Fusion

THE WILLIAM H. BEAUMONT MEDICAL RESEARCH HONOR SOCIETY | V. III, SPRING 2009

A Student-run Scientific Journal Serving The George Washington University Medical Center

about the society. . .

BEAUMONT LEADERS

The 2008–09 officers of the William H. Beaumont Medical Research Honor Society. Back row, from left: Rydhwana Hossain, MSII, editor; Sarah Brown, MSII, Journal Club director; Tayyab Khan, MSII, editor-in-chief; Elyse Katz, MSII, editor; and Lily Maltz, MSII, vice president. Front row, from Left: Amrita Karve, MSII, co-president; Talya Bordin-Wosk, MSII, co-president; and Kate Pickoff, MSI, editor.

Not Pictured: Andrew Degnan, MSII, editor; and Allison Spitzer, MSI, editor.

The William H. Beaumont Medical Research Honor Society is an honorary research society of medical students that was established in 1935 to honor Dr. William H. Beaumont, a pioneer in physiology research. The organization seeks to foster a continuing interest in biomedical research and to promote its value in the practice of medicine. As a part of this mission, the Society integrates current research topics in the curriculum; develops a research journal showcasing GW student research; makes available information on research opportunities and seminars throughout the area, including the William T. Gill Summer Fellowship for GW medical students; and highlights student and faculty research accomplishments at the annual GW Research Day.

VOLUME III SPRING 2009

Fusion is a publication of The George Washington University Medical Center's William H. Beaumont Medical Research Honor Society.

This research journal is published by students in collaboration with the Office of the Dean; Office of Health Research, Compliance and Technology Transfer; and Medical Center Communications and Marketing.

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ON THE COVER: *Complicated.*
A wound debridement in southern Iraq.

Photograph by Michael Martinez, MSII.

from the editors. . .

The George Washington University School of Medicine and Health Sciences (SMHS) is pleased to invite you to read the third edition of *Fusion*. Our 2009 edition showcases a spectrum of student research projects, including basic science research, clinical research, health policy, and research abroad. Leading faculty members at The George Washington University Medical Center have mentored the majority of the journal's student authors, from endeavors in a hookworm vaccine clinical trial in Brazil, to exploring cardiac pathophysiology, to spinal surgery at GW.

This year, we invited physicians' assistant students, medical students, and physical therapy students to submit their research. All 18 research projects presented in this issue were completed while students attended SMHS. Student officers of The William H. Beaumont Medical Research Honors Society were responsible for the editing and production of this year's journal. The editorial board included members from the medical school classes of 2011 and 2012.

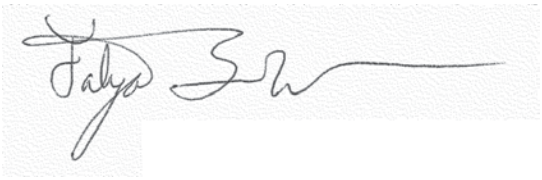
We also have an exciting new addition to the Beaumont Society's activities — a Journal Club. This year, we initiated monthly meetings to create an arena for students to discuss landmark research studies. These meetings are led by one of two members of the Beaumont Society and are moderated by faculty members including Dean James L. Scott, MD, FACEP; Dean W. Scott Schroth, MD, MPH; and Anne Hirshfield, PhD, our faculty advisor in the Office of Research. Journal Club meetings allow students to better understand the clinical relevance of scientific research and provide them with the opportunity to critically evaluate the strengths and weaknesses of various studies. We especially would like to acknowledge Sarah Brown, Journal Club director, for her exceptional job launching the Journal Club. We hope that Journal Club will continue to be an active and integral part of the Beaumont Society.

The Office of Student Opportunities, led by Cynthia Powell and Nancy Campbell, has been paramount in introducing students to research projects throughout the United States as well as internationally. One such opportunity, the William T. Gill Summer Fellowship, funded the basic science and clinical research projects of many students featured in this year's journal. The Dean's Office and the Office of Student Opportunities also have created the research track program, which helps students direct their career toward academic medicine.

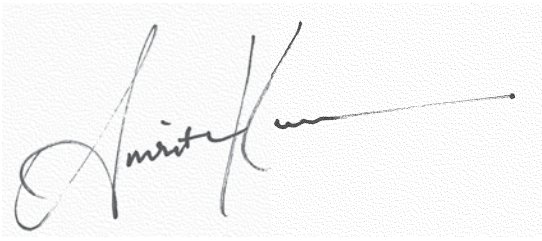
We are most grateful for the continual encouragement and support of Dean Scott, Dean Schroth, and Assoc. Vice President Hirshfield whose guidance helped shape the mission of the Beaumont Society, as well as the many dedicated faculty members at GW who mentored our peers. In particular, we would like to thank Thomas Kohout, in Medical Center Communications and Marketing, for his creativity, diligence, and enthusiasm in assembling this journal. Finally, we also thank our editorial board for its hard work.

Again, we invite you to share our enthusiasm for student research, and encourage our peers to continue careers in academic medicine to advance patient care in all dimensions, whether in basic science knowledge, clinical care, or health policy.

Enjoy *Fusion* 2009.



Talya Bordin-Wosk, MS II



Amrita Karve, MS II

TALYA BORDIN-WOSK, MSII

AMRITA KARVE, MSII

from the dean's office. . .

New endeavors, like breakthrough research, are inherently exciting. However, most of the time, those moments come after years of struggle by many dedicated individuals. *Fusion*, now in its third edition, came from dedicated work by a number of people as well. It is my pleasure to recognize just a few of those who dedicated their time and effort to bring this magazine to you.

This journal was conceived by medical students in the William H. Beaumont Medical Research Honor Society and I commend them for their work. Specifically, I want to recognize Talya Bordin-Wosk and Amrita Karve for their creativity and thoroughness in gathering, editing, and organizing these projects, and Thomas Kohout for serving as the managing editor. This journal would not exist without their hard work and vision. I also want to thank Associate Vice President for Health Research Anne Hirshfield, PhD, for her guidance and mentorship of the Beaumont Society and her encouragement of the students working on this journal. We also are indebted to the Office of Student Opportunities which arranged many of these projects for the students, and the generous supporters of that office. In addition, none of this would have been possible without the active involvement of so many of our faculty in the research and other activities of our medical students. To all of you, a collective thank you.

I take great pride in the scientific endeavors of our students, residents, faculty, and staff. I was especially delighted to read about their activities and accomplishments through the experiences in the pages of *Fusion*. I hope you share in that delight as you read on.

James L. Scott, MD
Dean, School of Medicine and Health Sciences,
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from the research office. . .

On behalf of the students of GW's William H. Beaumont Medical Research Honor Society, I welcome you to peruse the 2009 edition of *Fusion*, the third volume of a publication created by the members of this prestigious honor society. The Beaumont Society was established at the GW School of Medicine in 1935 to promote student research.

You will see from the following pages the depth of research experiences from our brightest students, not only on a local level but globally as well. The commitment to the mission of healing and discovery is evidenced in the work showcased throughout this publication. These students bring their excitement and passion for learning to you in a way that represents the opportunities for medical advancement in various fields. Thus, our citizens will reap the benefits of their labor in the years ahead.

Fusion is an entirely student run publication which further highlights the students' abilities as promising doctors and researchers. In addition, we salute the faculty advisors and all those who have mentored and assisted in any way to make this publication a reality. We also need to acknowledge the William T. Gill Summer Fellowship, which on a yearly basis, provides stipends to eligible students to gain valuable experience in research during the summer months.

Wherever their careers take them we know that the students of the Beaumont Society will make their mark in the medical field throughout the nation and world. I am honored to serve as the Society's faculty advisor and am happy to support these gifted students in their research endeavors.

Anne N. Hirshfield, PhD
Associate Vice President for Health Research, Compliance, and Technology Transfer

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Identifying the Cause of Ectopic Beats during the Initiation of Cardiac Arrhythmias

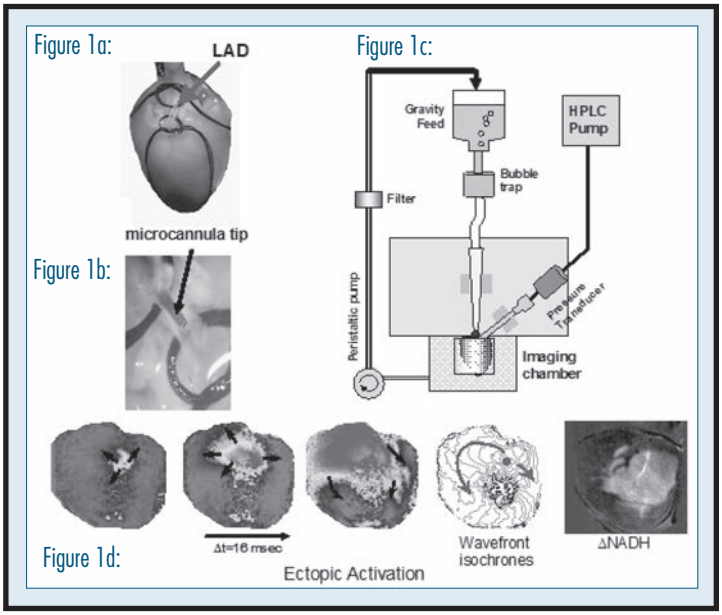

FIGURE 1: 1a and 1b: The LAD is delicately dissected and microcannulated with a polyimide tube, held in place with two sutures. Figure 1c: Custom arrangement designed to visualize electrophysiological signals, monitor flow, and record EKG signals, while keeping the heart viable. Figure 1d (images 1–4): Transmembrane potentials were imaged with the second camera to reveal the progression of action potentials throughout the epicardial surface of the heart. Figure 1d (image five): Low-flow reperfusion yields a very heterogeneous metabolic substrate that is prone to increased incidence of sustained arrhythmias.

Heart disease and its associated complications take the lives of more than 700,000 Americans each year, making it the leading cause of death in this country. It has long been understood that time is of the essence when an individual is in the midst of a myocardial infarction (MI), as the cardiomyocytes have only hours to survive without adequate oxygenation and lack the ability to regenerate once they begin to necrose. Therefore, current standards of care in cardiac catheterization have focused on rapid dissolution of coronary obstructions and restoration of blood flow to the myocardium using a variety of percutaneous coronary interventions (PCI).[1]

Directly related to this is a well-studied phenomenon known as "ischemic-reperfusion injury." Simply put, it is not only the deprivation of oxygen that leads to catastrophic metabolic and ionic changes within the myocytes, but also the restoration of blood flow which adds further insult to the already damaged cells. Even in patients who survive an MI, this phenomenon increases the risk of life-threatening sustained arrhythmias and must be carefully monitored.

At The George Washington University Medical Center, the cardiac research laboratory of Matthew Kay, DSBME, and Narine Sarvazyan, PhD, has developed a novel rat heart model of cardiac ischemia and reperfusion. With the model, coronary flow is carefully controlled to closely mimic *in vivo* MI conditions. The hearts of anesthetized rats are explanted and retrograde perfused through the aorta with an oxygenated nutrient solution that supports the organ for several hours. The Left Anterior Descending (LAD) artery is delicately dissected and microcannulated with a polyimide tube (Figures 1a and 1b). In this way, the flow of solution to the vascular bed supplied by the LAD is intricately controlled.[2]

The instrumentation is a custom arrangement designed to visualize electrophysiological signals, monitor flow, and record EKG signals, while keeping the heart viable (Furure 1c. A dual CCD camera system enabled us to collect near simultaneous data describing the metabolic and electrical conditions of the heart. We observed the geometry and degree of ischemia by imaging the fluorescence of NADH, which accumulates in the mitochondria of ischemic myocytes. After staining the heart with a potentiometric probe, transmembrane potentials were imaged with the second camera to reveal the progression of action potentials throughout the epicardial surface of the heart. At the end of the studies, imaging data were processed using custom algorithms. We then examined each data frame in detail to identify the electrophysiological origins of ectopic beats and any arrhythmogenic conditions (Figure 1d).[3]

By subjecting hearts to repeated phases of local ischemia, low-flow and full-flow reperfusion, we sought to support the working hypothesis that

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ECTOPIC BEATS *Continued from p. 6*

low-flow reperfusion yields a very heterogeneous metabolic substrate that is prone to increased incidence of sustained arrhythmias (Figure D, last image). If proven, one can begin to extrapolate this hypothesis to a clinical setting: if heightened excitability of certain myocytes during reperfusion is significantly arrhythmogenic, then perhaps catherization lab, emergency room, and even CPR protocols should be amended to consider elevated myocyte excitability resulting from reperfusion in addition to the complete restoration of blood flow to ischemic myocardium.

As the data continue to be analyzed, the lab is already performing experiments to complement the work that has been accomplished. We are studying a phenomenon known as "cardiac steal," and delving deeper into the origins of ectopic beats by studying how Purkinje fibers may participate in their formation. As the only lab in the world that uses a model and instrumentation such as this, the lab aims to continue refining its hypotheses and striving to investigate the problems that will bring clinically applicable science from the bench-top to the bedside.

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The Miracle Tree: Studying the Effects of Moringa Oleifera in Prostate Cancer

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According to a survey performed by the National Center for Cancer and Complementary Medicine (NCCM) in 2004, 62 percent of adults in the United States use some form of Complementary and Alternative Medicine (CAM). Excluding prayer used specifically for health, biologically-based therapies are the most popular CAM method used (22 percent).[1] Biologically-based therapies include supplements, which may contain ingredients such as botanicals, vitamins, minerals, etc., and are used for a variety of reasons, ranging from overall health maintenance to disease treatment. However, the full extent of the effectiveness and safety of most supplements is unknown, making them potentially harmful to consumers. The necessity for public knowledge has resulted in an increasing amount of research focused on studying the mechanisms and effects of botanicals. The purpose of my research was to study the effects of a botanical product, *Moringa oleifera*, on prostate cancer. *M. oleifera*, also known as "the Miracle Tree," is a pan-tropical tree that is used throughout the world in over 300 commercial and medicinal uses, including water purification and treatments for malnutrition, hypertension, inflammation, diabetes and cancer.[2] However, the mechanisms underlying its effects in prostate cancer are not known.

To mimic the microenvironment of human prostate cancer, prostate epithelial cancer cells were grown together with prostate stromal cells but separated by a basement membrane-like film that allowed for the passage of stromal mediators to epithelial cells. One significant stromal mediator is TGFβ1, which plays a role in cancer progression by inducing a reactive stroma that increases cell proliferation. TGFβ1 overproduction in the prostate is associated with increased tumor grade and the presence of metastases. An important hormonal mediator is dehydroepiandrosterone (DHEA), which has been shown to increase gene expression of prostate-specific-antigen (PSA — clinically used as a marker for prostate cancer) in this TGFβ1-induced reactive stromal environment.[3] The co-cultures of prostate epithelial and stromal cells were treated with TGFβ1 and DHEA. They were then treated with either ethanol or aqueous *M. oleifera* leaf extracts with the hypothesis that *M. oleifera* would reverse DHEA-induced PSA gene expression. The levels of PSA gene expression were quantified using RealTime RTPCR.

Combined results from five separate experiments found that, compared to controls treated

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with only DHEA and TGFβ1, addition of aqueous extract of *M. oleifera* to DHEA and TGFβ1 *increased* PSA gene expression in epithelial cells, while ethanol extract of *M. oleifera* to DHEA and TGFβ1 *decreased* PSA gene expression. These results suggest that ethanol and aqueous *M. oleifera* extracts contained different compounds that interacted with each other (by unknown mechanisms) to result in contrasting effects. This contrast between different extractions of *M. oleifera* demonstrates the major challenge in studying phytomedicines. Botanicals are complex mixtures of variable composition and unknown active compounds. Protocols for standardizing extract components to achieve reproducible results are still being developed. Nevertheless, the preliminary results of this research suggest that *M. oleifera* may have an effect on PSA gene expression in the prostate. However, more research is needed to determine whether *M. oleifera* is beneficial or detrimental in treating prostate cancer.

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The Effect of Daclizumab on FoxP3+ T Regulatory Cells in Multiple Sclerosis

Multiple Sclerosis (MS) is an inflammatory disease of the central nervous system characterized by lesions in the brain and spinal cord. Although there is no doubt that the disease is immune-mediated, the immune cells that are pathogenic and the antigen being targeted are unknown.[1]

Daclizumab therapy is one of many experimental therapies in MS currently being studied. The drug contains an antibody against the CD25 subunit of the IL-2 receptor (IL-2R). IL-2 is an important T cell growth factor that promotes T cell proliferation and differentiation.[2] Blocking CD25 significantly decreases the binding affinity of IL-2, shutting down the IL-2 signaling pathways of T cells.[3]

Only a small proportion of resting T cells express CD25 and have functioning high affinity IL-2R. The majority of these are FoxP3+ T regulatory cells (T-regs). T-regs are dependent on IL-2 for their *in vivo* proliferation and survival.[4] Therefore, one can hypothesize that blocking CD25 with Daclizumab, which leads to diminished consumption of IL-2 by T cells, may result in decreased *in vivo* proliferation of T-regs and/or their diminished survival. The question of T-reg dependency on IL-2 was addressed previously in mice, where it was shown that in the absence of IL-2, T-regs can use other cytokines, such as IL-7, to partially compensate for the lack of survival signal.[4] However, this question was never examined in humans. Consequently we wanted to assess numbers and *in vivo* proliferation of T-regs before and during Daclizumab therapy.

Cryopreserved peripheral blood mononuclear cells (PBMCs) from 26 MS patients from two separate clinical trials were used for an evaluation of surface molecules (CD3, CD4, CD8) and intracellular cytokine stain for FoxP3 and Ki67. FoxP3 is a T-reg marker and Ki67 is a proliferation marker, both of which bind to the chromatin in the cell nucleus.[2] Three separate time points were taken to examine the time course effects of Daclizumab treatment. We included evaluation of CD127 expression, a sub unit of the IL-7 receptor, to examine if T cells upregulate CD127 to compensate for the blocked CD25 and inability to utilize IL-2. Stained PBMCs were analyzed using the flow cytometer.

Results showed that while T-reg cell numbers decreased 8.1 percent ($p < 0.05$), Daclizumab had no significant effect on their *in vivo* proliferation. We did observe that of the proliferating T-reg cells, there was a 46 percent increase in CD127 expression following treatment with Daclizumab ($p < 0.05$).

Because CD25 on T-reg cells is blocked by Daclizumab, the cells are unable to utilize IL-2

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DACLIZUMAB

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(necessary for survival) and subsequently upregulate CD127 expression in an attempt to compensate for the blocked CD25. These findings indicate that human T-regs, like their animal counterparts, may be able to utilize another cytokine for growth and survival in the absence of IL-2. Despite the fact that Daclizumab mildly inhibits T-reg numbers *in vivo*, its overall effect on brain inflammation in MS is inhibitory.[5] This suggests that either FoxP3+ T-regs are not crucially important for MS disease process, or despite their lower numbers, their function is not significantly inhibited by Daclizumab. These conclusions will be tested in the future studies.

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Doxycycline-mediated Inhibition of Choroidal Neovascularization

Choroidal neovascularization (CNV) is a pathological process in which new blood vessels grow from preexisting vessels in the choroid through Bruch's membrane and invade the normally avascular subretinal space. These newly formed vessels contribute to the accumulation of blood in the subretinal space, a condition that leads to photoreceptor degeneration and death.[1] The formation of CNV complexes is the hallmark of exudative age-related macular degeneration (AMD).[2] Remodeling of the extracellular matrix plays a key role in neovascularization during migration and tubule formation of endothelial cells.[3] The delicate balance between matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9, and their inhibitors plays an

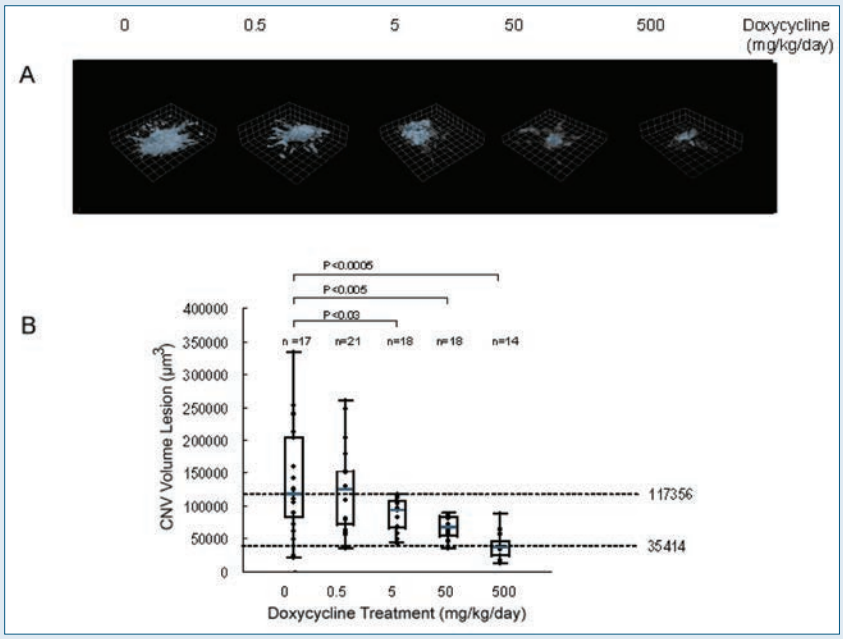

FIGURE 1: Effects of doxycycline on experimental CNV in rats. Rats were fed doxycycline enhanced water for seven days and then laser injury was performed to induce CNV. Seven days post-laser, animals were euthanized and endothelial cells were labeled with isolectin-IB4 in RPE/choroid flatmounts. Panel A shows representative images of isolectin-IB4 vessel staining (blue) in laser lesions from animals treated with the indicated doses of doxycycline. Panel B shows a graphical plot of the quantification of CNV lesion volumes of doxycycline-treated rats. Each point in this graph represents the average of three volume measurements per lesion. Five animals per condition were analyzed, and n represents the number of lesions. The median values are depicted in blue.

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DOXYCYCLINE

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important role in this remodeling.[4] Doxycycline is a broad spectrum antibiotic that has anti-angiogenic properties and inhibitory activity against MMPs.[5] We investigated the effect of doxycycline on CNV, on regulation of MMP-2 and MMP-9 and on control of anti-angiogenic pigment epithelium-derived factor (PEDF).

Doxycycline was orally administered to rats at 500, 50, 5, and 0.5 mg/kg/day, using non-treated animals as controls. After seven days of treatment, experimental CNV was induced using a ND:YAG 532-nm laser (Alcon). Seven days post-induction, animals were euthanized, and eyes enucleated. RPE/choroid flat-mounts were labeled with isolectin IB4 to determine CNV volumes using confocal microscopy and VolocityTM software.[6] Plasma protein levels of MMP-2, MMP-9, and PEDF were determined by ELISA. MMP catalytic activity was determined in solution using fluorogenic gelatin and peptide substrates, by gelatin zymography in SDS-PAGE and by in situ DQ-gelatin zymography in RPE/choroid sections.

Our results demonstrated that CNV volumes decreased logarithmically with doxycycline treatment. A dosage of 500 mg/kg/day caused a 70 percent ($p<0.0005$) inhibition of CNV compared to control animals (Figure 1). Doxycycline elevated PEDF levels in plasma, but did not affect the plasma pro- and active MMP-2 and MMP-9 levels. However, doxycycline inhibited the enzymatic activity of the purified MMP-2 and MMP-9 enzymes and the in situ gelatinolytic activities in RPE/choroid sections.

We concluded that doxycycline inhibited the progression of experimental CNV, elevated the plasma PEDF protein levels and inhibited the catalytic activity of MMP-2 and MMP-9 without affecting their protein levels. These results suggest a potential therapeutic role for doxycycline as an anti-angiogenic agent.

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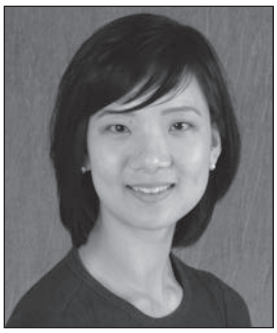
The Functional Link between Mitochondrial Biogenesis and Neuronal Differentiation: Clinical Implications for Mitochondrial Encephalopathies



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Mitochondrial encephalopathies are diseases caused by mitochondrial dysfunction due to impaired oxidative phosphorylation, resulting in decreased energy production in the form of ATP.[1] The brain, a highly energy-dependent organ, is especially vulnerable to the effects of the mitochondrial dysfunction in mitochondrial encephalopathies.[2] Mitochondrial diseases are especially challenging due to their diverse spectrum of manifestations and severity of the symptoms. It is also difficult to identify genetic mutations in mitochondrial or nuclear DNA. Clear basic pathogenetic mechanisms underlying mitochondrial diseases, as mitochondrial dysfunction gives rise to pleiotropic effects.[3,4]

As little is known about the functional link between mitochondrial biogenesis and neuronal differentiation, our study focused on the transcriptional regulation of mitochondrial biogenesis during the early steps of neurogenesis, more specifically during the transition from progenitors to differentiating neurons. We used the cellular paradigm PC12-ND6, a stable cell line engineered by Anne Chiaramello, PhD, Department of Anatomy and Regenerative Biology, The George Washington University School of Medicine and Health Sciences overexpressing the neurogenic transcription factor NeuroD6 (ND6), which is critical for the neuronal differentiation of progenitor cells during corticogenesis. It previously has been shown by Dr. Chiaramello's laboratory that NeuroD6 is, by itself, sufficient to promote spontaneous differentiation and neuronal survival.[5] Genomic studies revealed that NeuroD6 induces the expression of several mitochondrial genes implicated in oxidative phosphorylation and mitochondrial integrity.[6]

We investigated the functional link between NeuroD6 and the NDUFS4 subunit of the mitochondrial complex I, since our genomic studies showed increased expression NDUFS4 upon NeuroD6 expression. Furthermore, mutations in the NDUFS4 gene are frequently associated with mitochondrial encepalopathies.[7]

Our results provide the first demonstration that mitochondrial biogenesis occurs during the very early stages of neuronal differentiation, at the lamellipodia stage preceding axonal and dendritic outgrowth and development. Our results also revealed that NeuroD6 is able to induce mitochondrial biogenesis in a manner similar to NGF. Finally, we found that NDUFS4 expression levels increased concomitantly with NeuroD6-induced mitochondrial biogenesis. Thus, this work suggested that NeuroD6 may be a potential novel target for mutations associated with mitochondrial encephalopathies. Collectively, these results may provide molecular clues regarding the neuro-pathology associated with mitochondrial encephalopathies.

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Enhanced DNA Repair and Cellular Death Resistance: A Model for Early Events in Carcinogenesis

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How does a healthy, normally functioning cell become malignant? The regulation of cell death constitutes a critical response after carcinogenic genotoxin exposure.[1,2] It is believed that cell cycle arrest allows sufficient time for surveillance of damaged DNA, subsequent repair, and/or activation of apoptotic pathways. However, many of the early transforming events that occur in carcinogenesis are only now becoming better understood. Signaling pathways that regulate apoptosis are altered in the process of carcinogenesis, resulting in enhanced cell survival. Notably, cellular death resistance is thought to be at the foundation of neoplastic evolution. I learned many aspects of cellular death resistance and its potential relationship to the early stages of carcinogenesis while working in the laboratory of Susan Ceryak, PhD, MBA, associate research professor, Department of Pharmacology and Physiology, The George Washington University School of Medicine and Health Sciences. Her laboratory employs a clonal population of human fibroblasts that have survived genotoxin exposure and have acquired a general resistance to genotoxin-induced clonogenic lethality.[3,4] These cells have gained selective growth advantages that are characteristic of neoplastic cells, resulting in a survival phenotype. The goal of the laboratory is to identify the molecular mechanisms underlying this acquired resistance to genotoxin-induced cell death.

I tested the hypothesis that cellular death resistance following carcinogen exposure is associated with altered DNA repair. I analyzed DNA damage after genotoxin exposure in death-resistant and death-sensitive cells by using the "Comet" assay, a technique that measures DNA strand breaks.[5] The respective cell lines were exposed to a genotoxin (hydrogen peroxide), following which the cells were immersed in agarose on a glass slide, the nuclei were selectively lysed, and the cells were electrophoresed. During electrophoresis, broken DNA pieces migrated out of the nucleus, forming "comets," which were visualized with a fluorescent stain. Our experimental conditions specifically allowed for the detection of double-stranded DNA breaks, which are lethal if unrepaired.

In a series of experiments, 100 µM hydrogen peroxide exposure for 30 minutes caused extensive DNA strand breaks in both death-resistant and death-sensitive cells compared to the untreated control (Figure 1). However, when the cells were allowed to recover for three hours after removal of the hydrogen peroxide, the death-resistant cells showed markedly reduced DNA strand breaks, compared to the death-sensitive cells (Figure 2). These results suggest that the death-resistant cells undergo greater or faster DNA repair than the death-sensitive cells, which could be a key determinant of cellular death resistance. Current studies continue to investigate and

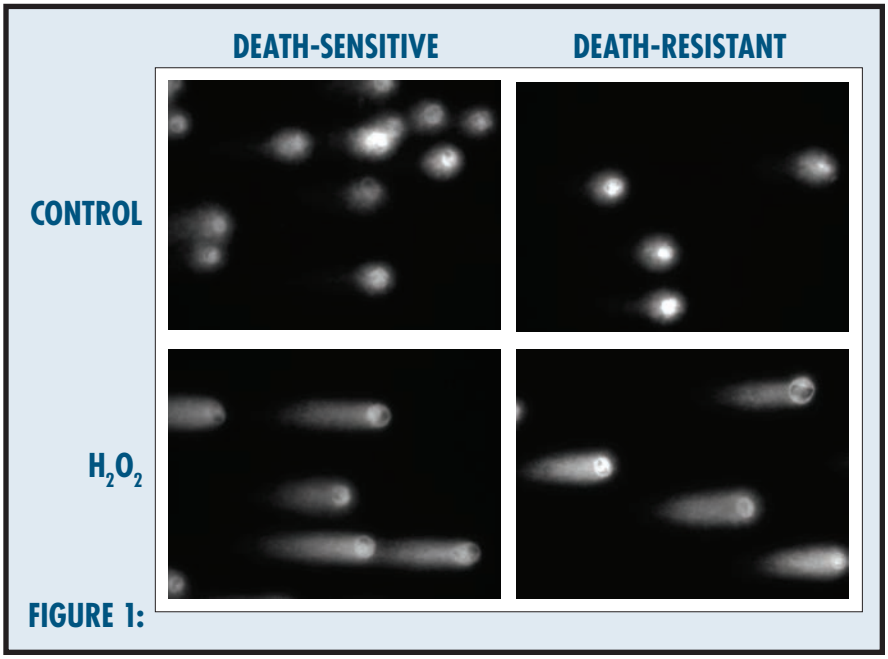

FIGURE 1: H_2O_2-induced comet tail formation in death-sensitive and death-resistant fibroblasts. Cells were incubated with 100 µM H_2O_2 for 30 min, and the cells were harvested and the cell membrane was permeabilized. The cells were then embedded in LM agarose on CometSlides and electrophoresis was carried out at 20 V for 30 min. The slides were then stained with SyBr Green to visualize cellular DNA and viewed with an Olympuslx70 fluorescent microscope.

CARCINOGENESIS *Continued on p. 13*

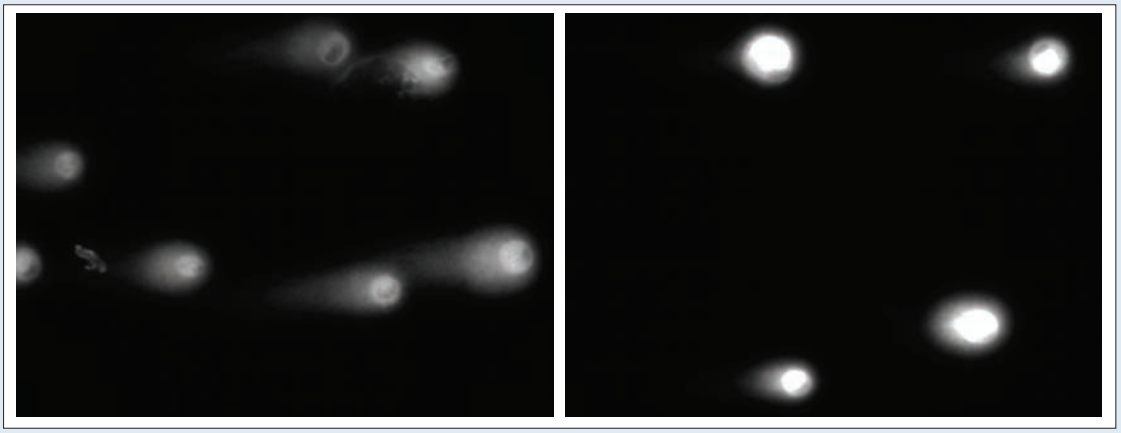

FIGURE 2:

FIGURE 2: Death-resistant cells repair DNA strand breaks faster than death-sensitive cells. Cells were incubated with 100 μM H_2O_2 for 30 min, at which time the cells were washed with PBS, the medium was replaced, and the cells were allowed to recover in the absence of H_2O_2 for three additional hours. At this time, the cells were harvested and DNA damage was assessed by Comet assay, as described in the legend of Figure 1.

CARSINOGENESIS

Continued from p. 12

confirm these preliminary data. Future studies will determine whether the enhanced DNA repair capacity following genotoxin exposure will lead to genomic instability in these death-resistant cells. The results from these present and future studies will have a substantial impact on our understanding of carcinogenesis and early oncogenesis as well as significant clinical implications related to experimental therapeutics.

I would like to acknowledge and thank Dr. Ceryak and Kristen Wright for their time and guidance throughout the summer, as well as the entire laboratory staff.

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Femoral Valgus With or Without Salter Osteotomy is Effective for Advanced Femoral Head Osteonecrosis in Pediatric Patients

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Osteonecrosis, or avascular necrosis (AVN), is the interruption of blood supply to cortical bone leading to ischemia, bone death, and the collapse of weight-bearing joints. The development of osteonecrosis in the pediatric and adolescent population is uncommon, but the destructive pathology of the condition necessitates a thorough evaluation of the best treatment options in this population. The vascular anatomy of the hip makes the femoral head particularly vulnerable to AVN following interruption of its blood flow through fractures of the femoral head and neck, slipped capital femoral epiphyses, developmental dysplasia of the hip, and in Legg-Calve-Perthes Disease, among a variety of other conditions.[1] In the pediatric and adolescent population, poor treatment outcomes have devastating implications with AVN accounting for 12 percent of all yearly hip replacements (including adult hip replacements). The treatment consensus in the current literature focuses on preserving an anatomic relationship between the femoral head and the acetabulum, with the explicit goal of avoiding hip replacement in the pediatric and adolescent population.

The current literature lacks a truly comprehensive examination of the use of a valgus osteotomy to treat osteonecrosis of the femoral head in the pediatric and adolescent population.

The most effective treatment method for AVN in the pediatric population has long been debated. Research has proven non-operative interventions such as bracing ineffective.[2] Surgical procedures including distraction arthroplasty, varus, and Salter osteotomies have been proven effective at delaying hip replacement by shifting weight bearing to the healthy portion of the femoral head.[3,4] The current literature lacks a truly comprehensive examination of the use of a valgus osteotomy to treat osteonecrosis of the femoral head in the pediatric and adolescent population. To explore the efficacy of a valgus osteotomy in treating AVN in the pediatric population, we conducted a retrospective study of 19 children treated with valgus with or without Salter osteotomies for advanced femoral head osteonecrosis.

In our cohort, the average age at time of surgery was 9.8 years with an average of 37.5 months of follow-up. Ten children underwent a valgus osteotomy while the remaining nine received a combination valgus and Salter osteotomy. In order to obtain quantifiable outcome measures, subjective determination of the children's pain and activity limitation was determined from chart review. Objective classification of the children's pre- and post-operative X-rays was performed. Goniometric measurements of flexion, abduction, adduction, internal, and external rotation were also obtained from the charts.

For many of the children in the study, the most telling subjective measure of their improvement following the valgus osteotomy was the significant decrease in pain related by the child. This decrease is best illustrated by the 10 cases in which pre-operative pain was moderate/severe and improved to mild/none following surgical correction. Similar findings were noted in activity limitation where seven children reported severe activity limitation pre-operatively and no limitation post-operatively. Significant improvement in abduction and external rotation was also observed.

The results of this study suggest that proximal femoral valgus osteotomy is an effective

FEMORAL VALGUS *Continued on p. 15*

FEMORAL VALGUS *Continued from p. 14*

treatment method for osteonecrosis of the femoral head in the pediatric population. This study is not a definitive statement on the efficacy of valgus osteotomy for avascular necrosis of the femoral head in children and adolescents. It is, however, a building block for future studies that can be aimed at determining the precise indications and contraindications for the procedure as well as better defining the device durability.

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Use of a "Hybrid" Locking Plate for Complex Metaphyseal Fractures and Nonunions about the Humerus

Anatomic location and bone quality are some factors that determine the complexity of fracture patterns. Proximal and distal humeral fractures and nonunions are examples of challenging injuries where the complex peri-articular anatomy, small fragment size of the short bony segments involved, and the osteopenic quality of the metaphyseal bone make stable fixation difficult.[1,2,3] Due to these anatomical difficulties, treating surgeons have sometimes been obliged to choose between treating fractures of the proximal and distal one-third of the humeral shaft with a smaller, "weaker" plate that allows more screws to be placed in the fracture segments, or a plate of larger diameter and greater implant strength with fewer screws in the short bony segments. Innovation in locking plate technology has improved our ability to treat fractures in osteoporotic bone and the development of "pre-contoured" or "anatomically correct" plate and screw designs has further advanced the ability to repair complex peri-articular fractures.

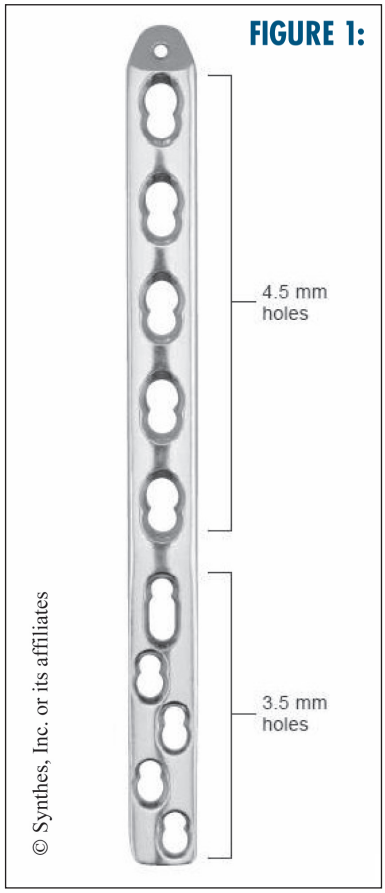

FIGURE 1:

The purpose of this retrospective study was to report on the use of a "hybrid" locking compression plate, the Synthes' Hybrid LCP Metaphyseal Plate (Figure 1, Metaphyseal Plate, Synthes, Paoli, Pa.), that possesses different size locking holes at each end, for the treatment of complex proximal and distal humeral shaft fractures and nonunions by a single surgeon at a regional academic trauma center. Our hypothesis was that treatment of difficult metaphyseal humeral shaft fractures with this novel implant would have higher union rates and lower complication rates than those previously reported for similar injuries treated with conventional locked plating.

HYBRID LOCKING PLATE *Continued on p. 16*

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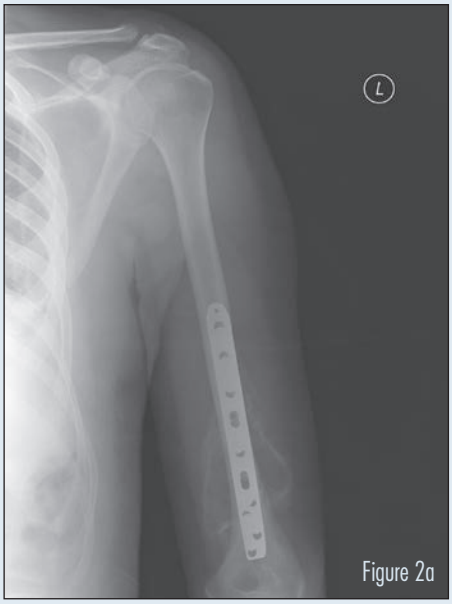
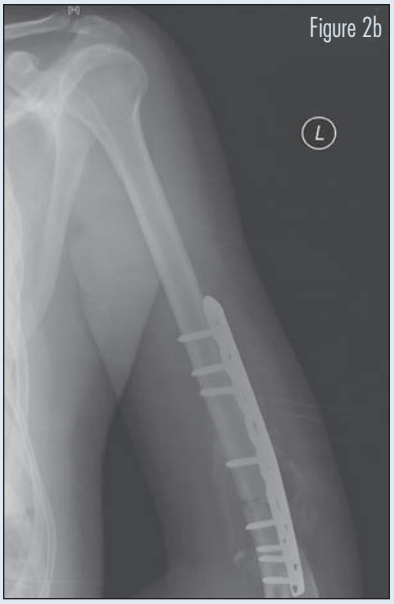
FIGURE 1: The Locked Metaphyseal plate utilized in this series.





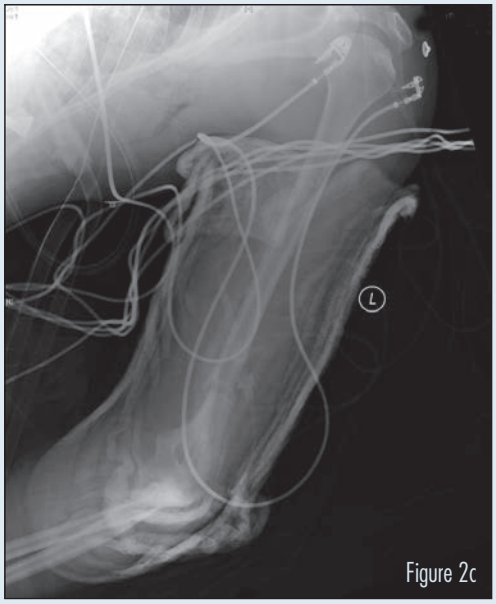


FIGURE:

2a. An AP trauma slot radiograph of a 32-year-old-male who sustained a high energy distal humeral shaft fracture and was treated with a synthes LCP metaphyseal plate.

2b. Post-operative AP radiograph at three months.

2c. Post-operative lateral radiograph at three months.

HYBRID LOCKING PLATE *Continued from p. 15*

Our cohort consisted of 21 patients with humerus fractures who were treated over a two-year period. Fourteen acute fractures and seven nonunions were identified in the study cohort. Our cohort consisted of six men and 15 women with a mean age of 49 years. All fractures were secured with a "hybrid" locking plate with a minimum of three 4.5mm screws on one side of the fracture and three 3.5mm screws on the other side (Figure 2). All patients were treated with a similar post-operative protocol for early range of shoulder and elbow motion.

All patients in our cohort healed their fracture or fracture nonunion demonstrating satisfactory functional and radiographic results. The mean time to union following surgical intervention was 4.5 months (Range 3-6). There were no implant-related complications and no small screw failures in this series. The metaphyseal locking plate seems to be ideal for situations in which it may be advantageous to place a greater number of screws within a small segment of bone, such as certain difficult fractures of the meta-diaphyseal humeral shaft.

We concluded that the Hybrid LCP Metaphyseal Plate is a useful new implant that maximizes the advantages of locked compression plating in the treatment of certain difficult proximal and distal one-third fractures of the humerus that are not amenable to traditional plate and screw fixation. Meta-diaphyseal upper extremity long bones may serve as the most ideal location for this implant. Our retrospective analysis provides clinical verification of our hypothesis and indicates that treatment of this category of fractures is a promising new application for the LCP Metaphyseal Plate.

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The Accuracy of a New External Anatomical Landmark on Visualization of Target Organs during the FAST Ultrasound Exam

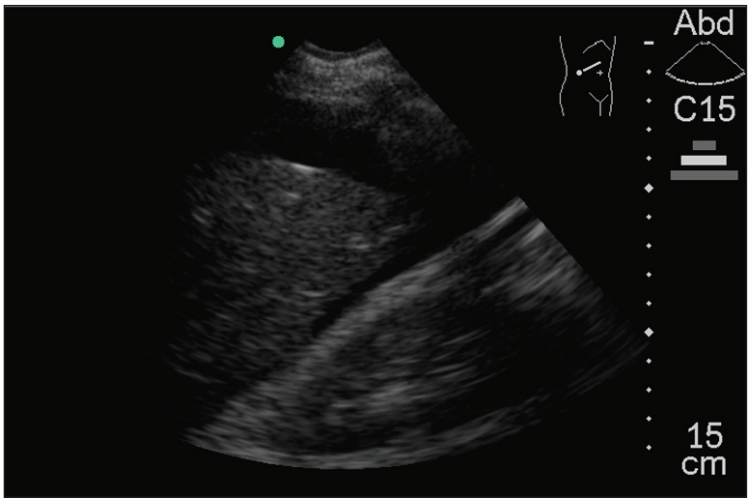

FIGURE 1: An ultrasound image of right upper quadrant showing a positive FAST result. Intraperitoneal fluid can be seen in Morrison's Pouch.

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Emergency Management Track
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The use of ultrasonography in trauma patients to assess the severity of intra-abdominal injury has become a frequent practice in surgery and emergency departments. With the development of the Focused Assessment with Sonography for Trauma (FAST) examination in 1996, surgeons and emergency physicians in the U.S. began using ultrasound as a faster, noninvasive method to evaluate abdominal trauma.[1] The FAST exam is currently used extensively as a diagnostic tool for detecting fluid in the pericardium and peritoneum, indicating serious injury (Figure 1).[2]

The current standard practice of ultrasound probe placement in the FAST exam for the right upper quadrant (RUQ) is to position the probe at the right midaxillary line between the 10th and 12th ribs where liver, right kidney, diaphragm, and Morrison's pouch should be identifiable in a coronal view. Similarly, the left upper quadrant (LUQ) probe position is at the left posterior axillary line between the 10th and 12th ribs where spleen, left kidney, diaphragm, and splenorenal recess should be identifiable. According to available literature, flawed techniques and provider inexperience often have detrimental effects on imaging procedure.[2]

The application of a simpler, more straightforward method of performing the exam using easily identifiable landmarks may improve technique and enable less experienced physicians to complete the exam faster and more precisely. The purpose of this study was to establish two external anatomical landmarks, the hepatorenal point (H point) for the RUQ and the splenorenal point (S point) for the LUQ, to be used by novice operators to accurately complete the FAST exam in an emergency situation to generate instantaneous images of the target organs that are diagnostically adequate in patients. The H point is the intersection of the horizontal subxiphoid line and right midaxillary line; while the S point is the intersection of horizontal subxiphoid line and left posterior axillary line.

In the first phase of the study, providers (medical students and residents) were trained to perform the FAST exam using the proposed external landmarks. Medical students also completed a pre- and post-test questionnaire to evaluate their learning curve. In the second phase, providers performed the FAST exam in the emergency department under the supervision of credentialed attending physicians. Adjustments necessary to obtain an adequate view of target organs away from the initial landmark-predicted points were recorded.

The site predicted by the landmark revealed the requisite organs at a rate of 82.4 percent at first attempt without need for further probe movement. There was a significant difference in precision rate between H and S points, at 89.6 percent and 75.2 percent respectively ($p < 0.01$). In patients with BMI>30, providers had more challenges in visualizing the LUQ target organs by following the landmark, however, providers with more than 10 previous FAST experiences did not encounter such difficulties.

In conclusion, the study results demonstrate that the H and S landmarks serve as simple and

ANATOMICAL LANDMARK *Continued on p. 18*

ANATOMICAL LANDMARK *Continued from p. 17*

precise guides that can facilitate the teaching and performance of the FAST exam in the hands of inexperienced providers, including pre-clinical and clinical medical students.

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Sexual Dimorphism in Humeral Bone Volume in the Young Adult Skeleton

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Bone volume is the result of a complex interaction of genetic as well as environmental factors.[1] For example, mechanical loading is an environmental factor that has been shown by experimental and observational data to affect bone size in a concept called "bone functional adaptation."[2] Data from the Functional Polymorphisms Associated with Human Muscle Size and Strength (FMS) study,[3] a multi-center study designed to evaluate the effects of genetic polymorphisms on bone geometry, fat volume, and muscle size and strength, offers the opportunity to investigate the relationship between bone volume and body weight, height, muscle size, and muscle strength (both isometric and isokinetic measurements), all potential sources of mechanical loading. The FMS study chose to investigate the humerus for several reasons, including its particular sensitivity for detecting genetic predisposition in bone and muscle health, as well as being a single bone and thus not subject to load sharing. It is also not weight-bearing and is therefore less affected by confounding factors such as fluctuations in weight or activity. However, the environment does play a substantial role in humerus bone development. The objective of this study was to find correlative evidence that bone volume is affected by factors of mechanical loading and then investigate how sex modulates that influence in a population of young, healthy adult males and females.

The young adult population of the FMS study gives a snapshot of the adult skeleton at the peak of bone growth in both males and females.

In this study measures of bone volume, muscle strength and size of the dominant and non-dominant humerus were determined in Caucasian subjects, male (n = 205) and female (n = 327). Subjects were between 18 and 40 years of age (males mean age = 23.9 ± 5.7, females = 22.8 ± 5.2). Muscle size (volume), and bone volume were determined from Magnetic Resonance Images (MRI) of the distal 9.6 cm of the humeral diaphysis using semi-automated software from Rapidia. Muscle strength was determined using both dynamic and isometric strength testing.

Both total bone volume and cortical bone volume was found to be highly correlated with weight in both males and females (Figure 1). Interestingly, the correlation was stronger between male bone size and weight (r^2 = 0.27) than in female bones (r^2 = 0.19). The difference between the sexes was even greater when comparing bone size to muscle strength, in both isometric and dynamic strength tests (Figure 2). Muscle volume is often considered representative of muscle strength in current research, however we found that while muscle volume did correlate with muscle strength, it did so at a lower than expected level, r^2 = 0.22, meaning only 22 percent of muscle strength could be accounted for by its size. In addition, they correlated differently when compared to bone size, with muscle strength correlating more strongly than muscle volume with bone size. Again, these relationships were found to be stronger in men than in women.

These results provide strong evidence that mechanical loading has a significant influence on bone volume. The young adult population of the FMS study gives a snapshot of the adult skeleton at the peak of bone growth in both males and

SEXUAL DIMORPHISM *Continued on p. 19*

FIGURE 1: Total Bone Volume vs. Weight in Females and Males.

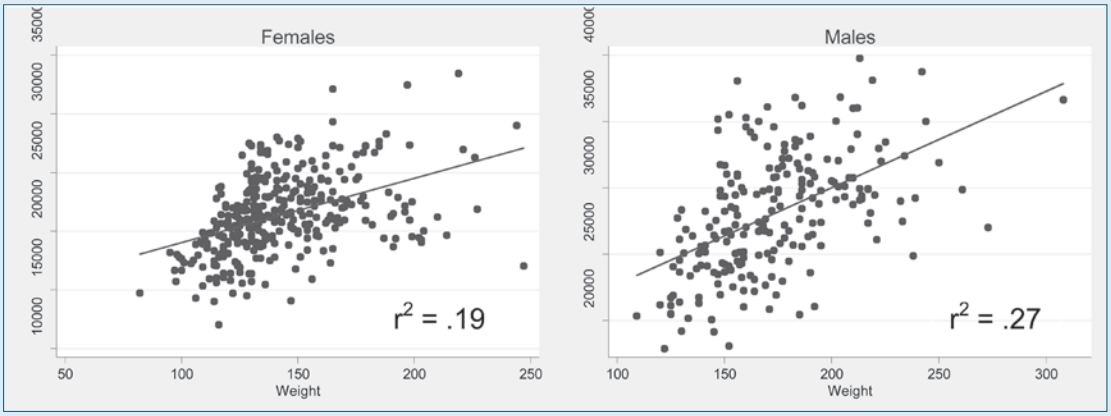
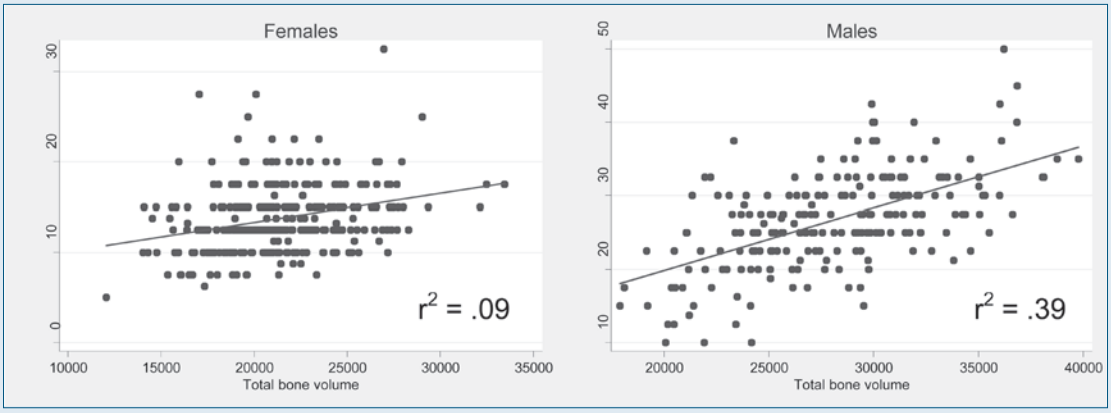


FIGURE 2: Dynamic Strength (one repetition max: 1-RM) vs. Total Bone Volume in Females and Males.



SEXUAL DIMORPHISM

Continued from p. 18

females. It also provides evidence of the end-result of the effects of mechanical loading factors including body weight and muscle strength, on the growth of the skeleton. Our study shows a clear difference within this population in the correlation between bone volume and the measurements of body weight and muscle strength between males and females, possibly showing the effects of sex on bone growth. Since females tend to stop adding bone on the periosteal surfaces after puberty, the stronger correlations found in this study between bone size and mechanical stress in males clearly reflect the difference in growth patterns between genders.

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BMP2 and BMP7: Combined Use in Complex Revision Reconstructive Spine Surgery

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Bone morphogenetic proteins (BMP), the only proteins known to have the osteoinductive capacity to generate new bone formation, were first isolated from bone 20 years ago.[1] BMPs are the focus of numerous studies seeking alternatives to the morbidity associated with autograft bone. In fact, studies have demonstrated that BMPs are a safe and effective alternative to allograft and autograft bone.[1,2] Two such BMPs, BMP 2 and BMP 7, are typically used independently. However, recent in vitro and animal studies have investigated a synergistic effect when BMP 2 and BMP 7 are combined.[3,4]

No clinical studies have investigated the use of combining BMP 2 and BMP 7 in lumbar fusions. Before addressing the efficacy of this combination in humans, it is important to establish the safety of combining these two materials in spinal surgery. This retrospective study aims to determine the prevalence of complications associated with combining BMP 2 and BMP 7 in posterolateral lumbar fusions. The study followed a cohort of patients who received a combination of BMP 2 and BMP 7 in posterolateral lumbar fusions. Medical records were reviewed to determine the prevalence of post-operative complications such as infection, inflammation, hematoma, nerve damage, ectopic bone formation, and allergic reactions. Potential post-operative complications were compiled from the package inserts of BMP 2 and BMP 7 as reported by their manufacturers. Patients were followed for an average of 4.5 months.

Cross-referencing the medical records of patients who had received BMP 2 and BMP 7 in posterolateral lumbar fusions revealed that 29 of 30 patients (97 percent) showed no post-operative complications associated with the combined BMP 2 and BMP 7 implant. One patient developed an infection at the surgical site that was cleared by oral antibiotics. There were no other cases of adverse events.

The results from this study suggest that patients who receive both BMP 2 and BMP 7 in a posterolateral lumbar fusion are not at any significant risk for complications arising directly from this combination. Future applications include studies seeking to measure the efficacy of this combination as well as to investigate other combinations that may be safely and effectively used to increase success rates in posterolateral lumbar fusions.

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Muscle Pain Detection Device in Properly Diagnosing Back Pain

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Regardless of their occupation, socioeconomic status, or ethnicity, patients can often experience back pain at various times in their lives. Back pain can range from minor to severe excruciating pain.

Unfortunately, there are a large number of patients whose pain is not alleviated by current physician interventions.

Dr. Norman Marcus, founder of The Norman Marcus Pain Institute (NMPI), challenges the well-accepted diagnostic and treatment tools for addressing back pain. In focusing on a more accurate way to diagnose, Dr. Marcus believes that the patients not benefiting from current methods of pain relief are not incurable but are instead misdiagnosed. Many physicians do not realize that back pain is the result of muscular injury as opposed to neurovascular. Dr. Marcus enhanced the techniques to evaluate and treat muscle pain taught to him by Hans Kraus, MD, father of sports medicine and back pain specialist to President John F. Kennedy.

Painful muscles may develop hardened areas of taut muscle fiber, or "trigger points," that are quiet at rest but if stimulated will produce pain. Various approaches have been suggested to treat these painful knots. The widely used method in determining the location of these painful nodules is manual pressure via hand palpations. At these points, physicians tend to inject 5 percent Lidocaine (numbing agent), steroids, or just a dry needle stick, which would all serve to relax the muscle and possibly the nerve.

Manual pressure to identify trigger points does not adopt consistent reliability between different examiners. In addition, the palpations are being conducted upon resting muscles, which will not elicit the accurate response as it would in an active muscle. In order to combat the aforementioned diagnostic difficulties, a Muscle Pain Detection Device (MPDD) has been formulated by Stevens Institute of Technology.[1] The MPDD, a small device, uses electricity to harmlessly promote a muscle contraction on selected muscles thus altering its activity, which in turn allows the physician to identify which muscles are inducing the pain.

By utilizing the MPDD, physicians can have access to a more reliable and valid evaluation of muscle pain. To investigate these results, a randomized controlled study of MPDD vs Manual Pressure was conducted. Forty patients with a three month history of back pain were divided blindly into two groups. Within each respective group, MPDD or Manual Pressure was utilized to identify the muscles creating the source of pain. All subjects were injected with 5 percent Lidocaine. Preceding the treatment along with one week and one month afterwards, the patients were given a physical exam and were asked to complete Oswestry and Visual Analogue Pain Scale questionnaires.

In order to combat … diagnostic difficulties, a Muscle Pain Detection Device (MPDD) has been formulated. The MPDD, a small device, uses electricity to harmlessly promote a muscle contraction on selected muscles thus altering its activity, which in turn allows the physician to identify which muscles are inducing the pain.

For both follow-up visitations, the MPDD group had an overall statistically significant improvement in mood, disability, and back pain ($P < 0.004 - 0.001$). At one month, there was a 53.2 percent reduction in pain in the Manual Pressure group compared to the astounding 82.5 percent ($P < 0.001$) pain relief in the MPDD group.

This study could revolutionize how back pain is perceived in the future. The MPDD should allow physicians to accurately diagnose the origin of the muscle pain and treat it more successfully. In appreciation to this investigation, back pain no longer has to possess an elusive diagnosis.

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Studying the Infant Brain at Rest: A Comparison of Functional Connectivity between Preterm and Term Infants

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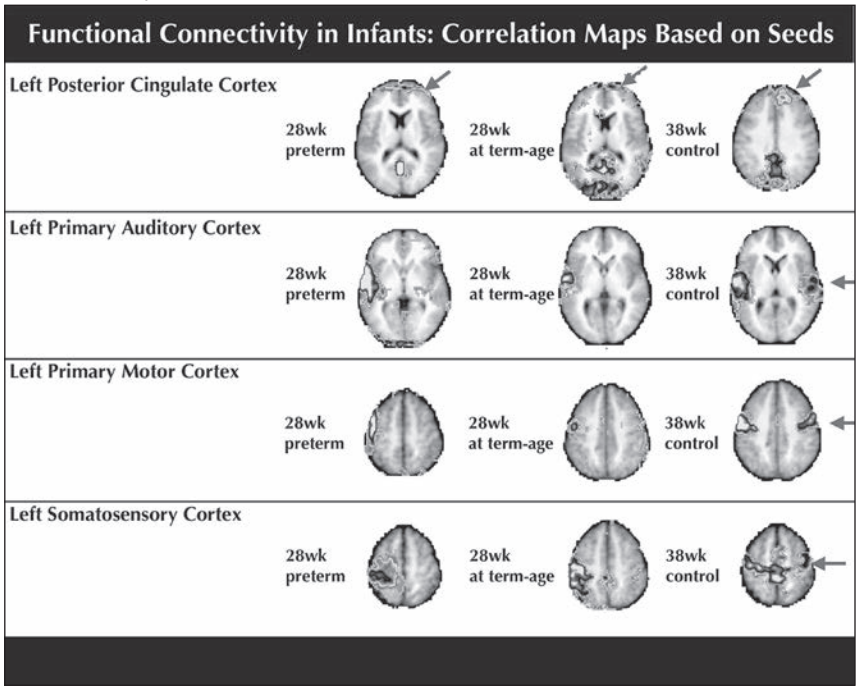
Preterm birth, defined as birth occurring earlier than 37 weeks gestational age (GA), is an increasingly important public health concern in the United States as the percent of preterm births has risen to 12.6 percent of all births in 2005.[1] Very preterm infants have significantly higher rates of cognitive impairment, sensory deficits, cerebral palsy, motor delay, and educational and social difficulties. Magnetic resonance imaging (MRI) examination for radiological abnormalities frequent in preterm newborns — intraventricular hemorrhage, periventricular leukomalacia, and other white- and grey-matter abnormalities — is predictive of these deficits.[2]

This study examined both preterm and term infants using a recently-employed imaging technique, functional connectivity MR, which examines spontaneous functional MRI (fMRI) signal fluctuations at rest. Functional connectivity has been extensively studied in adults and several regions have been identified as components of a "default mode network," or DMN. The DMN is a group of neuroanatomical regions that are thought to function together and have been hypothesized to form the basis of cognition and self-referential thought, as indicated by synchronous signal fluctuations identified by fMRI, PET, and EEG of resting individuals.[3] This resting-state network has been found to differ in children, who possess less connections than adults, and was shown to deteriorate in aging and Alzheimer's disease, thereby intimating its functional importance.[4,5] Only one study has been conducted in infants, who were preterm newborns scanned at term-equivalent age; this study found that infants did not appear to have a DMN similar to adults.[6]

Our study addressed two questions: first, do infants possess any elements of the DMN seen in adults, and second, do preterm infants show different patterns of resting-state activity? To answer these questions, we utilized a seeds-based correlational method, which looks at the fMRI time-course within a selected region of the brain and compares it to the rest of the brain. If a disparate region has a similar pattern of signal over time, it is thought to be functionally connected to the region chosen. Results are depicted in Figure 1.

We identified one component of the adult DMN in both preterm and term infants, the anteroposterior connection between prefrontal cortex and posterior cingulate cortex. This finding differs from that of a previous study in infants and indicates that infants may have greater maturation in the DMN than previously thought.[6] There was no apparent difference in resting state activity when comparing GA, but our study was limited to a set of 14 infants, four of which were term controls. Another important finding was that term infants showed greater interhemispheric correlations in functionally related regions such as the primary auditory, motor, and somatosensory cortices, whereas preterm infants had largely unilateral correlations. These findings have been demonstrated in EEG studies and suggest that, although interhemispheric anatomical connections exist much earlier, preterm infants may not have functional networks across hemispheres.[7] Functional connectivity

FIGURE 1: Representative examples of correlation maps generated by comparing seed regions (noted on left) to the individual subject's fMRI at-rest. Seeds placed in the posterior cingulate cortex showed functional connectivity with the prefrontal cortex (arrows) in all groups and indicates elements of the DMN. Term infants had greater bilateral connectivity shown in the primary auditory cortex, primary motor cortex, and somatosensory cortex.



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INFANT BRAIN *Continued from p. 22*

may facilitate a greater understanding of the mechanisms by which the brain functions and how the disruption of this connectivity reflects deficits in cognition and functional impairment seen in preterm infants later in life.

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Use of Trabecular Metal Implants in Anterior Cervical Discectomy and Fusion: A Two-Year Retrospective Study

Anterior cervical discectomy and fusion (ACDF) is a surgical procedure indicated in the treatment of compression injury in the cervical spine as a result of degenerative disc disease and herniated discs. ACDF is designed to restore strength and stability to cervical vertebrae through the removal of damaged or diseased intervertebral discs and insertion of fusion-promoting bone grafts or synthetic interbody cage devices. Successful vertebral fusion results in restored intervertebral disc height, alleviation of spinal cord compression, maintenance of cervical lordosis, and reduction in pain.[1]

Bone grafts, the implants of choice since the first ACDF was performed in 1953, have recently fallen out of favor due to complications such as graft expulsion, pseudarthrosis, and increased infection risk at the harvest site.[2] In particular, autologous bone grafts are associated with increased donor-site morbidity.[3] Allograft bone grafts, while eliminating donor-site complications, are associated with inferior fusion rates.[2] Synthetic interbody cages, such as trabecular metal implants, intend to eliminate the complications of bone grafts while providing the benefits of intervertebral fusion. The use of trabecular metal implants has increased in the past decade due to its comparable characteristics to cancellous bone.[1] Trabecular metal is a highly porous composite of tantalum metal and vitreous carbon with inherent osteoconductive characteristics, such as high volumetric porosity, high frictional characteristics, excellent corrosion and erosion resistance, and a low modulus of elasticity. These features, which closely resemble cancellous bone, enhance the biocompatibility of the metal and promote rapid vascularization and tissue infiltration at the site of fusion.[1]

The primary objective of this study was to validate the use of trabecular metal implants in ACDF by measuring and comparing preoperative and 24 months postoperative clinical and radiographic outcomes. The two-year mark was established to ensure spinal fusion could be successfully determined and degeneration had not occurred at levels above or below the implant (a common problem with allograft implants). Inclusion criteria focused on male and female patients, ages 30 to 90 years old, who underwent either a single or multi-level ACDF with trabecular metal implants during the study period from May 2004 to September 2008. The primary radiographic endpoint was evidence of fusion,

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which was determined by percentage of lucency at each vertebral body, Cobb angle analysis, change in hardware position, spinous process motion in flexion/extension views, and percent lordosis change at each vertebral level. Initial radiographic analysis indicated all 30 patients had complete fusion at 24 months with no need for revision surgery. Primary clinical endpoints were obtained comparing preoperative and 24-month survey scores from the SF-36, the Neck Disability Index (NDI), and the Visual Analog Scale (VAS). The SF-36 measures functional social, physical, and mental health using a 100-point scale. Initial survey data indicated improvements of 13.3 points and 13.2 points in the physical and mental component scores, respectively. The NDI, also using a 100-point scale, noted a 33.8-point reduction in neck disability. Additional analysis is ongoing, but the positive fusion rates and improved clinical outcome data suggest the use of trabecular metal implants in ACDF should be explored further as a viable alternative to bone grafts.

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Functional Connectivity of Language Processing Development

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Language dominance appears to be established between four to seven years, but few functional Magnetic Resonance Imaging (fMRI) investigations focus on development of functional connectivity of language networks in healthy children known to engage receptive (listening) and expressive (speaking) language regions. Functional connectivity is defined as the temporal correlation between spatially remote neurophysiological events.[1]

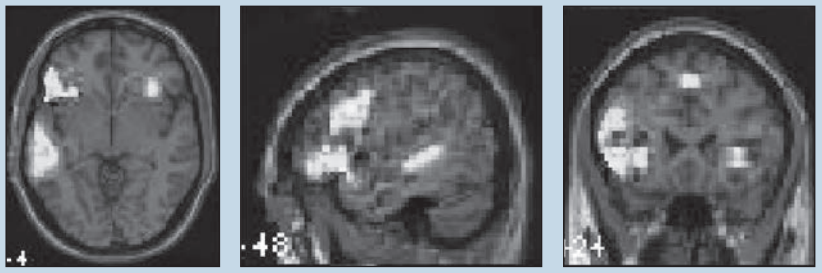

FIGURE 1: Group activation maps display areas of activation during the auditory description decision task. Regions of interest selected for study included the L-IFG (Broca's area), L-MFG, L-MTG and L-STG (Wernicke's area), and right sided homologues. Left-sided activation predominates, reflecting lateralization consistent with age-dependent development.

We performed a cross-sectional functional connectivity analysis on fMRI data collected from an auditory description decision task from 58 normally developing right handed children aged four to 12 years. The active condition requires a semantic decision based on a word definition while the control condition consists of reverse speech with tone identification. Regions of interest implicated in language-processing — the left inferior and middle frontal gyri (L-IFG and L-MFG), Wernicke's area (L-WA), and right hemispheric homologues — were examined. Functional connectivity was measured between the left networks and their right homologues. Paired regions were also compared across age groups, in relation to neuropsychological measures, and behavioral data.

Significant connectivity by age group interaction was found for L-IFG (p=0.03) and L-MFG (p=0.01). Post hoc analysis revealed age-related trends indicating 4–6 year-old children have less connectivity between L-IFG and L-WA than both 7–9 year-olds (p=0.08) and 10–12 year-olds (p=0.11). There was also an age-related trend for 4–6 year-old children having more connectivity between L-MFG and R-MFG than the 10–12 year-olds (p=0.07). We found strong connectivity between homologous regions that persisted across age groups (IFG r=0.6437; MFG

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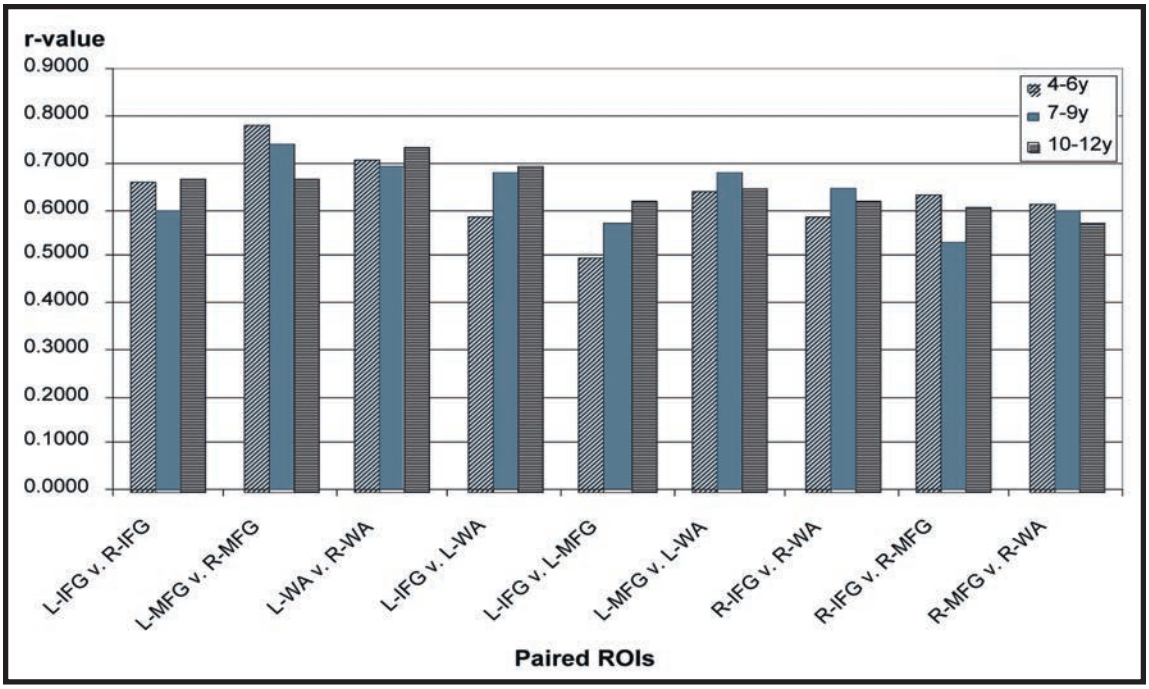

FIGURE 2: Relationship of age to connectivity (Pearson's r-value).

LANGUAGE PROCESSING *Continued from p. 24*

r=0.7207; WA r=0.7152). The L-IFG—L-WA connection positively correlated with expressive one word vocabulary (EOWV) (p=0.02) and trends were found for the WA and IFG homologues with other neuropsychological tests. There were no significant differences for behavioral measures.

The modest age-related findings reflect known anatomical and structural developments. Specifically, the greatest age-related changes occurred in the L-IFG and L-MFG, regions that demonstrate extended periods of maturation.[2] Additionally, the greater connectivity between left Broca's area and left Wernicke's area in the 7–9 and 10–12 year-olds relative to the 4–6 year-olds, reflects strengthening of that network, perhaps due to pruning of redundant or less utilized networks and consolidation.[3] This reveals a mature arcuate fasciculus, the primary connection demonstrated in anatomical studies.[4] The decrease in the L-MFG—R-MFG connection may represent the lateralization of attention and working memory functions that normally occurs with increases in age in right-handed individuals.[5] The protracted development of the frontal lobe may explain the significant age group interactions found in the L-IFG and L-MFG, while connections solidified outside this age range appear age-invariant.[6] Connectivity correlations with performance on the EOWV test may reflect the observation that Broca's area matures earlier than the more posterior language cortices[7] leading to more conspicuous age-related changes in the expressive task within this pediatric population. Strong connections between homologous regions suggest a great deal of cross-talk, even after laterality has been established, which may represent left networks modulating the activity of their right homologues, task-dependent recruitment, or inter-hemisphere connections that serve as a reserve in the setting of injury.

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The Relationship between Health Care Access and Disease Activity and Damage in a Multiethnic Cohort of Systemic Lupus Erythematosus Patients: The 1000 Canadian Faces of Lupus Study

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Systemic lupus erythematosus (SLE) is a chronic autoimmune disease, whose prognosis can be influenced by environmental factors. The importance of access to care and its effects on SLE outcome is not well defined in the literature and measures of access have varied widely between studies. The first five years of the Lupus in Minorities: Nature Versus Nurture (LUMINA) longitudinal study indicated a perceived difficulty in obtaining health care to be more frequently reported in SLE patients who subsequently died (21.4 percent) compared to surviving patients (3.8 percent, $p = 0.002$).[1] Treating early in disease onset and closer follow-up visits were hypothesized to contribute to improved survival in SLE.[2] Universal health care in Canada provides a unique setting for our investigation by minimizing effects of income on access. Therefore this study was designed to determine whether care barriers are associated with increased disease activity and damage in a multi-center, multiethnic Canadian SLE cohort. We also compared concordance between care barriers as reported by the patient and lupus specialist.

This was a retrospective study using data from 14 Canadian centers with annual visits from patients with SLE. The patients' prognosis was evaluated based on self-reported access to care. Access to care measures included availability of a family physician, financial barriers such as affording medication, and perceived difficulties in accessing rheumatology clinics and medications. Data including demographics, treatment, disease activity, and damage were analyzed. Damage was assessed using the SLICC ACR damage index developed by the American College of Rheumatology. Patient-reported access to care included availability of a family physician, financial barriers, and perceived difficulties in accessing rheumatology clinics and medications. We also compared patient's assessment of care barriers they believed they faced, to what barriers their physicians thought might be impeding the patient's access to care.

Our patient population extracted from the Canadian "1000 faces of Lupus" database consisted of 654 patients. It is important to note that all patients had access to a lupus specialist yet many still faced barriers to care. Just over half (50.8 percent) of the patients perceived a care barrier despite universal health care in Canada; 27.5 percent found cost of medication as a barrier to care, 19.6 percent reported waiting to see a rheumatologist as a barrier, 32 percent reported traveling to a rheumatologist's office as a barrier, 7.6 percent reported access to medication as a barrier and 8.6 percent reported cost of medical devices as a problem.

Doctors and patients did not seem to identify similar care barriers ($r = 0.09$). Doctors correctly identified only half the patients who had access to medication problems ($p = 0.003$). Lack of access to medication and costs were significantly associated with co-morbidity ($p < 0.001$, $p = 0.04$). Access to medication from the physician's perspective was positively associated with SLE Disease Activity Index ($p = 0.03$) and cost of medication ($p = 0.04$). Physician perceived barrier of access to medication was associated with SLE activity ($\beta = 1.14$, $p = 0.001$).

We concluded that access to a lupus specialist does not necessarily imply adequate access to health care. Care barriers are important for lupus outcomes and are grossly underestimated by physicians. In health care it is essential for physicians to consider and recognize barriers to care when trying to improve the health status of their patients. More emphasis in physician training needs to be placed on recognizing and accommodating for these barriers in patient care. This study was a "best case scenario" where lupus patients were seeing a specialist and thus were entered into a lupus registry. This is unusual in the lupus population and especially in the United States where there are gaps in health insurance and thus the barriers to care are likely to be far greater.

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A Neglected Tropical Disease: Hookworm Up Close and Personal in Americaninhas, Brazil

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Neglected tropical diseases (NTDs) impose a great burden on the global population, especially those living in poverty. Approximately one billion people are infected by one or more of the NTDs. Together, these diseases account for a greater portion of the total global Disability Adjusted Life Years (DALYs) than tuberculosis or malaria combined. Of these diseases, human hookworms infect up to 576 million people worldwide. The majority of these infections are by the Necator americanus and Ancylosoma duodenale species and occur mostly in sub-Saharan Africa, China, Southeast Asia, and Latin America.

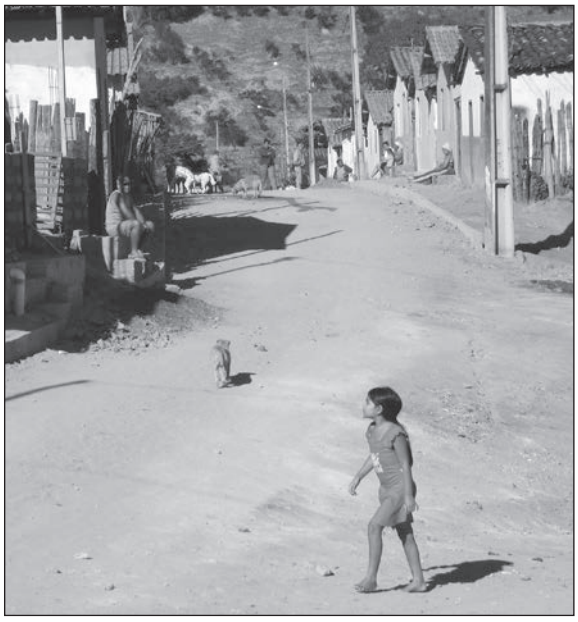
The impact of infection is greatest in children and pregnant women. Hookworm infection presents as severe anemia when the larvae embeds itself into the host intestinal wall, constantly causing cell injury and blood loss. There is stunted physical and cognitive development in children. Infected pregnant women have higher mortality and morbidity, as they are already anemic; the fetus is indirectly harmed.

Hookworm is easily treatable with oral medications such as Albendazole. However, high reinfection rates necessitates the development of an efficacious and cheap vaccine to be developed. Consequently, the Sabin Institute's Human Hookworm Vaccine Initiative (HHVI), in collaboration with The George Washington University and Fundação Oswaldo Cruz, is conducting a hookworm vaccine clinical trial in the rural town of Americaninhas, Brazil. Americaninhas is located in a rural area approximately 500km from HHVI's laboratory in Belo Horizonte.

My primary role at the Americaninhas site was to shadow the HHVI physician as she rotated between various clinics during her monthly visit to the area. Rural residents do not have ready access to care, therefore, both HHVI and non-study patients are seen. In contrast to how medicine is practiced in the United States, the HHVI physician focuses solely on the history and the physical exam. Lacking modern diagnostic tools, we used only a stethoscope, sphygmomanometer, and thermometer. I conducted the heart, lung, and abdominal exams. Many patients presented with conditions I will never see in the U.S. For instance, a family of eight was diagnosed with polyinfections of hookworm, ascaris, and schistosoma. A young woman complaining of joint pains and fever was diagnosed with dengue fever using only a sphygmomanometer. Virtually every patient we treated was infected with hookworm, underscoring the need for a vaccine.

The remainder of my summer was spent in the Belo Horizonte laboratory where I conducted Hemoquant and ELISA assays. Hemoquant quantifies hemoglobin in the feces of hookworm infected individuals. The primary goal of the project was to standardize the number of hookworm eggs to hemoglobin detected in feces by analyzing 1,000 fecal samples collected from patients living in Americaninhas and surrounding towns. Another project was to conduct ELISA assays and detect antibody response to a hookworm antigen.

My experience in Brazil allowed me the opportunity to interact with unique patients and work on a vaccine clinical trial while learning about the Brazilian culture, language, and health care system.



Above, Americaninhas, Minas Gerias, Brazil

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The Future of HIV Vaccine Research and Development

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The human immunodeficiency virus (HIV) has defied a quarter century of attempts to create a safe and effective vaccine.[1] More than 100 candidate vaccines have been evaluated in various stages of clinical trials; all of which have failed.[2] Recently, federal officials halted plans to advance a promising new HIV vaccine into a large clinical trial; the consensus being that researchers need to return to the bench before again proceeding to the bedside.[3] This current state of affairs has significant implications for future investment and progress in HIV vaccine research and development.

In order to work, a protective HIV vaccine must teach the body's immune system to recognize specific viral proteins and to attack both the virus itself as well as virally-infected cells. Early efforts focused on the HIV envelope proteins, which represent the targets of neutralizing antibodies produced by B-cells.[4] These viral surface proteins are often highly variable, however, owing to the highly mutagenic nature of HIV. This means that a vaccine must invoke an exceptionally broad immune response in order to be effective.[5] In more recent efforts, as the importance of T-cell responses in the control of HIV infection became appreciated, researchers broadened their scope to evaluate vaccines that incorporated the more conserved internal proteins of HIV.[4] Although only antibodies can actually prevent infections by neutralizing the virus itself, a vaccine that aims to harness T-cells would theoretically keep the viral load in check and prevent the progression from HIV infection to AIDS.[6] Over time, this strategy might even deplete the viral load due to incomplete replication of the virus within cells that are being killed. However, this approach is also inherently problematic because HIV selectively infects the CD4+ subset of T-cells, which are critical in initiating and maintaining an adaptive immune response.

Most of the early efforts in HIV vaccine research and development were spearheaded by the pharmaceutical industry, but as the likelihood of a quick success decreased, private investment began to wane.[7] At that time, HIV vaccine research began and continues to follow a somewhat atypical paradigm, with the public sector supporting a greater share of all phases of research and development. Throughout this period, the public sector has been trying to employ mechanisms to engage industry while continuing to support investigator-driven research within academia. In 2004, three-quarters of all global expenditures for HIV vaccine research and development were estimated to have come from the U.S. government.[4] Still, the National Institute of Allergy and Infectious Disease (NIAID) — the division of the National Institutes of Health (NIH) that undertakes most of the publicly supported HIV/AIDS research — devoted only 28 percent of its HIV research funding to HIV vaccine research efforts.

Scientific uncertainty appears to be the most important reason for low investment in HIV vaccine research and development.[7] There is an exceptionally poor understanding of the immune response needed for successful protection from HIV. Due to the inherent complexity of the situation with HIV, members of industry are largely unwilling to expose themselves to this degree of financial risk and potential for failure, especially when a substantial reward is unlikely. Vaccines, in general, have a more limited market and lower return on investment compared to therapeutic drugs. In this case, potential sales of all vaccines in the worldwide HIV space are estimated to represent only about $6.5 billion annually, or 2 percent of the global pharmaceutical market, an amount that is roughly equivalent to the sales of one successful ulcer drug.[8]

In order to increase private investment in this space, there will probably have to be a combination of push strategies, which reduce the cost and scientific risk of investment, and pull strategies, which guarantee a viable market. While push strategies are particularly important during this early stage of vaccine development, pull strategies will become important for poorer markets once the scientific problems have been solved. Unfortunately, the vicious cycle of HIV vaccine research and development will likely persist for quite some time, as scientific and fiscal barriers make industry reluctant to invest in the basic

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science research and clinical trials that are required to solve them.

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BASIC SCIENCE ABSTRACTS

CLINICAL PRACTICE ABSTRACTS

RESEARCH AND TRAVEL ABROAD ABSTRACTS

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Fusion

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Submissions from the classess of 2010, 2011, 2012, as well as the incoming class of 2013 for next year's edition of the journal will be accepted beginning September 2009. More information about the submission process will be provided during the summer of 2009. If students have any questions or comments, please contact the Beaumont Society at *gwbeaumont@gmail.com*.

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