative therapeutic strategy is to target cellular host factors that are used by the virus to complete its life cycle. One such potential target is telomerase. Telomerase is responsible for maintaining the telomeres, which are noncoding regions at the end of chromosomes that prevent loss of DNA after every round of replication. Previous studies reported that telomerase levels are increased in HSV-infected cells and that the telomerase pathway alters sensitivity to HSV-dependent apoptosis.

Hypothesis: We hypothesized that by inhibiting telomerase we would be able to interfere with the life cycle of HSV-2.

Methods: To determine if telomerase plays a role in the life cycle of HSV-2, HEp-2 cells were infected at a multiplicity of infection of 5 in the presence or absence of MST-312. MST-312 is a compound pre-

viously shown to be capable of blocking telomerase activity in vitro and in cell culture systems. Samples were then sonicated to release virus particles, and a plaque assay was performed to measure viral replication. Viral protein expression was measured by immunoblots.

Results: We saw a reduction of viral replication as measured by plaque assay from samples that were treated with the telomerase inhibitor. We also found a reduction in the accumulation of the late viral protein, VP22.

Conclusion: Together, these results led us to conclude that MST-312 has a negative impact on the HSV-2 life cycle. A similar repression of the virus life cycle was observed when HEp-2 cells were infected with several laboratory-derived strains or a recent clinical isolate of HSV-1 in the presence of MST-312. Although our results did not address the mechanism through which the MST-312 acts, these results suggest further research is warranted.

S11

Activity of Xanthine Dehydrogenase and Xanthine Oxidase in White Adipose Tissue

Ryan P. McCafferty, OMS II; Bradley Sevy, BS; Margaret Hower, HSDG; Maria Soukup, BS; Sabrina Vance, HSDG; Kristie Bridges, PhD

West Virginia School of Osteopathic Medicine, Lewisburg

Background: Obesity is associated with elevated serum uric acid (UA) levels, and it has been proposed that UA is a causative agent in the development of metabolic syndrome. However, it is also possible that elevated UA reflects changes in the purine catabolic pathway that are more problematic than the UA itself. The last 2 steps in UA production are catalyzed by xanthine dehydrogenase/xanthine oxidase (XDH/XO). This enzyme is initially expressed as XDH, which donates electrons to nicotinamide adenine dinucleotide. Conformational changes reversibly convert it to the XO form, which uses oxygen as an electron acceptor and generates oxygen radicals. Xanthine dehydrogenase can also be irreversibly converted to XO through proteolytic cleavage. It has been shown that inflammation causes conversion of XDH to XO in epithelial cells and that XDH activity is required for maturation of 3T3-L1 cells into adipocytes. Therefore, inflammation associated with obesity may increase XO activity and oxygen radical production in adipose tissue. Although XDH/XO has been detected in 3T3-L1 cells, its expression and activity in adipose tissue has not been investigated.

Objective: The goals of this study were to confirm that XDH/XO is expressed in rat and human white adipose tissue (WAT) and determine if it is found in the XDH, full-length XO, or cleaved XO form.

Hypothesis: Xanthine dehydrogenase and XO will be expressed in WAT with an XDH:XO ratio similar to that reported for rat liver (1:1 to 5:1).

Methods: A fluorescent assay monitoring XDH/XO conversion of pterin to isoxanthopterin in the presence of different electron acceptors was optimized using extracts from 3T3-L1 cells. White adipose tissue was removed from 5 female rats and extracts were prepared. Xanthine dehydrogenase/xanthine oxidase activity was measured and protein expression was investigated through Western blotting. Expression studies were also done with human WAT lysates purchased from a commercial vendor. This work was exempt from institutional review board review.

Results: As expected, there was increased XDH/XO expression and activity in mature 3T3-L1 adipocytes vs preadipocytes. Rat WAT expressed both forms of the enzyme with an average XDH/XO activity ratio of 2.17, but there were no detectable amounts of cleaved XO. Only the full-length version of XDH/XO was detected in human WAT lysates.

Conclusion: Xanthine dehydrogenase/xanthine oxidase is expressed in both rat and human WAT, primarily in the full-length form. Future studies can determine if inflammation associated with obesity leads to conversion of XDH to XO resulting in oxidative damage and altered adipose function.

S12

Dynamic Properties of the Porcine Lumbar Spine

Robert L. Zondervan, MA, OMS II;
John Popovich, PhD; Vilok Vijayanagar, BS;
Clark Radcliffe, PhD; Peter Reeves, PhD

Michigan State University College
of Osteopathic Medicine, East Lansing

Background: Recent work has shown that dynamic properties (stiffness and damping) of a human cadaveric lumbar spine increase as axial compressive load increases. This is an important attribute that helps provide stability to the spine. The stiffness characteristics of porcine specimens have been shown to be comparable to the human spine; however, the dynamic properties have yet to be investigated.

Hypothesis: Our study hypothesis will test if stiffness and damping of the porcine lumbar spine will increase with axial compressive loading.

Methods: Five fresh frozen porcine lumbar functional spine units (FSUs) were obtained and dissected of all non-osteoligamentous structures. The superior and inferior vertebrae of the FSUs were potted in a polyurethane resin. The potted FSUs were mounted in a 0.9% NaCl bath maintained at 22°C and that contained protease inhibitors. A custom-built pendulum arm was attached to the superior aspect of the FSU. Functional spine units were allowed 2 hours to equilibrate in the bath under a compressive load of 672 N and preconditioned in the sagittal plane 6 times every 15 minutes to mimic physiologic motion. After the equilibration period, FSU were tested with 4 axial compressive loads (440 N, 672 N, 899 N, 1123 N); each weight was tested 3 times. During testing, the specimen was flexed to 5° and allowed to swing unconstrained. Angular motion in flexion/extension was tracked, and bending stiffness and damping were calculated by fitting a mathematical model to the experimental data. Linear regression models were used to determine if bending stiffness and damping increases linearly with axial compressive load.

Results: Like the human FSU, the porcine FSU acted as an underdamped vibrating elastic system. Stiffness in flexion/extension was linearly correlated with compressive load (R^2=0.985, P=.007). Damping in flexion/extension was also linearly correlated with compressive loads (R^2=0.986, P=.007).

Conclusion: This preliminary investigation into the dynamic properties of the porcine spine demonstrates a linear correlation in both stiffness and damping as axial compression increases, which is consistent with human cadaveric spines. The implications of this finding is that porcine specimens, which are more cost efficient and structurally homologous, may be used as analogs to the human lumbar spine in dynamic testing. Methodology developed in this study will be used to design subsequent investigations on intact and damaged FSUs.

♦S13

Cystine/Glutamate Antiporter Regulates Indoleamine 2,3-dioxygenase Enzymatic Activity and Protein Levels in Human Monocyte-Derived Dendritic Cells

Matthew J. Copeland, OMS II[1];
Evan S. Stern, BS[2]; Mildred L. Mattox, BS[2];
Bonny L. Dickinson, PhD[2]

[1]Biomedical Sciences, West Virginia School of Osteopathic Medicine, Lewisburg; [2]West Virginia School of Osteopathic Medicine, Lewisburg

Background: The cystine/glutamate antiporter controls the biosynthesis of the major cellular antioxidant glutathione by transporting cystine, the rate-limiting precursor of glutathione synthesis, into the cell in exchange for glutamate. We recently demonstrated that the antiporter regulates dendritic cell (DC) expression of indoleamine 2,3-dioxygenase (IDO), an immunosuppressive enzyme that has emerged as a key regulator of peripheral immune tolerance. As the suppressive effects of IDO are largely mediated by DCs, a detailed understanding of how IDO is regulated in these cells is critical for

♦ Abstract that won first or second place in the 2012 SOMA Student Poster Competition.

the design of targeted strategies to induce robust immune tolerance.

Hypothesis: We tested the hypothesis that the cystine/glutamate antiporter controls IDO enzymatic activity by regulating IDO protein synthesis.

Methods: Dendritic cells were prepared from normal human monocytes using standard techniques. Indoleamine 2,3-dioxygenase enzymatic activity was measured using a colorimetric assay to quantify kynurenine in culture medium, and data were verified by high performance liquid chromatography. Antiporter activity was blocked with L-homocysteic acid, a competitive inhibitor of the antiporter, or by incubating DCs in medium devoid of cystine/cysteine. Protein levels of IDO were examined by immunoblot and normalized to glyceraldehyde-3-phosphate dehydrogenase by densitometry. Protein synthesis was blocked by treating DCs with cycloheximide.

Result: Enzymatic activity of IDO was significantly increased when antiporter function was inhibited with LHC or following DC incubation in cystine/cysteine-free medium, and this finding correlated with an increase in IDO protein levels. N-acetyl-L-cysteine, a potent antioxidant, reversed the increase in IDO activity observed after antiporter blockade. Blocking protein synthesis with cycloheximide decreased both IDO enzymatic activity and protein levels in DCs and interfered with the increase in IDO enzymatic activity and protein levels observed when antiporter function was blocked.

Conclusion: Our data suggest that the cystine/glutamate antiporter plays a critical role in regulating IDO enzymatic activity in DCs by regulating cellular redox and controlling IDO protein synthesis. These findings highlight a role for redox as a critical control point in the regulation of IDO expression and activity in DCs and suggest that perturbation of redox homeostasis may adversely affect immunity by promoting the generation of IDO-competent DCs.

S14

Axonal Architecture of Molecular Layer Heterotopia of the Cerebellar Vermis

Sarah E. VanDine, OMS I;
Raddy L. Ramos, PhD

New York Institute of Technology College of Osteopathic Medicine, Old Westbury

Background: The cerebellum is vulnerable to neurodevelopmental defect. There are many disorders associated with reduced neuronal proliferation resulting in hypoplasia or agenesis of the cerebellum, as well as disorders involving defective neuronal migration and altered lamination of cerebellar neurons. Many mouse mutants, like the C57BL/6 mouse strain, as well as knock-out mice, exhibit malformations in the molecular layer of folia VIII and IX of the cerebellar vermis, known as molecular layer heterotopia (MLH). Previously, our laboratory demonstrated that heterotopia are composed primarily of granule cells, Golgi cells, and GABAergic interneurons and are indicative of neuronal migration defect.

Hypothesis: We hypothesized that the axonal architecture is altered in MLH and documented the presence of glutamatergic, noradrenergic, cholinergic, and catecholaminergic axons in heterotopia.

Methods: C57BL/6, C57BL/10, and GFP-THY1 reporter mice were perfused and fixed, and brains were cryopreserved and sectioned in the sagittal plane. Gold-chloride histochemistry and immunocytochemistry for myelin basic protein (MBP) was used to demonstrate the presence of myelinated axons in MLH. Immunocytochemistry using the primary antibodies serotonin transporter (5-HTT) and tyrosine hydroxylase (TH) was used to reveal serotonergic and catecholaminergic axons.

Results: Myelinated axons are almost exclusively found in the granule cell layer and, in cases where heterotopia formed a bridge of cells spanning the molecular layers of folia VIII and IX (between an area devoid of pia), myelinated axons also crossed these 2 folia. GFP-labeled axons were present in

heterotopia and also exhibited terminal rosettes, a characteristic feature of mossy fibers, suggesting that glutamatergic spinal cord and/or brainstem projections innervate heterotopic granule cells. We also demonstrated TH labeled axons and 5-HTT-labeled fibers, which indicates that catecholaminergic and serotonergic axons are present in MLH. In every case of folia-spanning malformations, GFP-, TH-, and 5-HTT-labeled axons could also be observed crossing the molecular layers of both folia VIII and IX through putative breeches in the pia axons. These data demonstrate that axonal architecture is indeed altered in MLH. In addition, a case of MLH in the Allen Brain Atlas stained for acetylcholinesterase (AchE), showed AchE-labeled Golgi cells, which suggests that cholinergic axons are found in MLH.

Conclusion: Our data indicate that heterotopic neurons in MLH receive diverse sources of afferent projections. However, it still remains unknown whether and how intracerebellar circuits are reorganized as a result of MLH or if there are behavioral consequences of MLH. Further histological examination will be necessary to determine possible negative effects on cerebellar physiology and function.

S15

Role of P-cadherin in Modulation of Growth Factor Signaling Pathways

James T. Johns, OMS II[1]; Desseree Lysne, BS[1]; Michelle Perkins, MS[1]; Darren Cuevas, BS[1]; Matt Van Dam, BS[1]; Kathryn Lawson, PhD[2]

[1]Midwestern University/Arizona College of Osteopathic Medicine, Glendale; [2]Biochemistry, Midwestern University, Glendale, Arizona

Background: The role of the calcium-dependent cell adhesion protein P-cadherin in oral tumor development has not been well characterized. Histological studies have found that P-cadherin expression increases when normal epithelial cells become dysplastic. E- and N-cadherin have both been shown to bind to and affect growth factor–related signaling.

Hypothesis: We hypothesized that P-cadherin may be increasing growth factor signaling in a similar manner to increase tumor development.

Methods: To investigate this hypothesis, 2 oral squamous carcinoma cell lines (SCC1 and SCC22A) were engineered to alter P-cadherin levels. These cells were treated with growth factors to identify P-cadherin–dependent alterations in signaling. We also investigated the effects of P-cadherin on expression of SNAI1 (commonly referred to as snail), a transcription factor that has been shown to increase tumor progression. Additional characteristics of tumor progression were analyzed, including epithelial cadherin expression, cell growth, and cell motility.

Results: Both P-cadherin overexpressing cell lines showed increases in basal expression of snail compared with controls. When treated with insulin-like growth factor (IGF), the P-cadherin overexpressing SCC1 cells exhibited even greater increases in snail levels, particularly after 2 hours of treatment, compared with controls. To investigate whether the rapid increase in snail protein levels may be a result of increased protein stability, we examined an inactivating phosphorylation (Ser-9) of the snail regulatory protein glycogen synthase kinase beta (GSK-3b). Phosphorylation of Ser-9 in GSK-3b, induced by IGF, was unchanged in cells overexpressing P-cadherin. In SCC22A cells, the inhibition of either the Akt or mitogen-activated protein kinase (MAPK) pathways resulted in decreased snail expression, indicating these pathways are both necessary for P-cadherin–mediated increases in snail levels. After IGF treatment, P-cadherin overexpressing cells from both lines displayed time-dependent decreases in P-cadherin level. Overexpression of P-cadherin did not affect cell proliferation but did increase cell motility.

Conclusion: These data suggest P-cadherin plays an active role in promoting tumor aggressiveness in oral squamous carcinoma cells by altering growth factor receptor signaling, expression of downstream effectors, and cell behavior.

S16

Foundations of Alzheimer Bioenergetic Vulnerability in Young Adult APOE ε4 Carriers

Luke B. Henderson, OMS III; Randall Allred, BS; Andrew B. Wolf, BS; Mona Alex, BS; Nikki Konkowski, BS; Jon Valla, PhD

Midwestern University/Arizona College of Osteopathic Medicine, Glendale

Background: In vivo imaging studies using fludeoxyglucose positron emission tomography have shown that carriers of the ε4 allele of apolipoprotein E (APOE ε4), the major susceptibility gene for the development of late onset Alzheimer disease (AD), demonstrate regional brain bioenergetic deficits in young adulthood, which mimic regional brain energy deficits found in AD. Subsequent analysis of postmortem brain tissue from young-adult carriers of APOE ε4 revealed significant reductions in neocortical mitochondrial functional activity in the same regional (posterior cingulate cortex) and laminar (superficial lamina most affected) patterns of vulnerability as were seen in AD. These deficits in brain energy metabolism may represent the earliest detectable signs of AD-related pathology in APOE ε4 carriers, with significant effects seen decades prior to the typical onset of clinical symptoms in AD. It is not known what mechanism confers this bioenergetic vulnerability to APOE ε4 carriers or how it affects the risk of developing AD in the future.

Hypothesis: We hypothesized that these declines in functional measures represent metabolic downregulation in APOE ε4 carriers, possibly because of a lower level of synaptic activity and neural connectivity.

Methods: We are currently evaluating gene and protein expression and neuronal morphology in subsets of the same young-adult APOE ε4 carriers and noncarriers previously analyzed. Frozen postmortem brain tissue from young adults (age 18-45 years) was genotyped for APOE status. Posterior cingulate cortex samples were homogenized in sucrose-Tris-ATP buffer (20% tissue). Mitochondria were isolated, lysed, and samples run in 4-12% Bis-Tris protein gels and transferred to PVDF membranes. Membranes were probed with an antibody cocktail representing each of the 5 electron transport chain (ETC) complexes (Mitosciences/Abcam) and visualized by chemiluminescence.

Results: Preliminary results indicate a pronounced downregulation of both mitochondrial- and nuclear-encoded subunits of the ETC, most prominently of cytochrome oxidase (complex IV).

Conclusion: We are currently working to verify that this finding represents downregulation and not a defect in ETC function. Further, we have begun to assess neuronal and synaptic morphology toward the hypothesis that APOE ε4 carriers develop less, or lose neocortical dendritic complexity synaptic contacts, or both, leading to the apparent downregulation of bioenergetic pathways in higher-order brain regions.

Acknowledgments: This work was supported by Midwestern University and the Arizona Alzheimer's Consortium and NIA AD Clinical Core (P30 AG019610).

S19

Analysis of Angiogenic Biomarkers in Pancreatic Cancer

Christopher T. Than, OMS II; David Strom, PhD

Des Moines University College of Osteopathic Medicine, Iowa

Background: Pancreatic cancer is a devastating disease that is known to be the fourth leading cause of cancer-related deaths throughout the world. It is accepted that tumor proliferation requires a series of crucial events, one being angiogenesis. Angiogenesis, the development of new blood vessels, provides tumor cells the means of receiving nutrients, removing cellular waste, and a possible route for metastasis.

Hypothesis: We hypothesized that tumor samples with a higher grade or stage of cancer would express higher levels of angiogenic biomarkers, cellular products describing a cell's biological state. The relative expression levels of biological markers for each tumor sample were attempted to be correlated with tumor information such as cancer stage, grade, and prognosis.

Methods: In this study, our methods involved homogenizing pancreatic cancer tumor samples using silica beads and a bead beater. Then the homogenized samples were tested for the expression of 55 angiogenic biological markers using the R&D Proteome Profiler Array-Human Angiogenesis Array Kit. The degree of biomarker expression was then measured using the Bio Rad ChemiDoc XRS+ System.

Results: After collecting the biomarker expression levels from each tumor, we concluded that cancerous pancreatic tissues expressed high levels of a few angiogenic biomarkers. All tumor samples had consistently high levels of angiogenic biomarker, an angiogenesis promoting factor. Another interesting finding was with the matrix metalloprotease 9 (MMP-9) biomarker; MMP-9 is an enzyme known for its ability to breakdown basement membranes and extracellular matrix, which aids in the process of metastasis. Levels of MMP-9 varied between relatively low levels to very high levels. The study surprisingly also showed high expression levels of serpin F1, also known as pigment epithelium-derived factor, and tissue inhibitor to metalloprotease-1, TIMP-1. Serpin F1 has anti-angiogenic properties, while TIMP-1 is known for inhibiting metalloprotease and promoting cell proliferation.

Conclusion: These biomarkers would be promising with further research and future correlation with tumor information.

S20

Characterizing Differences in Left Ventricular Performance in Young and Old BALB/c Mice Using Transthoracic Echocardiography

Ryan R. Kahl, OMS II[1]; Kenneth C. Klein, BS[2]; Matthew J. Copeland, BS[2]; Evan S. Stern, BS[2]; Jeremiah X. Karrs, BS[2]; Bethany Hampton, BS[2]; Brian N. Griffith, PhD[2]; Judith A. Maloney, PhD[2]

[1]West Virginia School of Osteopathic Medicine, Lewisburg; [2]Department of Biomedical Sciences, West Virginia School of Osteopathic Medicine, Lewisburg

Background: Transthoracic M-Mode echocardiography is a reliable method for assessing left ventricular function and mass. Laboratory mice are a valuable tool for evaluating cardiac function in vivo because of the ease by which they are bred and genetically manipulated.

Hypothesis: Older BALB/c mice will exhibit decreased ejection fraction (EF) and fractional shortening (FS) and increased posterior wall thickening (PWT) and left ventricular mass (LVM) when compared with younger BALB/c mice, with males having proportionally higher values in all 4 criteria as compared with females.

Methods: Four groups of mice were evaluated: 32- to 36-week-old male and female mice were defined as old and 9- to 11-week-old male and female mice were defined as young. Mice were given an intraperitoneal injection of 30 to 60 mg/kg pentobarbital, and heart rate and oxygen saturation were monitored. A 15-6L linear ultrasound transducer was used with a SONOS 5500 Ultrasound machine set at 2 cm depth. A parasternal short axis view of the left ventricle at papillary muscle level was identified in 2-dimentional mode and measured using M-Mode. Measurements were taken during diastole and systole of interventricular septum, left ventricular inner diameter, and left ventricular posterior wall and then used to assess left ventricular function.

Results: Young male mice exhibited a higher EF than old male mice (90.3±0.2% vs 83.6±2.0%, $P<.02$). Young female mice exhibited a higher EF than old female mice (92.8±1.5% vs 84.3±1.2%, $P<.01$). Young male mice exhibited a higher FS than old male mice (54.1±0.3% vs 45.6±2.1%, $P<.01$). Young female mice exhibited a higher FS than old female mice (59.1±2.8% vs 46.2±1.4%, $P<.01$). No significant difference was seen in all groups concerning PWT. The only significant difference in LVM was seen between young male mice and young female mice (0.12±0.01 g vs 0.074±0.01 g, $P<.03$).
Conclusion: Older BALB/c mice exhibited decreased EF and FS when compared with younger BALB/c mice. Young male mice exhibited increased LVM compared with young female mice.
Acknowledgment: This research was funded by a West Virginia School of Osteopathic Medicine Intramural Grant.

S21

Effects of HSP70 and HSP90 Inhibition on an In Vitro Model of HIPEC

Samantha Hoy, OMS I[1]; Christina R. Moynihan, OMS II[2]; Ryan Huffman[3]; Mike Stencel, OMS I[3]; Jessica Boettcher, OMS III[4]; Aruna Hawkins, OMS III[4]; Bryan Larsen, PhD[5]; David Strom, PhD[5]

[1]Physiology/Pharmacology, Des Moines University College of Osteopathic Medicine (DMU-COM), Iowa; [2]Oncology Community Service Chair, DMU-COM, Iowa; [3]Physiology/Pharmacology, DMU-COM, Iowa; [4]DMU-COM, Iowa; [5]Marian University College of Osteopathic Medicine, Indianapolis, Indiana

Background: Hyperthermic Intraperitoneal Chemotherapy (HIPEC) is a procedure that uses the combination of heat and chemotherapy to kill malignant cells of peritoneal carcinomatosis that may not be completely removed during cytoreductive surgery. One effect of heating cells is the induction of chaperone proteins, such as HSP70 and HSP90, which aid in protecting the cell from death during environmental stress.
Hypothesis: Inhibition of HSP70 and HSP90 will enhance the effects of Hyperthermic Intraperitoneal Chemotherapy (HIPEC) in an in vitro model.
Methods: Experiments were performed with HeLa cells grown under standard conditions, except for some experimental cells that were transferred to a 41°C humidified CO_2 incubator to mimic HIPEC conditions. Flow cytometry was used to measure apoptosis, as defined by the sub-2N population of cells when stained with propidium iodide. Western blot analysis was used to quantify levels of HSP70, HSP90, and HSP90 targets.
Results: HeLa cells provided an accurate model of the HIPEC procedure in vitro. Our results also indicated that HSP90 is induced during HIPEC treatment and that inhibition of HSP90 does not enhance cell death by Doxorubicin. The results of HSP70 inhibition are currently being explored.
Conclusion: We can accurately mimic HIPEC in a laboratory setting using HeLa cells shifted to 41°C for 100 minutes. Using this model we have examined expression of HSP70 and HSP90, and have determined that although HSP90 is induced during HIPEC and should enable greater survival during cellular stress, inhibition of this chaperone protein does not enhance cell death in the presence of Doxorubicin.

S22

Pseudo-Pregnancy Reduces Susceptibility to Ischemia-Induced Ventricular Fibrillation in the Isolated Female Rat Heart

William G. Simpson, OMS II[1];
Hugh Clements-Jewery, PhD[2]

[1]West Virginia School of Osteopathic Medicine, Lewisburg; [2]Biomedical Sciences, West Virginia School of Osteopathic Medicine, Lewisburg

Background: More than 300,000 deaths per year in the United States are attributed to sudden cardiac death occurring in the context of coronary heart disease. Sudden death is most commonly the result of ventricular fibrillation (VF) occurring spontaneously without warning during periods of myocardial ischemia. Development of effective and safe therapies for prevention of VF requires reliable models of ischemia-induced VF. The isolated rat heart is one such model, but because the vast majority of studies use males, the influence of the endogenous reproductive hormonal environment on susceptibility to ischemia-induced VF in the isolated female rat heart is poorly characterized.

Objective: We investigated the extent to which pseudo-pregnancy, a state that can be induced in female rats that is associated with a prolonged diestrus phase maintained by progesterone, altered susceptibility to ischemia-induced VF in isolated female rat hearts compared with a group of male rat hearts.

Methods: Thirteen female, normally cycling Sprague-Dawley rats were mated with vasectomized male rats to induce pseudo-pregnancy. Estrous cycle stage and occurrence of pseudo-pregnancy were determined by daily vaginal lavage. The pseudo-pregnant state was indicated by a prolonged diestrus phase. After 7 to 10 days of pseudo-pregnancy, hearts were excised under sodium pentobarbital-induced anesthesia and perfused at 37°C with modified Krebs solution containing 3 mM K in the Langendorff mode. Myocardial ischemia was induced in each rat heart by occluding the left main coronary artery. The incidence of VF during a 30-minute period of myocardial ischemia in these hearts was determined from the electrocardiogram and compared with that in 12 male isolated rat hearts.

Results: In male rat hearts the incidence of ischemia-induced VF was 100% (12 of 12), while in the hearts from pseudo-pregnant rats the VF incidence was 62% (8 of 13; $P=.0391$). Ischemic zone size was similar in the male and female rat hearts (44% and 46% respectively; $P=$NS). There were no differences in the time to onset of VF, the total duration of VF, heart rate, QT interval, or coronary flow between the 2 groups.

Conclusion: The results indicate that a prolonged pseudo-pregnant state diminishes susceptibility to ischemic VF. Because this state is maintained by progesterone, this finding suggests that progesterone may be partly responsible for this chronic effect. However, the precise mechanisms involved remain to be determined and require further exploration.

S26

NKT Cell–Dependent Modulation of OPN Receptors in RSV Infection

Sherman Wu, MS, OMS III[1];
Natalie Gevorkian, BS[1]; Kainath Durre, MS[1];
Mahboob Qureshi, MD PhD[2]

[1]Touro University Nevada College of Osteopathic Medicine, Henderson;
[2]Immunology, Touro University Nevada College of Osteopathic Medicine, Henderson

Background: Neonatal respiratory syncytial virus (RSV) infection can predispose individuals to asthma later in life. Involvement of myeloid (m) and plasmacytoid (p) dendritic cells (DC) and natural killer T (NKT) cells has been reported. Also, osteopontin (OPN) modulates the immune response to RSV in neonatal lungs.

Hypothesis: Respiratory syncytial virus infection in neonates modulates OPN receptor expression on DC, T, and B cells in an NKT cell–dependent mechanism and thus contributes to the development of hyperreactive airway and asthma.

Methods: We infected 5-day-old BALB/c and NKT$^{-/-}$(CD1d$^{-/-}$) mice intranasally with RSV and examined OPN receptor (CD44 and CD51/61) expression on lung mDC (CD11b$^{lo/-}$CD11chiCD45R$^{lo/-}$) and pDC (CD11b$^{lo/-}$CD11c$^{lo/-}$CD45R^{hi}), T (CD3^{+}), and B (CD19^{+}) cells by flow cytometry at designated postinfection days. Control animals received buffered saline.

Results: Expression of CD44 and CD51/61 decreased on mDC, pDC, T, and B cells in RSV-infected BALB/C pups compared with the control pups particularly early on infection (day 2 and 4 after infection). On the other hand, these OPN receptors increased expression in the RSV-infected NKT$^{-/-}$ pups compared with the infected BALB/C mice and uninfected NKT$^{-/-}$ pups. This augmented expression was abolished by day 10 after infection.

Conclusion: Our results suggest that OPN receptor expression is modulated by RSV infection; as indicated by reduced CD44 and CD51/61 expression on lung DC and lymphocytes in the RSV-infected pups. In addition, expression of these receptors is dependent on NKT cells, as RSV-infected NKT$^{-/-}$ pups had significantly increased CD44 and CD51/CD61 expression on DC and lymphocytes. Moreover, this modulation was predominantly observed during active RSV infection and was abolished by day 10 after infection. Thus, decreased OPN receptor expression during RSV infection diminishes the action of endogenous OPN allowing skewed polarization of DC and hence, T cell response (previous studies showed skewed polarization toward pDC during RSV infection and reversal of this phenomenon upon exogenous OPN administration). Increased expression of OPN receptors in absence of NKT cells signifies the inhibitory role of NKT cells. Therefore, nullifying the action of NKT cells (thus, augmenting the action of endogenous OPN) and/or administration of exogenous OPN warrant further investigation as new candidates for therapeutic strategies against RSV-associated asthma.

S27

Effects of Epstein-Barr Virus LMP2A on Fas-Mediated Apoptosis in a Murine B Cell Lymphoma

Samira Hussain, OMS III[1]; Ryan Incrocci, BS[2]; Michelle Swanson-Mungerson, PhD[2]

[1]Microbiology-Immunology, Midwestern University/Chicago College of Osteopathic Medicine, Downers Grove, Illinois; [2]Midwestern University/Chicago College of Osteopathic Medicine, Downers Grove, Illinois

Background: The majority of the human population is latently infected with Epstein-Barr virus (EBV). Latent Membrane Protein 2A (LMP2A) is an EBV protein expressed during latency and in EBV-associated malignancies. It enhances B cell lymphoma survival and acts as a BCR mimic by initiating similar signal transduction pathways, including the PI3K/Akt pathway.

Hypothesis: Because of the ability of LMP2A to protect B cell lymphomas from apoptosis, we tested if LMP2A regulates Fas expression and whether it protects a murine B cell line, A20, from Fas-mediated apoptosis through PIK3 activation.

Methods: Fas expression was measured by incubating LMP2A-expressing and control A20 cell lines with a fluoresceinated anti-Fas antibody followed by flow cytometry. Additionally, both cell lines were stimulated by anti-Fas antibody and analyzed by flow cytometry for levels of Fas-mediated apoptosis using Annexin V staining. Finally, LMP2A-expressing and control A20 cell lines were incubated in the presence of a PI3K inhibitor and analyzed for Fas-mediated apoptosis.

Results: We found that the LMP2A-expressing cell line showed increased Fas expression and were more susceptible to Fas-mediated apoptosis when compared with the control cells. There was also an increase in Fas expression and Fas-mediated apoptosis in the LMP2A-expressing cells compared with control cells when incubated with a PI3K inhibitor.

Conclusion: These results show that LMP2A expression increases B cells' susceptibly to Fas-mediated apoptosis by increasing Fas expression. Additionally, under the conditions used, LMP2A does not use PI3K to modulate Fas-mediated apoptosis in B cells.

♦S28

Relationship Between Time Spent Outdoors and Health in Older Adults

Suvi Neukam, OMS III[1]; Jacqueline Kerr, PhD[2]; Katie Crist, MPH[2]; Keri Wasilenko, MPH[2]

[1]University of New England College of Osteopathic Medicine, Amherst, New Hampshire; [2]Center for Wireless and Population Health Systems, University of California San Diego, La Jolla

Background: As a preliminary investigation of the MIPARC study (Multilevel Intervention for Physical Activity in Retirement Communities), the baseline relationship between time spent outdoors and physical activity was assessed. Older adults are the least active cohort in our population, and their sedentary behavior places them at greater risk for myriad physical, social, and cognitive health problems. A positive relationship between time spent outdoors and physical activity (PA) was determined from a literature search. Additionally, both time spent outdoors and PA independently have been shown to be beneficial for various aspects of health, including quality of life (QOL), cognitive function, depression, and fear of falling.

Hypothesis: With these relationships in mind, we proposed that increased time spent outdoors is correlated to greater QOL and cognitive function in addition to reduced depression and fear of falling.

Methods: Sixty-four residents from 3 retirement communities in San Diego County, average age 84 years, received and wore device belts carrying geographic information positioning systems and accelerometers for 7 days. Positional information and PA intensity were collected, respectively, from the devices. Additionally, participants completed 2 cognitive function tests (Trails A & B, Symbols Search) and a survey assessing QOL, depression, and fear of falling. A bivariate correlation analysis was applied to explore the relationship between PA intensity, PA intensity while outdoors, and total minutes outdoors to the aforementioned health outcomes.

Results: A significant (.05) relationship in the expected direction was attained between PA intensity and QOL, cognitive function (Trails B), and fear of falling. Interestingly, no significant relationships existed between PA intensity while outdoors and the various measures, although they were all in the expected directions. Finally, daily minutes outdoors did not produce a relationship that was significant or in the expected direction with any of the measured outcomes. On the basis of these outcomes, we have determined that PA is important for health, but perhaps where it occurs—indoors vs outdoors—is less important.

Conclusion: Further studies may explore explanations for the observed lack of relationship between outdoor PA and the measured health outcomes, such as a higher baseline level of vitamin D in the participants. This study introduced the use of geographic information positioning systems methodology in an outdoor PA study, offered a novel investigation of outdoor PA as it relates specifically to older adults, and demonstrated that PA is related to health.

♦ Abstract that won first or second place in the 2012 SOMA Student Poster Competition.

♦S30

HIF Prolyl-Hydroxylase-3 Plays a Key Role in Epithelial to Mesenchymal Transition

Trenton Place, OMS III[1]; James Mezhir, MD[2]; Frederick Domann, PhD[3]

[1]Molecular and Cellular Biology, Midwestern University/Chicago College of Osteopathic Medicine, Downers Grove, Illinois; [2]Surgery, University of Iowa Carver College of Medicine, Iowa City; [3]Radiation Oncology, University of Iowa Carver College of Medicine, Iowa City

Background: Epithelial-to-mesenchymal transition (EMT) is a developmental process by which epithelial cells lose cell-cell adhesion and develop an invasive, migratory phenotype. Stimuli that promote EMT, such as TGF-β, have been shown to promote tumor metastasis, implicating EMT as a driving force behind metastatic disease. Our laboratory has recently discovered a correlation between a mesenchymal phenotype in cancer cells and loss of HIF prolyl-hydroxylase-3 (PHD3) expression. Prolyl-hydroxylase-3 is highly expressed in hypoxic primary tumors, and it was previously thought only to negatively regulate the hypoxia-inducible transcription factor. However, recent literature supports a more diverse, and largely unexplored role of PHD3 in cell signaling.

Hypothesis: In light of our correlative observation between PHD3 silencing and a mesenchymal phenotype in cancer cells, we hypothesized that PHD3 is an inhibitor of EMT.

Methods: We used the Madin-Darby Canine Kidney (MDCK) epithelial cell line, a well-established model for the study of EMT. For EMT induction, MDCK cells were exposed 10 pg/mL TGF-β, a known inducer of EMT. To study the effects of PHD3 overexpression on the induction of EMT, we created stable PHD3-overexpressing cells using retroviral transduction of a PHD3 transgene, or vector control. Quantitative real-time polymerase chain reaction analysis of genes involved in EMT (Downregulation of E-cadherin; upregulation of Zeb1), and characterization of cell morphology were used to confirm EMT.

Results: We found that induction of EMT by TGF-β in MDCK cells resulted in a significant 55-fold reduction of PHD3 mRNA expression ($P=.003$), with a similar decrease in PHD3 protein levels. Surprisingly though, when we attempted to block TGF-β–induced EMT by stably overexpressing PHD3, the cells underwent a striking EMT morphological change in the absence of TGF-β, with more than a 90-fold reduction in E-cadherin expression ($P<.01$) and a 2.4-fold upregulation of Zeb1 ($P=.03$)

Conclusion: Our data suggest that PHD3 is a novel, potent initiator of EMT. However, PHD3 does not appear to be required for EMT maintenance. In fact, once EMT is established, PHD3 is significantly downregulated. Current work is aimed at establishing the mechanism by which PHD3 induces EMT and discerning why PHD3 is downregulated during EMT maintenance. These data will provide novel insight into the mechanisms promoting EMT and may aid in the development of chemotherapeutic drugs that target tumor metastasis.

S31

NFκB Activation Prevents Ischemia/Reperfusion Injury in Cardiac Myocytes

Kenneth C. Klein, OMS II; Ryan R. Kahl, BA; Bethany Hampton, BS; Bonny L. Dickinson, PhD; Judith A. Maloney, PhD; Brian N. Griffith, PhD

Department of Biomedical Sciences, West Virginia School of Osteopathic Medicine, Lewisburg

Background: Cardiomyocyte injury is a complication of reperfusion therapy following myocardial infarction. The pathophysiology of ischemia-reperfusion (IR) injury is complex and involves the interplay of both proapoptotic and antiapoptotic pathways culminating in necrotic and autophagic cardiomyocyte death. The transcription factor nucle-

♦ Abstract that won first or second place in the 2012 SOMA Student Poster Competition.

ar factor κB (NFκB) has emerged as a key biochemical target of IR injury; however, its precise role is a matter of intense debate. In vivo data suggest that NFκB activation during IR injury is cardiotoxic while other studies suggest that NFκB activation during ischemia results in cardioprotection.

Hypothesis: The transcription factor NFκB, activated by both ischemia and reperfusion, is cardioprotective, and inhibiting NFκB transcriptional activity will sensitize cardiomyocytes to IR-induced cell death.

Methods: Murine HL-1 cardiomyocytes underwent simulated ischemia (2 hours) followed by simulated reperfusion (3 hours) and the effect of NFκB inhibitors (QNZ, JSH-23, or caffeic acid phenethyl ester [CAPE]) on cell death was assessed with annexin-V/propidium iodide staining by flow cytometry. HL-1 cells were treated with NFκB inhibitors during reperfusion or during both ischemia and reperfusion.

Results: Inhibiting NFκB transcriptional activity with QNZ caused a significant dose-dependent increase in IR-induced cell death when administered to HL-1 cells during reperfusion. Moreover, when QNZ was administered during both ischemia and reperfusion, IR-induced cell death was further increased beyond that observed when QNZ was administered during reperfusion alone. This finding suggests that NFκB transcriptional activity during both ischemia and reperfusion may contribute to its cardioprotective effect. However, CAPE (an IκB inhibitor) and JSH-23 (an NFκB translocation inhibitor) had no affect on IR-induced HL-1 cell death when administered during reperfusion, suggesting that the activation and translocation of NFκB into the nucleus during ischemia may be sufficient for cardioprotection.

Conclusion: The studies presented here suggest that NFκB is activated during ischemia and reperfusion and exerts cardioprotective effects. These data lend insight into the role of NFκB during IR injury and suggest that therapies aimed at mimicking the protective effects of NFκB may reduce cardiomyocyte cell death during reperfusion therapy.

Acknowledgment: This research was funded by an intramural grant from the West Virginia School of Osteopathic Medicine.

♦S32

Analysis of the Role of v99 in Hermaphrodite Development

Eric Goldwaser, OMS II

University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine, Stratford

In humans, the 3 Gli proteins are transcription factors that play critical roles in development and carcinogenesis. Nematodes have a single Gli protein known as TRA-1, which plays a central role in sexual development. We are studying how TRA-1 is regulated to learn more about Gli proteins. This process is simple, because many nematodes have evolved self-fertile hermaphrodites through modifications to the sex determination pathway.

In *Caenorhabditis briggsae*, the *she-1* gene acts upstream of tra-1 to allow XX animals to make sperm and become hermaphrodites. Because mutations in the *she-1* gene are temperature sensitive, suppressors can be selected by growing worms at 25°C, where she-1 mutants are unable to self-fertilize. One of the suppressors we identified, *v99*, interacts genetically with the Tip60 histone acetyltransferase (HAT) complex. Our hypothesis is that the *v99* gene regulates the interaction of TRA-1 with the Tip60 complex. To test this model, we need to clone and characterize the *v99* gene.

Cloning of this gene requires us to map it to a precise location on chromosome III. We have been identifying and studying genetic recombinants between *v99* and markers located along the chromosome in order to assess its location. Most of these markers are single nucleotide polymorphisms (SNPs) which are numerous and easy to characterize by DNA sequencing or by restriction enzyme digests. So far I have isolated 24 recombinants. Primers were designed for SNPs and indels

♦ Abstract that won first or second place in the 2012 SOMA Student Poster Competition.

along chromosome III and amplified with recombinant DNA from 2 *C briggsae* strains—AF16 and HK104—with known marker mutations. Results thus far have allowed us to narrow down the location of *v99* to a region of 2.5 Mb on chromosome III. There are several known genes in this region that are being used as candidates for further analysis. Once the identity and location of *v99* is clear, TALEN's can be designed to create deletions in the target gene and to allow us to study its role in sex determination and the regulation of TRA-1.

S34

Developmental and Functional Significance of D-Dopachrome Tautomerase in Parkinson Disease

Edlira Yzeiraj, OMS III; Emily Forbes, BA; Joerg R. Leheste, PhD; Brian H. Hallas, PhD; German Torres, PhD

Biomedical Sciences, New York Institute of Technology College of Osteopathic Medicine, Old Westbury

Background: D-dopachrome tautomerase (D-DT) is a homolog of macrophage migration inhibitory factor. This homolog is present in many organs; however, few studies have mapped the anatomical distribution of this enzyme in the brain. Because D-DT plays a critical role in inflammation, we tested whether D-DT activity could be used as a potential biomarker for Parkinson disease (PD). Although much of the neuropathology of PD is well understood, the causes that lead to disease onset remain unclear. Clinical and animal studies point to inflammation of the brain as a major end-result factor in PD.

Hypothesis: We tested whether D-DT levels could be used as biomarkers of the neurodegenerative disease using a well-established mouse model of PD: the aphakia (pitx3 -/-) mouse. We compared levels of D-DT in brain and blood with those of wild-type mice during several stages of development. We hypothesized that levels of D-DT would be higher in the PD mouse relative to normal age-matched control mice.

Methods: To detect D-DT by immunoblotting, brain samples were separated into nuclear and cytoplasmic components. Samples were probed with anti-sera for mouse D-DT and visualized by chemiluminescence. Total RNA was extracted from brain and blood samples using RNeasy Mini Kits. Data from quantitative polymerase chain reaction (PCR) were analyzed using 2^-ΔΔCt methods. Data were reported as relative values (ie, 100%) from β-actin. Differences between groups were assessed with student *t* tests. Data were reported as means ± SEM (standard error of the mean).

Results: We detected bands migrating to approximately 13 kDa by Western blotting. Next, we measured levels of D-DT in the pitx3 -/- brain and compared them with those of controls. We also measured blood levels of D-DT between these 2 genotypes. The mean-fold change in expression of D-DT relative to β-actin was 6-fold higher in midbrain and prefrontal cortex from aphakia mice compared with controls ($P<.05$). The mean fold change in cerebella of aphakia mice was 3-fold higher compared with controls ($P<.01$). Quantitative polymerase chain reaction analysis of blood samples yielded 39% less D-DT expression in aphakia mice relative to controls ($P<.01$).

Conclusion: Our data show D-DT located to areas of the brain normally associated with PD pathology. The quantitative polymerase chain reaction results support our working hypothesis that levels of D-DT are significantly modified in animals with PD-like symptoms suggesting that levels of D-DT could be used as biomarkers of disease onset and disease progression.

♦ Abstract that won first or second place in the 2012 SOMA Student Poster Competition.

S36

Extrachromosomal Circular DNA in Parkinson Disease

Emily Forbes, OMS III; Kristin DiGregorio, BS, MA; Edlira Yzeiraj, BA; Brian H. Hallas, PhD; German Torres, PhD; Joerg R. Leheste, PhD

New York Institute of Technology College of Osteopathic Medicine, Old Westbury

Background: The Human Genome Project revealed a total of only about 23,000 human genes, and we have since learned that phenotypic diversity and variation are based on creative use rather than quantity. The formation of extrachromosomal circular DNA (eccDNA) from repetitive genomic patterns (eg, tandem repeats, satellite DNA) follows this paradigm and is common to all eukaryotes. In yeast for example, ribosomal DNA (rDNA) circles are established regulators of fecundity and replicative lifespan. In humans, generation of eccDNA species by means of homologous recombination is frequent during early developmental stages but drastically declines in adults, where it is seen as a sign for genomic instability increasing the risk of somatic mosaicism and apoptosis.

Objective: To investigate identity and distribution of human eccDNA species in established human cells and compare results to pathological brain tissue derived postmortem from individuals with a common age-linked disease affecting the central nervous system. Specifically, we intended to determine if the pattern of human eccDNA species could be indicative of Parkinson disease (PD).

Hypothesis: The pattern and distribution of eccDNA species are tied to replicative age and human health. Age-linked pathologies, such as PD, are therefore expected to have a unique eccDNA signature.

Methods: In vitro studies were carried out in human HEK-293 cells under standard conditions. Postmortem human mesencephalic tissue (substantia nigra) of PD patients and controls was obtained from The National Neurological Research Specimen Bank (Los Angeles, California). Cells/tissues were sequentially treated to remove linear DNA, RNA, and protein before eccDNA purification. For eccDNA pattern analysis, samples were amplified, cloned, sequenced, and analyzed using polymerase chain reaction.

Results: HEK-293 cells revealed diverse eccDNA species (average, 611 base pair [bp]) equally spanning across the human genome. Approximately 95% consisted of non-coding DNA, whereas about 5% encoded DNA exons. Repetitive elements (Alu, retrotransposons, retroviruses) were commonly included (approximately 75%). In PD midbrain samples, the diversity of eccDNA species (average, 494 bp) was reduced and at least 50% contained coding exons including park2. Repetitive elements were less common (approximately 50%).

Conclusion: With PD as an example, we are demonstrating that an age-linked pathologic process of the brain can have a disease-specific eccDNA signature.

S37

Central Retinal Artery Atherosclerosis: An Indicator of Carotid and Coronary Artery Disease

Matthew J. Schear, OMS III[1]; Brian Beatty, PhD[2]

[1]New York Institute of Technology College of Osteopathic Medicine, Old Westbury; [2]Anatomy, New York Institute of Technology College of Osteopathic Medicine, Old Westbury

Background: The retinal examination has been a measurement for monitoring vascular disease for a long time. However, there remains a lack of understanding about the pathogenesis underlying the relationship between microvascular and macrovascular disease.

Hypothesis: We hypothesized that atherosclerosis in the retinal circulation would correlate to atherosclerosis in the carotid and coronary arteries.

Methods: Twenty-eight cadavers (15 males, 13 females) were dissected, obtaining 1 orbit, 1 carotid artery, and 1 coronary artery from each. The specimens were sectioned and stained for histologic analysis by light microscopy using Hematoxylin and Eosin, Verhoeff's Elastic, and Gomori's Trichrome. The degree of atherosclerosis was graded from absent, or I (least severe) to VIII (most severe) according to the current American Heart Association guidelines.

Results: A positive correlation was found between the central retinal artery and carotid artery (r=0.212), the central retinal artery and the coronary artery (r=0.310), and the carotid artery and coronary artery (r=0.451).

Conclusion: Evaluation of the retinal vessels is a useful way to follow the contribution of atherosclerosis to the pathogenesis of macrovascular disease. By establishing the correlation of pathology of the central retinal artery to the carotid and coronary arteries, this suggests that measuring atherosclerosis in retinal vessels can influence decisions about cerebrovascular and heart disease treatment.

S38

Block of the Cardiac Potassium Channel HERG by Extracellular Hydrogen, Calcium, and Magnesium

Shaun Rafael, OMS II; Eric Lau, OMS II; Ryan Frasier, OMS II; Vincent Carbone, OMS I; Alan Miller, PhD

Touro University College of Osteopathic Medicine, Vallejo, California

Background: The human ether a-go-go–related gene (HERG) encodes a cardiac potassium channel that is important in the repolarization of the action potential. A reduction in the number of HERG channels has been implicated in long QT syndrome, which in some cases can degenerate into the lethal arrhythmia torsades de pointes. Many patients present with abnormal serum electrolyte levels due to a variety of conditions including gastrointestinal dysfunction, renal and endocrine disorders, diuretic use, alcoholism, and aging. Changes in extracellular electrolytes and extracellular pH have been shown to reduce HERG channel function.

Hypothesis: The hypothesis of this project is that extracellular H+ extracellular calcium, and extracellular magnesium block the HERG channel.

Methods: Experiments were performed using 2-electrode voltage clamping of Xenopus oocytes expressing either wild-type HERG or the HERG mutant S631A, located in the outer pore of the HERG channel. Complementary RNA was injected into enzymatically defolliculated oocytes and currents were recorded 1 to 5 days after injection.

Results: Changing extracellular potassium from 0 mM to 20 mM resulted in a greater decrease in WT HERG current due to an increase in either extracellular calcium, magnesium, or hydrogen. Additional experiments showed that in 0 mM potassium, an increase in extracellular calcium resulted in a greater reduction in wild-type HERG current compared with the reduction seen with the mutant S631A.

Conclusion: Although the mechanism by which extracellular calcium, magnesium, and extracellular pH reduce current through HERG channels is not clear, one plausible explanation is pore block by these cations. The dependence of the permeant ion on the decrease in current due to either calcium, magnesium, or pH, as well as the change in current reduction with the outer pore mutation S631A, suggest that potassium, calcium, magnesium, and hydrogen may interact at the outer mouth of HERG. There is evidence that the outer mouth of the HERG channel is different than other voltage-gated potassium channels and thus these interactions may be unique to the HERG channel. This study has implications for an increased risk of cardiac arrhythmias in patients with hypokalemia.

B18*

Stretched Dermal Fibroblasts Direct Acetylcholine Receptor Expression and Clustering on Skeletal Muscle Cells

Michael Hicks, PhD[1]; Thanh Cao, BS[2]; David Campbell, BS[2]; Paul Standley, PhD[2]

[1]Department of Molecular and Cellular Biology, Arizona State University, Phoenix; [2]Department of Basic Medical Sciences, University of Arizona, Phoenix

Background: Manual manipulation targeting fascia is often used to restore mobility and functional recovery of damaged tissue. Increased acetylcholine receptor (AChR) expression, clustering at the postsynaptic membrane of skeletal muscle, or both may regulate functional recovery by decreasing the depolarization threshold needed for muscle contraction.

Hypothesis: We hypothesized that stretched fascia induce fibroblasts secretion of paracrine mediators that regulate the AChR on skeletal muscle.

Methods: We have previously developed in vitro models of repetitive motion strain (RMS), myofascial release (MFR), and a combined RMS+MFR using fibroblasts seeded on Bioflex plates. C2C12 myoblasts were grown on non-deformable glass coverslips for 24 hours and then transferred juxtapose to the fibroblasts in Bioflex wells at diffusible range for secreted cytokines. Fibroblasts were then subjected to the strain paradigms mentioned above. At 96 hours after strain, C2C12 coverslips were analyzed by Western blot for AChR expression or by fluorescent labeling with α-bungarotoxin for AChR clustering. Eight images per coverslip were analyzed for AChR cluster number per mm^2 and cluster sizes by means of an automated software platform CellProfiler. C2C12 myoblasts in uniculture served as a negative control. Each independent co-culture served as a sample (N=3), and student *t* tests were used to determine statistical significance ($P<.05$).

Results: We found the presence of fibroblasts increased C2C12 AChR expression by 130% vs C2C12 in uniculture ($P<.05$). Our preliminary data also showed RMS and RMS+MFR increased AChR expression by 22% and 46% vs nonstrained co-culture, respectively. AChR cluster numbers reached 127±9 per mm^2 for nonstrain co-culture as compared with 37±8 for uniculture groups ($P<.05$). However, we did not see a statistically significant difference in cluster number (114-148/mm^2) or cluster fluorescent area (3,750-5,350 μm^2) among any of the stretched co-culture groups.

Conclusion: These results show that fibroblasts drastically upregulate AChR expression and clustering on skeletal muscle cells. As strain affects fibroblast cytokine secretion, these data suggest that fibroblasts may regulate muscle functionality through paracrine mediators. We measured small increases in C2C12 AChR expression in response to repetitively strained fibroblasts. More experiments are being conducted to determine the correlation and mechanisms of mechanical strain-induced skeletal muscle AChR.

Acknowledgments: American Osteopathic Association, Arizona Biomedical Collaborative.

*Abstract B18 was submitted through the regular abstract submission process but was omitted from the August 2012 publication of abstracts.

Medical Education
S18

There's an App for That—Medical Smartphone Usage and Perceptions Among Medical Students and Practicing Physicians

Andrew C. Buchholz, MPH, OMS IV[1]; Brittany Perry, OMS IV[1]; Danielle Cooley, DO[2]; Lucia Weiss, MS[3]

[1]University of Medicine and Dentistry of New Jersey-School of Osteopathic Medicine (UMDNJ-SOM), Stratford; [2]Department of Family Medicine and Department of Osteopathic Manipulative Medicine/Neuromusculoskeletal Medicine, UMDNJ-SOM, Stratford; [3]Department of Family Medicine, UMDNJ-SOM, Stratford

Background: Smartphones—cellular telephones running computer applications—have become mainstream, including in the health care setting. However, little formal research has been performed to examine it. This study aimed to examine usage among medical students and physicians in a statewide medical university.

Hypothesis: That medical students will be more likely to have the technology, will have fewer perceived barriers to it, and will have recommended it for patient use more often.

Methods: An institutional review board–approved 23-item survey eliciting demographics, smartphone use, and perceptions was developed by the research team. An e-mail invitation was sent to all medical students and practicing physicians in 3 medical schools. All submissions were anonymous, collected through SurveyGizmo, and analyzed with SPSS.

Results: A total of 544 surveys were submitted, 347 of which were completed and analyzed. Of these respondents, 93.9% had smartphones, with no significant difference between students and physicians (95.2% vs 92.5%, $P=.356$). Of those with the technology, 82.9% stated they have used it at least once in a clinical setting. Respondents perceived fast access to information to be the greatest benefit to mobile medical technology (96.6%), as well as simplified access (75.5%) and calculations (70.8%). Barriers to using this technology included price (43.4%), uncertainty about available applications (39.4%), and inexperience (23.4%). There was no significant difference between students and physicians with regard to inexperience (21.2% vs 21.9%, $P=.887$) or uncertainty (37.1% vs 35.8%, $P=.806$). Concerning patient-centered applications, only 24.5% of respondents have recommended them to patients, with fewer students having done so than physicians (16.4% vs 29.6%, $P<.0001$). However, assistance with lifestyle modification (78.8%), increased adherence to treatment plans (73.8%), and promoting patient self-efficacy (57.0%) were agreed upon as potential benefits. Perceived barriers to recommending this technology were added cost to the patient (52.6%), concerns about self-diagnosis (47.7%), and lack of training (34.9%), with no significant differences found between the groups.

Conclusion: The data from this study suggest smartphone use is prominent in the health care setting and indicates strong agreement regarding its benefits and barriers. Integrating smartphone education into the medical curriculum and continuing education courses would be beneficial to health care providers.

S24

Students' Attitudes, Preparedness, and Expectations of the SOMA Clinical Presentation Curriculum

Rosalee Nguyen, MS, OMS II

A.T. Still University-School of Osteopathic Medicine in Arizona, Brooklyn, New York

Background: The A.T. Still University-School of Osteopathic Medicine in Arizona (ATSU-SOMA, or SOMA) opened its doors in 2006 to a new generation of osteopathic medical students and provides a unique and innovative way of learning medicine. The A.T. Still University-School of Osteopathic Medicine in Arizona integrates basic and clinical sciences and incorporates them into early clinical exposure as a 1-3 model. The foundation of SOMA's curriculum is rooted in 120 probable clinical case presentations seen by a primary care physician. Although SOMA has been implementing the Clinical Presentation Curriculum (CPC) for the past 6 years, there is currently not enough written information for prospective students to fully understand the integrated curriculum. However, the preliminary data regarding how SOMA students are performing in the didactic and clinical phases of the CPC seem promising.

Objective: This study aimed to be a source of feedback for the university and a reference for perspective students who are looking to access the effectiveness of using inductive reasoning (IR) with scheme navigation as a foundational tool for learning medicine. The main objective of this study was to report students' perspective on the CPC.

Methods: We compared first-year osteopathic medical students and fourth-year osteopathic medical students and determined the perceived effectiveness of the scheme presentation model and its application by means of students' attitudes, preparedness, and expectations of the CPC. The study was exempted by the A.T. Still University Institutional Review Board. Electronic surveys were sent to 106 first-year osteopathic medical students and 86 fourth-year osteopathic medical students, respectively. A total of 109 students responded to the survey with 69 responses (65.1%) from first-year osteopathic medical students and 40 responses (46.5%) from fourth-year osteopathic medical students. A typical 5-level Likert scale was used, and the data analysis was completed by means of SPSS software. "Positive" results indicated an average of mean ratings approaching or exceeding 3.5 out of 5, and "negative" results indicated an average of mean ratings below 3.5 out of 5.

Results: Our data suggest that the SOMA CPC is an effective method and tool for delivering undergraduate medical education. The first-year osteopathic medical students consistently had stronger positive responses compared with the fourth-year osteopathic medical students with significant differences of 95% or more.

Conclusion: Our study provides the SOMA faculty with ways of improving their current methods of teaching and facilitating the use of inductive reasoning skills with scheme-based navigation to prepare students for the challenges of being clinicians. An inductive scheme-based approach is one way of improving the delivery of undergraduate medical education around the country.