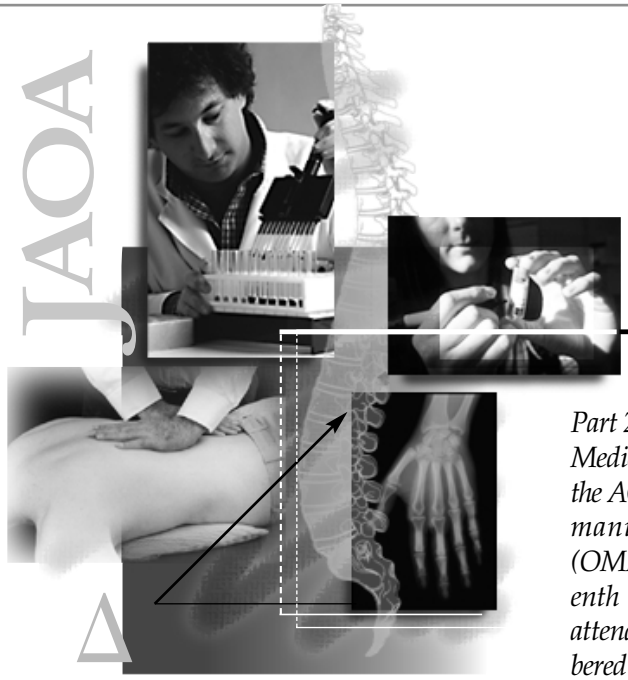

Forty-seventh Annual AOA Research Conference Abstracts, 2003: Part 2

Part 2 contains abstracts in the Poster Session on Basic Sciences and Medical Education. Part 1 (August issue) contained abstracts in the AOA Research Fellowships, and poster presentations on osteopathic manipulative medicine/osteopathic principles and practice (OMM/OPP) and Clinical Studies to be presented at the Forty-seventh Annual AOA Research Conference. For the convenience of attendees, abstracts appear in their scheduled sequence and are numbered for easy reference.

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Basic Science

B01

Short chain fatty acids affect ketogenesis in ultra marathon runners

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Purpose To determine whether a diet enriched in short chain fatty acids provided through the consumption of high-fat cheeses affects ketone production in ultra marathon (ultra distance) runners.

Methods Nine participants provided random specimens throughout the Australian 6 Day Race [an international invitational held at Colac (Melbourne) November 17-23, 2002]. Urinalysis was done using Bayer Ketostix®. The degree of ketogenesis was graded as 0, 5, 15, 40, 80, and 160 mg/dL, and then documented along with total hours of running (within 60 minutes) and distance. Two runners consumed a diet high in soft cheeses having an 80% minimum fat content. Other runners in the study consumed their usual diet, which did not contain high-fat cheeses.

Results The runners that consumed high-fat cheeses did not form ketones, whereas runners using their established diets, i.e., those not containing high-fat cheese, formed ketones between the 28th and 91st hours. Ketone production was found to be independent of runner performance, however. There were no gastrointestinal or other side effects and no indication that race performance was impaired.

Conclusions We conclude that consumption of a diet enriched in short chain fatty acids in the form of high-fat cheese prevents ketogenesis in ultra distance runners. Whether performance was affected by the high-fat cheese diet could not be determined from the available data. Ketone production did not depend upon performance; both top runners and less-competitive participants produced ketones.

Acknowledgement Supported by intramural resources of the OMM Department

B02

Isolation and Identification of Hepatitis A Virus in Shellfish with Reverse Transcriptase Polymerase Chain Reaction

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Hypothesis: RNA extraction, reverse transcription and polymerase chain reaction (RT-PCR) and electrophoresis are sensitive enough to detect hepatitis A virus (HAV) in infected mussel tissue under laboratory conditions.

Materials and Methods: The first phase of this study developed a protocol to detect HAV. RT-PCR (EZ rTth Applied Biosystems) and HAV primers were used to amplify HAV RNA. HAV and (+) control λ-phage virus were amplified. The PCR mix contained dH_2O, 10μM primers, buffer, 4 dNTPs, $Mn(OAc)_2$ and DNA Pol rTth. Thermal cycling was conducted in a Perkin Elmer Thermal Cycler. The protocol was as follows: 65°C for 20 minutes linked to 40 cycles of 94°C/1 min, 64°C/1 min, and a final 72°C/7 min cycle. RT-PCR products were electrophoresed in 2% agarose-ethidium bromide gels in TAE buffer. The gel images were digitized and graphically analyzed. The second phase of this study used mussel tissue containing HAV. These mussel samples were extracted with freon and the aqueous fraction was collected. A Qiagen protocol was used to isolate total RNA and it was subjected to RT-PCR and gel electrophoresis. Mussel tissue containing λ–phage was processed in parallel with the mussel-HAV RNA to serve as the positive control. The Thermal Cycler was programmed to: 65°C/45 minutes, 94°C/2 min, 35 cycles of 94°C/1 min, 60¡C/1 min, and a final 72°C/7 min cycle. After the cDNA products were amplified as above, they were subjected to a nested PCR. The thermal cycler program used was: 94°C/2 min, 28 cycles of 94°C/1 min and 60°C/1 min, and a final cycle of 72°C/7 min.

Results: A linear relationship was observed between the concentration of viral RNA and gel band pixel count when digitally analyzed. The RNA extraction experiments showed that HAV RNA in the mussel tissue can be isolated and amplified. The nested PCR procedure amplified HAV cDNA amplicons so that gel bands could be readily visualized.

Conclusion: This procedure allowed us to detect as few as 1,000 tissue culture infective doses ($TCID_{50}$) of virus per ml of sample.

This research was supported by a UNECOM Dean s Research Fellowship

B03

ANALYSIS OF NUCLEOSOME UNWINDING BY DNA HELICASES

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In an effort to understand how nuclear processes occur in the context of repressive chromatin structures found *in vivo*, we have investigated the abilities of the *E. coli* UvrD protein and human Bloom's Syndrome helicase (hBLM), to unwind nucleosomal substrates *in vitro*. UvrD is a DNA repair helicase whose eukaryotic counterpart is mutated in patients with the skin cancer disorder, Xeroderma Pigmentosum. Human BLM is a DNA helicase involved in maintaining genomic stability that is mutated in patients with Bloom's Syndrome, a disease predisposing them to multiple defects including cancer. Our hypothesis is that helicase-promoted unwinding through chromatin substrates will occur at reduced rates relative to naked DNA due to the limited accessibility of DNA to the helicase. We have used a model mononucleosome template that consists of a 172 bp EcoRI fragment containing the 5S rRNA gene, a sequence that can position a nucleosome. Both the UvrD and BLM helicases were incubated with ^{32}P-labeled naked DNA and mononucleosome templates in the presence of Mg-ATP, and then single-stranded DNA (ssDNA) products were separated from double-stranded DNA (dsDNA) substrates using native PAGE gel electrophoresis followed by autoradiography. Our results demonstrate that the UvrD helicase is able to unwind DNA assembled into a mononucleosome. We have preliminary evidence that the BLM helicase can react on both naked DNA and nucleosomal templates to produce ssDNA as well as an additional higher molecular weight species. We are currently attempting to resolve the nature of latter product. These studies will help establish how clinically relevant helicases contend with chromatin structure and what mechanisms the cell uses to modify chromatin structure so that DNA-related enzymes can gain access to them.

The authors gratefully acknowledge funding support provided to J.G.Y. and H.R.Z. by Midwestern University.

B04

THE EFFECT OF FLUOXETINE AND OLANZAPINE ON IMMEDIATE-EARLY GENE EXPRESSION AND A STUDY OF THEIR BEHAVIORAL AND ENDOCRINE PROFILES

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It is hypothesized that drug augmentation therapy is effective in the management of treatment-resistant depression because of long-term adaptive changes in multiple synaptic endpoints that arise from chronic use of an anti-depressant with an anti-psychotic agent. In this study, we examined various modes of gene expression in rats treated acutely with either fluoxetine (10mg/kg) or olanzapine (5mg/kg) and chronically with a combination of the two drugs; as well as changes in motor behavior patterns and endocrine profiles.

Representative rat brain sites including the hippocampus and piriform cortex previously expressing the immediate-early gene transcription factors (i.e. ERK, pCREB and FOS) in response to either fluoxetine (32.1 ± 10.2) or olanzapine (58.0 ± 9.1) administration, showed significantly less ($P \leq 0.05$) induction of these transcription molecules when exposed to either drug augmentation therapy or inactive vehicle solutions (13.2 ± 2.5): typical values expressed as the mean ± SEM of pCREB positive neurons per mm² of the piriform cortex.

Furthermore, fluoxetine used individually (1.100 ± 0.007) or in conjunction with olanzapine (1.20 ± 0.03; $P \leq 0.05$) elicited pronounced, cataleptic-like behavior–as evidenced by an animal behavioral rating scale–manifested by motor immobility; whereas olanzapine treatment produced only minimal changes in spontaneous behavioral activity (3.60 ± 0.12; $P \leq 0.05$). Finally, we illustrated that the combination of fluoxetine and olanzapine sensitizes peak adrenal corticosterone secretion (blood hormone levels of vehicle-treated animals compared to levels with both psychotropic drugs, respectively: 9.9 ± 3.5 ng/ml, 19.614 ± 0.129 ng/ml) without creating significant differences ($P > 0.05$) in serum glucose levels (glucose levels for control animals compared to combined drug exposure: 194.5 ± 21.7 mg/dl, 187.3 ± 15.4 mg/dl).

Our experimental results provide new insight into how fluoxetine and olanzapine operate at the cellular level to realize a therapeutic advantage in patients with treatment-resistant depression.

This study was supported in part by a 1999 and 2001 award from the National Alliance for Research on Schizophrenia and Depression (NARSAD).

B05

EXTRACELLULAR RECORDINGS FROM BRAINSTEM NEURONS

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The purpose of these experiments was to record from extracellular neurons within the brainstem of anesthetized, artificially ventilated rats. Specifically, we wanted to record the change in firing frequency of (1) barosensitive neurons within the nucleus tractus solitarius (NTS), (2) somatosensory neurons within the dorsal column nuclei and (3) respiratory-related neurons.

Male rats (250-300 g) were initially anesthetized with Ketamine + Xylazine. The femoral artery and vein were cannulated in order to monitor blood pressure and inject drugs, respectively. A cannula was inserted caudally into the trachea to enable breathing. A second cannula was inserted into the trachea rostrally to be used in initiating the diving response. Temperature was measured via a rectal thermometer and maintained with a heat lamp. In some cases an inflatable snare was placed around the descending aorta. The rat was placed in the stereotaxic apparatus, given curare/pancuronium and artificially ventilated and two electrodes were placed around the anterior ethmodial nerve (AEN). Anesthesia was continued with either chloralose or urethane. The AEN electrodes were connected to a stimulator, and when stimulated, produced the diving response. The nasopharyngeal cannula was also used to initiate the diving response by passing either ammonia vapors or water through the nasal passage. Phenylephrine (PE) and sodium nitroprusside (SNP) were injected (IV) to increase or decrease blood pressure, respectively. Inflation of the aortic snare also increased arterial blood pressure.

Experiments were conducted analyzing the diving response and the baroreflex with various techniques (stimulation of nasal passages, electrical stimulation of AEN, inflation of aortic snare, injection of PE and/or SNP). Numerous neurons were recorded from various locations in the medulla. Within the NTS, we recorded from barosensitive cardiovascular neurons, as well as respiratory neurons. Within the dorsal columns nuclei (nucleus gracilus and nucleus cuneatus) we recorded neurons that responded to somatosensory stimulation. Lesions were made in the location of the specific neurons. The rat was then perfused with 4% paraformaldehyde. The brain was removed, fixed in a sucrose solution and sliced at 50 microns on a microtome. The sections were histologically stained with neutral red. Brain sections were serially reconstructed to determine location of the lesion site.

B06

De-Aggregation of Glycated α-Crystallin by Carnosine

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Carnosine is an efficacious treatment for cataracts. It is suggested that the observed reversal of lens opacity is due to carnosine's anti-oxidant and anti-glycation properties. This mechanism, however, infers protection of protein damage rather than dispersal of cataractous material. We propose an alternate mechanism of cataract reversal by carnosine and provide evidence to support this mechanism. Since glycation of lens α-crystallin contributes to cataractogenesis, we used this model to study carnosine's proposed effect. Protein glycation is a process by which proteins are non-enzymatically modified by sugars. When α-crystallin was incubated with a glycating agent, methylglyoxal, the percent opacity of the solution as measured by light transmittance increased. Addition of carnosine decreased the opacity back to baseline levels. Methylglyoxal also caused protein aggregation as measured by 90° light scattering. Following the addition of carnosine light scattering decreased back to baseline levels, suggesting a reversal of glycation-induced aggregation. In conclusion, we propose and provide evidence for an alternate mechanism to explain cataract reversal by carnosine. (This study was supported in part by a grant from the Division of Research at UHS)

B07

Anti-Crosslinking Activity of Anserine

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Carnosine is a histidine dipeptide (β-alanyl-*L*-histidine) found in high concentrations (20 mM) in neural tissue. Carnosine prevents toxicity of neural cells and inhibits the nucleation and aggregation of β-amyloid, which is the model for Alzheimer's disease. The protective properties of carnosine involve prevention of protein crosslinking that occurs following glycation, which is the unwanted reaction of sugars with proteins. Anserine, the methylated form of carnosine, is also found *in vivo* however its anti-crosslinking properties have not been studied. We studied the anti-crosslinking activity of anserine to determine whether it may contribute to neuroprotection. Our glycation model included using glycating agents (i.e. glyceraldehyde) to modify a target protein as measured by subunit crosslinking. Anserine showed anti-crosslinking activity; however, carnosine was a more effective anti-crosslinking agent than anserine, suggesting the importance of the histidinyl imidazole. The methylation of the N-1 nitrogen of the imidazole ring decreases carnosine's anti-crosslinking activity, presumably by altering its anti-oxidant properties. In conclusion, our observations suggest that anserine is an effective anti-crosslinking agent and that the methylation of carnosine's imidazole ring plays a crucial role in moderating anti-crosslinking activity. (This study was supported in part by a grant from the Division of Research at UHS)

B08

P53 Upregulation During Estrogen Treatment Of Osteoblasts

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This study was undertaken to gain an understanding of the role of p53 expression during estrogen treatment of osteoblasts. It is now known that estrogen activates pathways that involve plasma membrane signaling in addition to its classical receptor mediated changes in gene expression. P53 is a tumor suppressor gene whose main role is to prevent accumulation of DNA damage by triggering apoptosis in damaged cells. Estrogen is known to be anabolic for bone and is important to prevent osteoporosis. We have shown p53 expression to be up regulated by 17-B-estradiol (E_2). This increase does not require E_2 to enter the cells, indicating perturbation of membrane signaling by E_2. Accordingly, activity of ERK1 and 2 was increased, suggesting involvement of the MAP Kinase pathway. We also tested for p53 activity after shorter intervals of treatment with E_2, and found it to be increased within 10 minutes of E_2 treatment. While this increase appeared to be a result of membrane signaling, it did not occur through activation of the MAP Kinase pathway. The significance of p53 activation by E2 and the possible mechanism of action of E2 will be discussed.

This work was supported by a partial summer research fellowship from Midwestern University to EM.

B09

Morphometric Variation in African Ape Lumbar Vertebrae

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Purpose: To assess 1) how different parts of lumbar vertebrae change with increasing body size during growth in two closely related primate species of differing body size, and 2) whether or not *Pan* and *Gorilla* lumbar vertebrae share similar growth trajectories for their bony dimensions but differ in terminal (adult) size.

Materials and Methods: Digital photographs of lumbar vertebrae were taken from an ontogenetic, cross-sectional collection of wild-shot *Pan troglodytes troglodytes* (common chimpanzee) and *Gorilla gorilla gorilla* (western lowland gorilla) at The Cleveland Museum of Natural History. Dimensions were digitized and measured using SigmaScan Pro 4.0. Using Systat 10.0, all of the measurements were log-transformed and ordinary least-squares (OLS) regression was used to describe growth trajectories of vertebral dimensions relative to body size. An Analysis of Covariance (ANCOVA) was used to test for differences between *Pan* and *Gorilla* in the slope and y-intercepts of the growth trajectories.

Results: For both *Pan* and *Gorilla* there are no changes in neural dimensions of the lumbar vertebrae with size during growth. The slopes of most of the somatic dimensions of the lumbar vertebrae are near isometry (slope = 0.5) during growth, i.e. the dimensions increase in proportion to body size. However, spinous process dorsal projection increases at a faster rate than body size. For interspecific patterns of the lumbar vertebral dimensions examined, the growth trajectories of *Pan* and *Gorilla* are significantly discordant due to a downward shift of the *Gorilla* trajectory from *Pan*.

Conclusions: Vertebral foramen dimensions do not correlate with body size. Most of the vertebral somatic dimensions conform to predictions of isometry during growth. However, the disproportionate increase in spinous process dorsal projection with body size is perhaps indicative of certain biomechanical demands on the vertebral column at larger body sizes. The consistent shift in the *Pan* and *Gorilla* growth trajectories away from one another may indicate some functional difference between chimpanzees and gorillas in posture and/or locomotion. This study on our closest living relatives can help us better understand the function and evolution of the human vertebral column.

B10

Title: Amino acids regulate blastocyst implantation.

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Hypothesis: Amino acid signaling via the mammalian target of rapamycin (mTOR) is needed for development of trophoblast protrusive activity in mouse blastocysts *in vitro* (Martin and Sutherland, Dev. Biol., 240, 182-193, 2001). Such trophoblast motility initiates blastocyst implantation *in vivo*. While some studies indicate that leucine and arginine are most critical to development of trophoblast protrusive activity (Van Winkle, Biol. Reprod., 64, 1-12, 2001), the ability of these amino acids to initiate such development in the absence of other amino acids has not been tested. Materials and methods: Delayed implantation mouse blastocysts were cultured *in vitro* to measure amino acid uptake and ability to support trophoblast outgrowth. Results: Either 200 μM leucine or 10 μM arginine induced trophoblast motility, albeit two days later than all amino acids. This development was less frequent ($p < 0.01$) in 10 μM arginine than in 200 μM leucine. Fifty to 200 μM arginine was toxic to the embryos, but this toxicity was reversed when 100 or 200 μM leucine also was present. In this regard, arginine unexpectedly inhibited its own uptake via system $b^{0,+}$ in blastocysts at greater than 20 μM arginine ($p < 0.01$), and such inhibition might be reversed by concomitant incubation with leucine. Preincubation of blastocysts with both leucine and arginine for 15 min did not, however, reverse the resultant effects of arginine on its own transport. Preincubation of blastocysts with arginine for 3 h increased arginine transport, whereas it reduced leucine transport via system $b^{0,+}$. Conclusions: Leucine fosters development of trophoblast motility in the absence of other amino acids probably owing to its ability to initiate mTOR signaling. Arginine (10 μM) may partially circumvent leucine-initiated mTOR signaling by serving as a substrate for synthesis of the putative downstream signaling molecules, putrescine, spermidine, spermine and nitric oxide. The relatively small amount of trophoblast outgrowth supported by the latter substances or by arginine alone may indicate that additional downstream signaling molecules also are needed for optimum development of trophoblast motility.

B11

Effects of Androgens and Antiandrogens on Cell Cycle Regulation in the Thymus

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It has long been suspected that androgens alter the course of autoimmune diseases. It is plausible that an environmental antiandrogen, such as the DDT metabolite, DDE, could interfere with the signaling mechanisms of androgens, rendering affected individuals more susceptible to immune dysfunction. Our hypothesis predicts that androgens and the environmental antiandrogen DDE signal the immune system by regulating cell cycle mediators in the thymus. Our experiments were done with a mouse model. Thymuses from the mice were either treated *ex vivo* with dihydrotestosterone (DHT) or DDE. Western blotting was used to assess expression of cell-cycle regulated proteins expressed at different cell cycle phases, including AIM-1, Chk2, PKC delta, and PTEN. Our results indicated that DDE increased expression of PTEN, a tumor suppressor that arrests cells in G1. While DDE treatment resulted in less expression of Chk2, DHT treatment caused increased expression of this G1 to M phase protein. DDE and DHT both increased the expression of PKC delta, which can result in cell cycle arrest. Conversely, DDE and DHT treatment reduced expression of AIM-1, a protein that accumulates at the G2/M interphase.

In conclusion, we observed androgen and antiandrogen regulation of thymus cell cycle regulators. Therefore, cell cycle regulation in the thymus is a biochemical mechanism through which androgenic and antiandrogenic signals impact the immune system.

B12

Low Levels of Ethanol Restore Cytokine Production in Aged Mice After Injury

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Multiple factors influence mortality and sepsis after traumatic injury. Advancing age and high blood alcohol levels are two factors associated with increased risk of negative outcomes. However, low blood alcohol levels, while not extensively studied in relation to traumatic injuries, have been found beneficial in relation to several other medical conditions. Using a murine model, the present study seeks to determine whether the presence of a low level (60 mg/dl) of ethanol at the time of injury alters the post injury immune response of aged mice. After burn injury, aged mice have decreased production of Th1 and Th2 cytokines relative to young (2 month old) & aged (18 month old) sham-injured mice. Relative to young burn-injured mice, aged burn-injured mice have a significantly greater suppression of Th1 cytokines and similar production of Th2 cytokines. In particular, the production of the Th1 cytokines IL-2 and IFNγ were significantly suppressed in aged burn-injured mice relative to young sham-injured mice (43% and 47% decrease, respectively). However, the production of the Th2 cytokines IL-4 and IL-10 decreased to similar levels in both young and aged burn-injured mice relative to their sham-injured counterparts. Interestingly, exposure to a low physiologic level of alcohol prior to burn injury improved the cytokine production in aged burn-injured mice. Production of IFNγ and IL-4 doubled in aged mice that received ethanol prior to burn-injury, relative to those that received vehicle. Additionally, the level of IL-2 and IL-12 rose by 50% and 40%, respectively. Ethanol did not influence the production of cytokines in aged sham-injured animals. These results suggest that low circulating levels of alcohol leads to restoration in cytokine production after injury in aged mice. Mechanisms responsible for this these cytokine changes are currently under investigation. This work was supported by NIH AA12034 & AG18859.

B13

DIFFERENTIAL EXPRESSION OF E-CADHERIN AND N-CADHERIN ALONG THE RAT NEPHRON. W.C. Prozialeck, Ph.D. and P.C. Lamar, B.S. Department of Pharmacology, CCOM/Midwestern University, Downers Grove, IL 60515.

E-cadherin and N-cadherin are Ca^{2+}-dependent cell adhesion molecules that play important roles in the maintenance of renal epithelial cell-cell junctions and epithelial polarity in various segments of the nephron. Recent students from several laboratories indicate that the multiple cadherins are expressed in the kidney and that the patterns of expression vary markedly along the nephron. However, most of these previous studies have focused on the mouse and other species; to date, few studies have examined the patterns of cadherin expression in the rat kidney. In the present study, we have employed dual immunofluorescent labeling procedures that utilized specific antibodies against either E- or N-cadherin, along with antibodies against markers for specific nephron segments, to characterize the patterns of cadherin expression in frozen sections of adult rat kidney. The results showed that N-cadherin was the predominant cadherin in the proximal tubule, but was essentially absent in other nephron segments. By contrast, E-cadherin was most abundant in the distal tubule, collecting duct and some medullary segments, but was present only at very low levels in the proximal tubule. Additional results revealed different patterns of N-cadherin labeling along various segments of the proximal tubule; the S1 and S2 segments exhibited a fine threadlike pattern of labeling at the apical cell surface, whereas the S3 segment showed intense labeling at the lateral cell-cell contacts. These results indicate that E- and N-cadherin are differentially expressed along the rat nephron and that the patterns of localization are generally similar to those previously described in studies on mouse kidney. Supported by Grant #R01 ES006478 from the NIEHS.

B14

Effects of Extracellular Magnesium on Block of the Cardiac Potassium Channel HERG. Randy Flores OMSI, David Tunnel OMSI, Melissa Pearce OMSI, and Alan Miller Ph.D. Dept. of Basic Sciences, Touro University College of Osteopathic Medicine, Vallejo, Ca 94592

Cisapride is a prokinetic gastrointestinal drug known to block the cardiac potassium channel HERG. Block of HERG is an unwanted side effect which can, in some cases, prolong the QT interval and lead to the potential lethal arrhythmia Torsades de Pointes. Cisapride block of HERG under different extracellular magnesium concentrations was studied in Xenopus oocytes using the two-electrode voltage clamp. Drug block was monitored every 6 seconds just after a voltage change from $^{+}$20 mV to $^{-}$60 mV. In 0 mM Mg^{2+} and all cisapride concentrations tested, the current level at the beginning of the $^{-}$60 mV pulse decreased with successive pulses and reached a steady state level that depended on drug concentration. In 20 mM Mg^{2+} and 1 μM and 10 μM cisapride, the current level at the beginning of the $^{-}$60 mV pulse also decreased to a steady state level that depended on drug concentration. However, in 20 mM Mg^{2+} and 100 nM cisapride, the current level at the beginning of the $^{-}$60 mV pulse initially increased with successive pulses and then decreased with further pulsing to a steady state level. In 20 mM Mg^{2+} and 100 nM cisapride, steady state current normalized to the current during the first pulse was 1.1 ± 0.1, whereas in 0 mM Mg^{2+} and 100 nM cisapride the normalized steady state current was 0.8 ± 0.1. At all other concentrations tested the steady state current level was similar in both 0 mM Mg^{2+} and 20 mM Mg^{2+}. These results suggest that at least at low cisapride concentrations the extracellular magnesium concentration can influence HERG block by cisapride.

B15

"Travel" in Rhesus Macaques: A Dominant Substrate Associated Locomotor Activity. J.P. Wells, Ph.D., J.E. Turnquist, Ph.D.[2], West Virginia School of Osteopathic Medicine, Division of Structural Biology, Lewisburg, WV 24901; [2] University of Puerto Rico, Department of Anatomy, San Juan, Puerto Rico 00936.

This study was conducted at Cayo Santiago, part of the Caribbean Primate Research Center. Substrate was characterized structurally. Age known cadaver material was studied as to segment morphology including length, weight, and center of mass location. A substrate associated locomotor and postural inventory was collected with a total of 6,551 observations of Infant I, Infant II, Juvenile, and Adults identified.

As a behavioral category "travel" accounts for approximately ½ of Infant I total activity time. Adults travel the least and thus are more sedentary. Most of this activity takes place on the ground or on broad substrate bases in Zone I and on the proximal regions of Zone II in Type I trees: Adults commonly travel on horizontal branches of up to 16 cm. when arboreal and 76% of the time they travel terrestrially. Young animals, particularly juveniles, use the smaller and more flexible supports (2 to 4 cm.) for travel learning to locomote and negotiate the complex arboreal setting.

Selection of substrate type, zone, grade, and diameter correlate well with age during travel. Young infants and older more sedentary adults seek wide and safe bases of support. As the segment center of mass shifts more proximally and caudally in the developing infant, its dependence on stable bases of support decreases.

B16

Enhanced Medial Collateral Ligament Regeneration Using Porcine Small Intestinal Submucosa. M.M. DelBaggio, BS, P.J. Chubb, BS, T.D., Dailey, BS, C.H. Greene, PhD., Ryan M. Smith, BS. Philadelphia College of Osteopathic Medicine Department of Biomedical Sciences, Philadelphia, PA 19131.

Synthetic materials currently used for ligament prosthesis can provoke serious complications such as infection, rejection, and lack biocompatibility. Porcine small intestinal submucosa (SIS) is an acellular collagen-based resorbable scaffold that has been successfully used in many graft applications and may not share these disadvantages. This study was designed to provide additional information concerning the performance of this material when used as a medial collateral ligament prosthesis in a rabbit model.

Seven New Zealand White Rabbits were divided into two groups: SIS group (n=5) and control group (n=2). The MCL of the right hind limb in the SIS group was exposed and transected with a scalpel at the joint line. The two ends of each transected MCL were then bridged with an SIS graft inserted between and attached to the ends of each transected ligament. The MCL's of the right hind limb of the control group were transected, approximated and sutured in the same manner but without the use of SIS. All the rabbits were allowed unrestricted cage activity. The grafts were explanted at postoperative day 42 and presented for light and scanning electron microscopic visualization.

Preliminary observations indicate that the SIS supports ligament regeneration in the MCL based on microscopic examination. However, statistical confirmation must await completion of the healing period for all animals. It is anticipated that future studies will be directed toward testing the integrity of this repair over extended periods of weight-bearing activity.

This project was supported with intramural funding by the Department of Biomedical Sciences, Philadelphia College of Osteopathic Medicine.

B17

EFFECTS OF DIET ON PMN FUNCTION IN MYOCARDIAL REPERFUSION INJURY

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Polymorphonuclear leukocytes (PMN's) contribute to ischemia/reperfusion (I/R) injury of cardiac tissue through the production of inflammatory mediators and reactive oxygen species (ROS). We investigated the role of diet in the function of PMN's during myocardial reperfusion injury in isolated perfused hearts. Weanling Sprague-Dawley rats were fed a purified fat free diet supplemented with lipids containing one of the following: Diet I - high saturated fat; Diet II – 18:2(ω-6); Diet III – (ω-3) ethyl esters [20:5, 22:6, etc]. Following six weeks of feeding PMN's were isolated. Membrane phospholipid analysis showed PMN's from Diet III had increased incorporation of 20:5, ω-3, and a decrease in 18:2 and 20:4 (ω-6) when compared to Diets II. PMN's were infused, during the first five minutes of reperfusion, into the coronary inflow tract of isolated hearts from rats fed a standard Purina chow diet. At 2h following I/R, there was no significant difference in cardiac function between hearts perfused with PMN's from either diet group. However, coronary flow was significantly higher in hearts perfused with PMN's from Diet III at 30 minutes post ischemia when compared to Diet II ($p<0.05$). The conversion of L-tyrosine to dityrosine was measured as an index of ROS production. Hearts perfused with PMN's from Diet III had a significantly higher production of ROS during the first 7 minutes of reperfusion ($p<0.05$) compared to PMN's from Diet II. However, neutrophil infiltration into left ventricular tissue was significantly decreased in hearts perfused with PMN's from Diet III compared to both Diets I & II ($p<0.05$). Our data indicates that ω-3 dietary lipids (Diet III) alter PMN membrane structure. The alteration in membrane structure is associated with an increased coronary flow and an enhanced ROS production during early reperfusion and a reduced neutrophil infiltration into cardiac tissue.

B18

SULFONAMIDES INHIBIT THE GROWTH OF CANCER CELLS BY TWO MECHANISMS.

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Carbonic anhydrase (CA) catalyses the production of bicarbonate ($CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$), which is the obligatory form of substrate for a number of essential carboxylation reactions in several metabolic pathways, including gluconeogenesis, synthesis of certain amino acids, lipogenesis and pyrimidine synthesis. There is evidence that a low flux through these pathways may be accommodated by the uncatalysed rate of bicarbonate provision, whilst metabolic conditions demanding a higher level of flux require the participation of CA.

We have examined the hypothesis that specific sulfonamide inhibitors of CA also inhibit the growth of cancer cells, and investigated the mechanism by which this may occur. The cell surface, transmembrane CA isozymes, CA IX and CA XII are over-expressed in many tumors, including those of VHL disease and the majority of renal clear cell carcinomas and are now considered to be diagnostic of renal cancers. These isozymes may act to acidify the extra-cellular milieu, thereby creating a microenvironment that is conducive to cancer cell growth. The specific CA inhibitor acetazolamide (DIAMOX), at concentrations of 10μM and less, has been shown to inhibit the invasive properties of several human cancer cell lines.

We have demonstrated that specific sulfonamide inhibitors of CA isozymes also inhibit the growth in culture of cells derived from a range of human cancers. Acetazolamide inhibited the growth in culture of cell lines that have been shown not to express CA IX or CA XII, but at concentrations 10 to 30 times higher than those required to inhibit cell invasion. Furthermore, no inhibition was observed when the CA IX and CA XII-deficient cells were incubated in media containing nucleotide precursors. These data suggest that the anti-cancer effect of a higher concentration of CA inhibitors, on cells lacking the surface isozymes, may be ascribed to inhibition of the synthesis of nucleotides required for the rapid cell replication that is characteristic of cancer cells.

We are currently examining the effects of CA inhibitors on the growth rate of human renal cancer cells implanted into immunodeficient mice.

The support of the Hess-Roth-Kaminsky Urological Foundation is gratefully acknowledged.

B19

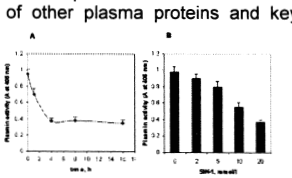
Spermine and spermidine inhibit structural and functional changes induced by glycation on proteins. A. Gugliucci, MD, PhD and T Menini, MD MS. Biochemistry Laboratory, Division of Basic Sciences, Touro University College of Osteopathic Medicine, Vallejo, CA 94592.

Hypothesis. An intense search for synthetic new antiglycation agents for prevention of diabetic complications is ongoing. A somewhat neglected avenue is the search for endogenous compounds that may inhibit the process and be a source of protodrugs. Based on their ubiquity, their polycationic nature, their essential role in growth, their relatively high concentrations in tissues, and their high concentrations in sperm, we hypothesized that polyamines inhibit glycation and that might be one of their so far elusive functions.
Methods and Results We employed two approaches: in the first, we monitored structural changes on histones and ubiquitin incubated in the presence of glucose-6-phosphate, fructose, methyglyoxal (MG) or glyceraldehyde (0.6-2 mmol/l) for 0-96 h, in the absence or presence of spermine, spermidine, carnosine or aminoguanidine (0.6-10 mmol/l). Glycation was monitored by SDS-PAGE and immunoblotting. Polyamines inhibit glycation-induced dimer and polymer formation as well as generation of AGE immunoreactivity. In the second approach we monitored functional impairment of catalytic activity of antithrombin III and plasminogen. Protection is afforded against glycation by hexoses, trioses and dicarbonyls AGE precursors and is comparable to those of aminoguanidine and carnosine.
Conclusions. In this study we demonstrate a potent antiglycation effect of physiological concentrations of the polyamines spermine and spermidine. These data support our hypothesis for a new role for polyamines in biology as natural antiglycation agents, that needs to be addressed in future in vivo studies. ***Sponsored by Touro University.***

B20

Human plasminogen is highly susceptible to peroxynitrite inactivation. A. Gugliucci, MD, PhD and T Menini, MD MS Biochemistry Laboratory, Division of Basic Sciences, Touro University College of Osteopathic Medicine, Vallejo, CA 94592.

Hypothesis.
Diabetes is a hypercoagulable state and diabetic patients show increased nitrosative stress as evidence for three-fold increases in plasma nitrated tyrosine have recently been shown. In previous work we documented that glycation decreases plasminogen activity in human plasma. Based on the considerations above we hypothesize that nitration of plasminogen could impair its catalytic properties and be another factor in diabetic thrombogenicity.
Methods To test this hypothesis in this study we addressed the effects of SIN-1 (a peroxynitrite generator) on human streptokinase-induced plasmin activity. Given the link between glycation and oxidation we also explored whether peroxynitrite enhances the effect of fructose (1-5 mmol/l) and glucose (5-50 mmol/l) on plasminogen.
Results As depicted in the figure, we provide evidence that plasminogen but not antithrombin III is quickly inactivated by exogenously generated peroxynitrite (0-20 mmol/l SIN-1), in a time and dose dependent manner. The effect occurs even when the molar ratio of other plasma proteins and key antioxidants is respected. In our system, peroxynitrite did not enhance the effect of the sugars. Preincubation of the sugars with peroxynitrite also failed to produce any effect.

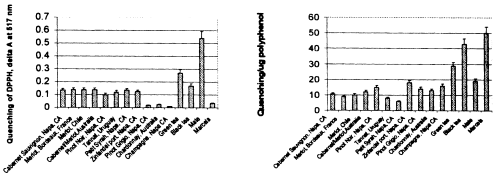


Conclusions. Our results suggest that in conditions and times approaching the in vivo situation, plasminogen is more susceptible to peroxynitrite damage than to carbonyl damage. Plausibly, nitration of tyrosine should play a critical role in either conformational or functional changes. If proven in ulterior in vivo studies, this factor would provide another mechanism by which nitrosative stress participates in diabetic complications. ***Sponsored by Touro University.***

B21

Free radical quenching activity of Achyrocline satureioides: comparison with other herbal teas and wines. *S Behtash OMS II, L Spieler OMS II, and A. Gugliucci, MD, PhD. Biochemistry Laboratory, Division of Basic Sciences, Touro University College of Osteopathic Medicine, Vallejo, CA 94592.*

Hypothesis. Previous work from our lab demonstrated Achyrocline satureioides (AS) extracts ability to inhibit LDL and whole plasma copper-induced oxidation. In this work we extend our observations to direct measurement of the free radical scavenging activity of this herb and compared it with well studied antioxidant beverages.
Methods. The free radical scavenging capacity of AS, green and black tea and wine samples was analyzed by using the 1,1-diphenyl-2-picrylhydrazyl assay (DPPH) as described [Malterud 1993]. Total polyphenol concentrations in our samples was determined spectrophotometrically with the phosphomolybdic-phosphotungstic acid reagents (Folin-Ciocalteau) using quercetin aglycone as a standard [Vinson 20 01].



Results. As shown in the figure, all of the samples studied scavenge free radicals, their respective potencies being Ilex paraguariensis >green tea >black tea >red wines> AS> white wines. When the activity was corrected for polyphenol content in each sample, the relative potencies were: AS >black tea>green tea>Ilex paraguariensis >red wines> white wines.
Conclusions. Extracts of AS possess the highest ratio of free radical scavenging activity/polyphenol content. AS is a non caffeinated herbal preparation that could then be an alternate, source of potent natural antioxidants devoid of unwanted stimulatory effects.
Sponsored by Touro University

Medical Education

M01

The Use of the NBOME Shelf Examination as a Discipline Outcome Assessment by M.A. Kilmore, Ph.D.[1]; W.H. Terry, Ph.D.[1]; E.P. Finnerty, Ph.D.[1]; L. Shen, Ph.D., MPH.[2] [1]Des Moines University-Osteopathic Medical Center, Physiology/Pharmacology Department, 3200 Grand Avenue, Des Moines Iowa 50312, [2]NBOME, 8765 W Higgins Rd., Ste 200, Chicago IL 60631

A requirement for accreditation is to have outcome goals with objective measurements of success with data that can be used to identify and correct deficiencies in curriculum. One of the outcome goals of the Physiology/Pharmacology Department at DMU, is to prepare the students to perform in the upper half of COMLEX I. Both of these disciplines are taught throughout the first and second academic years, with the largest bulk constituting a course at the end of first year where an NBOME discipline shelf examination was administered as a final examination to all students. In addition, the examination was administered to approximately 30 selected students near the end of their second year. These second year students equally represented the entire grade point distribution as determined from the first year class standing. The NBOME graded the exams and prorate the score, based upon statistics achieved from students using the exams over the years. Each student received a score from which an average and S.D. were obtained. The NBOME prepared a key word phrase for each question which recorded the percent of our students who responded correctly compared to the national average. The key word phrases allowed the questions to be grouped into various subject areas ie, cardiovascular, respiratory, autonomics, endocrine, etc. The average total score and the subject area results have been collected from classes covering eight years. The second year students consistently improved in physiology compared to their first year score. However, the pharmacology score was consistently lower when taken in the second year. These results were consistent for all subject areas except for cardiovascular pharmacology which had an improvement. Also, results showed that one area, endocrine physiology, had consistently better performance than other areas, except for 2002. Other subject areas in both physiology and pharmacology were very similar in performance. A correlation study of performance in year one and year 2 courses, the discipline shelf examinations and COMLEX scores revealed that the shelf examinations had the best correlation. Overall, the use of the NBOME shelf examination is an excellent objective mechanism to evaluate the physiology and pharmacology courses to determine preparedness for COMLEX I.

M02

Teaching the Osteopathic Musculoskeletal Exam (OME) for Patients in the Hospital Setting: Assessing House Staff Competence

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Hypothesis: 1) House staff self-reported competence in the hospital based OME would increase following completion of the "OME Module," and 2) the quality of information recorded on the OME form would improve.

Methods: The "OME Module," was administered to house staff from four hospital sites. A Mann-Whitney U test was performed to compare house staff pre and posttest responses for each of the self-efficacy questions. A random medical record OME audit was conducted where the "OME Module" was presented to the house staff (N=25). The findings of this audit were compared to a pre "OME Module" baseline random medical record OME audit (N=33). All audited medical records were from patients discharged with a diagnosis of COPD.

Results: A total of 52 house staff completed the pre and post assessment. The Mann Whitney U test showed that house staff self-assessment of competence statistically increased ($P < .05$). The random medical record OME chart audit demonstrated improved documentation of somatic dysfunction from a baseline of 44% to 84%.

Conclusions: Providing the "OME Module" significantly improved house staff self-assessment of competence in completing the OME on patients in the hospital setting. The quality of data on the OME form improved following "OME Module" implementation; however, other factors may have contributed to this outcome. Further study needs to be done to clarify the direct impact of the "OME Module" on the quality of OME form documentation across multiple disease areas of acutely ill hospital patients.

M03

Assessing Curricular Effectiveness in Measuring House Staff Competence in Osteopathic Principles and Practice (OPP)

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Hypothesis: The hypotheses of this study were 1) disseminating "OPP Provider Modules" would be an effective way to train and assess house staff competence in OPP, and 2) house staff self-reported competence would improve following completion of the "OPP Provider Module."

Methods: Two workshops were administered to train clinical faculty in the use of the "OPP Provider Modules." The "Modules" used two assessment methods to determine the competence of the house staff: 1) a six question pre and post self-efficacy assessment that was completed by learners, and 2) a critical actions behavioral checklist of live performance.

Results: 55 clinical faculty were trained in the use of the "OPP Provider Modules." In examining the impact of one of the "Modules" where N=124, house staff demonstrated competence in the critical actions checklist. A Mann-Whitney U test, a conservative type t-test, was performed to compare the pre test and the posttest responses for each of the six questions. For all independent comparisons between the pre test and posttest rank orders on individual questions, each question demonstrated improvement statistically significant at $P < .0001$.

Conclusions: Disseminating "OPP Provider Modules" was an effective way to train and assess house staff competence in OPP. House staff self-assessment of competence improved significantly as a result of the "OPP Provider Modules." Future modules will require further study with more rigorous evaluation measures regarding house staff competence in OPP.

M04

Osteopathic Manipulative Treatment (OMT) Protocols in the Hospital Setting: A Template for Implementation

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Hypothesis: Providing Directors of Medical Education (DMEs) with a detailed template for implementing disease-specific OMT protocols would increase the utilization and documentation of OMT in the hospital setting.

Methods: An OMT Protocol Implementation Template (OMT-PIT) was developed and distributed to DMEs at three SCS hospital sites as a pilot project. The template provided step-by-step instructions on how to implement OMT protocols for specific conditions in the hospital setting. Audits of randomly selected medical records with corresponding primary diagnoses were performed on patients admitted before and after the OMT-PIT was introduced.

Results: At each of the three pilot sites, medical record review yielded no documented OMT at baseline. After the OMT-PIT was introduced, OMT was documented on 10% of charts at two sites and on 20% of charts at the third site.

Conclusions: Preliminary results demonstrate that providing DMEs with a detailed template for implementing disease specific OMT protocols will increase the utilization and documentation of OMT in the hospital setting for selected DRG categories. Additional strategies are being developed to increase the frequency of OMT protocol utilization for selected DRGs.

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M05

Implementation of Interactive, Web-Based Tools on a Pocket PC

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This work is an extension of our efforts to use computer technology for curricular advancement in those areas that are of particular significance to the osteopathic profession. We have demonstrated that our computer-based, interactive teaching strategy, with real-time feedback and self-assessment exercises, facilitates student learning as measured by higher scores on midterm and final examinations (Academic Medicine, 77:263-265, 2002). We now suggest that utilization of wireless technology will further enhance the educational experience by enabling students to have rapid access to syllabi, course assignments, reference works, and other course-related materials.

We have ported our visualization software (http://hal.bim.msu.edu) to a Windows® platform PDA device (Toshiba Pocket PC, e740) so that concepts such as single-segment vertebral dysfunction, can be reviewed/studied at the learner's convenience. We have also used a multiple bit-rate, intelligent streaming strategy to enable us to efficiently compress video content. Doing this makes it possible to save up to 60 minutes of lecture materials to a removable 64MB Secure Digital (SD) memory card that plugs into the Pocket PC.

We suggest that utilization of wireless technology, similar to that integrated into the Toshiba Pocket PC, will offer students and faculty increased opportunity to assess education materials at their office, their home, and in a growing number of public locations.

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